UNIVERSITY OF CALIFORNIA SAN DIEGO

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Hungry Hungry Hippocampus: Effects of overweight/obesity and risk for obesity on hippocampal structure and functioning in children and adolescents

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Clinical Psychology

by

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The dissertation of Zoe Mestre is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATION

I dedicate this dissertation to my wonderful parents Daniel and Cheryl who have always supported and encouraged me, my life partner Tyler who pushes me every day to be a better version of myself, my sister Melissa, who has been there for me through all the ups and downs, and my brother Eliot for reminding me of what's important in life. I could never have gotten to this point without their love, guidance, and firm determination to see me thrive and grow into the clinician and scientist that I am today.

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Eichen, D., **Mestre, Z**., Strong, D., Rhee, K. E., & Boutelle, K. (2020). Defining and identifying predictors of rapid response to pediatric obesity treatment. *Pediatric Obesity 15(2):e12621. doi:* 10.1111/ijpo.12621

Zlatar, Z. Z., Hays, C.C., **Mestre, Z.**, Hays, C.C., Bangen, K. J., Liu, T. T., Kerr, J., & Wierenga, C. E. (2019). Dose-dependent association of accelerometer-measured physical activity and sedentary time with brain perfusion in aging. *Experimental Gerontology, 125*(2019): 110679. doi: 10.1016/j.exger.2019.110679

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Mestre, Z. L., Bischoff-Grethe, A., Eichen, D. M, Wierenga, C. E., Strong, D., & Boutelle, K. N. (2017). Hippocampal atrophy and altered brain responses to pleasant tastes among obese compared with healthy weight children. *International Journal of Obesity*, *41*(10), 1496-1502. doi: 10.1038/ijo.2017.130.

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Mestre, Z. L., Melhorn, S. J., Askren, M. K., Tyagi, V., Gatenby, C., Young, L., Mehta, S., Webb, M. F., Grabowski, T. J., & Schur, E. A. (2016). Effects of Anxiety on Caloric Intake and Satiety-Related Brain Activation in Women and Men. *Psychosomatic Medicine, 78(4), 454-64. doi: 10.1097/PSY.0000000000000299*

Askren, M. K., McAllister-Day, T. K., Koh, N., **Mestre, Z.,** Dines, J. N., Korman, B. A., Melhorn, S. J., Peterson, D. J., Peverill, M., Qin, X., Rane, S. D., Reilly, M. A., Reiter, M. A., Sambrook. K. A., Woelfer, K. A., Grabowski, T. J., & Madhyastha, T. M. (2016). Using Make for Reproducible and Parallel Neuroimaging Workflow and Quality-Assurance. *Frontiers in Neuroinformatics, 2*(10), 2. doi: 10.3389/fninf.2016.00002. eCollection 2016.

Rane, S., Koh, N., Boord, P., Askren, M. K., Gatenby, J. C., Madhyastha, T**., Mestre, Z**., & Grabowski, T. J. (2016). Cerebrovascular reserve as a marker of vascular pathology and cognitive status in older adults. *Alzheimer's & Dementia*, *12*(7), 502–503. doi: http://dx.doi.org/10.1016/j.jalz.2016.06.997

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Madhyastha, T. M, & **Mestre, Z. L**. (2015). Using GNU Make for Neuroimaging Workflow. Research, doi: 10.13140/RG.2.1.3015.7920, 2015-07-02 T 01:18:01 UTC

MANUSCRIPTS IN PREPARATION

Mestre, Z., Virzi, N., Manzano, M., Strong, S., Malcarne, V., & Boutelle, K. Translation of the Food-Cravings State Questionnaire in pediatric children with overweight and obesity.

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Mestre, Z., Bischoff-Grethe, A., Wierenga, C. E., Strong, & D., Boutelle, K. N. Differences in hippocampal structure and hippocampal-dependent memory in children at high or low risk for obesity

POSTER PRESENTATIONS

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Osuna, J., **Mestre, Z.,** Thomas, K., Harys, C, Campbell, L, & Wierenga, C. (2019, February). *The relationship between arterial stiffness, APOE genotype, and cognition in cognitively normal older adults*. Poster presented at the International Neuropsychology Society conference, New York, New York.

Manzano, M., **Mestre, Z**., Appleton-Knapp, S. L., & Boutelle, K. N. (2018, November). *Self-reported cravings during exposure to highly craved foods in children with overweight and obesit*y. Poster presented at the Obesity Week, Nashville, Tennessee.

Mestre, Z., Osuna, J., Wierenga, C.E., & Zvinka, Z. (2018, October). *Positive effects of body mass index on memory performance, irrespective of activity level, in healthy aging older adults*. Poster presented at the National Academy of Neuropsychology, New Orleans, Louisiana.

Mestre, Z., Eichen, D. M,, Bischoff-Grethe, A., Wierenga, & C. E., Boutelle, K. (2017, July). *Greater BMI in children is associated with reduced fornix white matter integrity*. Poster

presented at the annual meeting of the Society for the Study in Ingestive Behaviors, Montreal Canada.

Kurniadi, N., **Mestre, Z.,** Liang, J., & Boutelle, K. (2017, February). *Specificity of disinhibition in overweight and obese children*. Poster presented at the annual meeting of the International Neuropsychological Society, New Orleans, Louisiana.

Mestre, Z., Bischoff-Grethe, A., Wierenga, C. E., Strong, & D., Boutelle, K. (2016, November). *Hypersensitivity and slower rate of habituation to pleasant food tastes in reward brain regions in obese compared to healthy weight children*. Poster presented at the annual meeting of the Society for Neuroscience, San Diego, CA.

Mestre, Z., Melhorn, S.J., Mehta, S., Webb, M., Tyagi, V., Grabowski, T., & Schur, E.A. (2014, July). *Does anxiety affect food perception and intake in women and men?* Poster presented at the annual meeting of the Society for the Study of Ingestive Behaviors, Seattle, WA.

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Richards, T., Grabowksi, T., Askren, K., Boord, P., Yagle, K., **Mestre, Z.,** Reitz, F., Welker, O., Gulliford, D., Young, L., Collins, E., & Berninger, V. (2013, November). *Functional and structural connectivity across levels of language in children with dysgraphia*. Poster presented at the Society for the Neurobiology of Language, Annual Meeting, San Diego, CA.

Askren, K., **Mestre, Z.,** Yagle, K., Grabowski, T., Richards, T., & Berninger, V. (2015, July). *Altered functional connectivity in functionally defined language networks in children with dyslexia*. Poster presented at the Organization for Human Brain Mapping, Annual Meeting, Honolulu, Hawaii.

Richards, T., Robinson, P., Askren, K., **Mestre, Z**., Grabowski, T., Yagle, K., & Berninger, V. (2015, July). *Diffusion tensor imaging in learning disabilities following computerized instruction in children*. Poster presented at the Organization for Human Brain Mapping, Annual Meeting, Honolulu, Hawaii.

Yagle, K., Richards, T., Askren, K., Beers, S., **Mestre, Z.,** Grabowski, T., & Berninger, V. (2015, July). *Eyetracking during sentence processing in an fMRI study of children with learning disabilities*. Poster presented at the Organization for Human Brain Mapping, Annual Meeting, Honolulu, Hawaii.

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Mestre, Z., Bischoff-Grethe, A., Wierenga, C. E., Strong, D., & Boutelle, K. (2016, July). *Altered brain responses to pleasant taste and hippocampal atrophy in obese children*. Oral Presentation at the Society for the Study of Ingestive Behaviors, Annual Meeting, Porto, Portugal

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ABSTRACT OF THE DISSERTATION

Hungry Hungry Hippocampus: Effects of overweight/obesity
and risk for obesity on hippocampal structure and functioning in children and adolescents

by

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Doctor of Philosophy in Clinical Psychology

University of California San Diego, 2021
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Children with obesity (OB), relative to healthy-weight (HW), have reduced hippocampal volume and lower performance on hippocampal-dependent memory tasks. Little is known about whether differences occur across the weight range or in HW children. This three-paper

dissertation aimed to 1) replicate and build upon prior volumetric findings in children with OB, relative to HW; 2) evaluate differences in hippocampal volume and tissue biology in youth across the weight range; and 3) examine differences in hippocampal structure and hippocampal-dependent memory in HW children at high-risk (HR; two overweight/OB parents) or low-risk (LR; two HW parents) for obesity. **Study 1** used magnetic resonance imaging (MRI) and measures of eating and eating habits in 25 8- to 12-year-old children (13 HW, 12 OB) to examine group differences in hippocampal volume and activation during a functional MRI taste task and explore associations between hippocampal volume and activation and eating/eating behaviors. Children with OB, versus HW, showed reduced left hippocampal volume and greater response to taste in three clusters within the left hippocampus. Moreover, activation within the hippocampus was positively associated with eating and eating behaviors. **Study 2** used MRI in 102 adolescents across the weight range to examine the association between standardized BMI (BMIz), hippocampal volume, and tissue biology. BMIz was negatively associated with T2-weighted hippocampal signal intensity in bilateral hippocampi, suggesting differences in tissue biology. **Study 3** used MRI, hippocampal-dependent memory tasks, and measures of eating and eating habits in 82 HW children (41 HR, 41 LR) to examine group differences in hippocampal volume and memory and explore whether volume and memory were associated with eating behaviors. HR children, relative to LR, had smaller left hippocampal volumes and lower performance on a hippocampal-dependent word memory task. Moderator models suggested that HR children, relative to LR, may already be showing patterns similar to children with OB. Collectively, these studies demonstrated differences in hippocampal structure and functioning in HW children at HR for OB, relative to LR (**Study 1)**, and in children with OB, relative to HW

(Study 2). Moreover, **Study 3** findings suggest that, during adolescence, hippocampal differences related to increased weight reflect variations in tissue characteristics.

CHAPTER 1:

Integrated Introduction

A state of overweight/obesity in children is defined as having a body mass index (BMI) at or above the 85th percentile for age.[1] According to a recent review, the rate of childhood obesity in the United States from 1968 to 2016 has more than tripled from 5 to 18.5 percent.[2] Obesity is associated with significant health complications, including cardiovascular disease, Type II diabetes, osteoarthritis, cancer, and overall poor quality of life.[3] Obesity is also associated with neural changes related to hyperphagia and cognitive impairment.[4] In adults, increased weight is associated with damage in memory regions (i.e., hippocampus), faster rates of cerebral atrophy, and increased risk of dementia.[5-8] Moreover, obesity in childhood tracks well into adulthood as children with obesity are 6-7 times more likely to become adults with obesity, relative to children with healthy weight.[9] In fact, weight patterns appear to be determined by the age of 11 years old.[2] In order to address the obesity epidemic, it is essential to identify potential risk and maintenance factors for this disease.

Obesity is a multifaceted disease associated with many causes including food palatability, genetic susceptibility, maternal BMI and employment, decreased energy expenditure, and increased sedentary behavior (e.g., increased screen time).[1,10-13] One well-known factor related to a state of obesity is overeating (i.e., an imbalance between energy intake and expenditure).[1,2,10,11,14] It has been suggested that increased caloric intake may be related to decreases in the costs of food due to advances in technology.[2,15] However, more recent research has shown that certain neural structures (e.g., limbic and para limbic regions) may also be implicated in eating/overerating.[16,17] More specifically, a small number of studies have shown

that brain regions responsible for higher order cognitive functions, such as the hippocampus, are crucial in the initiation and termination of eating.[18,19]

The hippocampus, a structure within the limbic system, may play a role in overeating and weight gain through its involvement in feeding behaviors and weight regulation. This region has been shown to perceive and process hunger and satiety signals, as well as initiate and terminate eating in response to perception of hunger and satiety.[18-21] Furthermore, the hippocampus regulates food intake by detecting learned signals which are paired with eating and the consequences of eating (i.e., Pavlovian conditioning, operant conditioning, negative occasion setting).[18,20-22] Thus, damage to the hippocampus has been shown to increase food intake by impairing inhibitory control related to memories of the rewarding consequences of eating (i.e., impaired negative occasion setting; hippocampal-dependent process involved with learning to resolve predictable ambiguities) and by reducing the ability to detect hunger and satiety signals.[19-21,23]

Animal research has shown that damage to or inactivation of the hippocampus impairs perception and processing of interoceptive signals of energy states which then increases food-seeking behaviors and food intake, and decreases the postprandial inter-meal interval (i.e., amount of time from the end of one meal to the beginning of the next).[20,22-24] In humans, patients with bilateral hippocampal damage show difficulties in identifying hunger and satiety, and consume several meals consecutively if allowed.[25] The hippocampus may also contribute to overeating and weight gain due to its role in memory, specifically memories related to eating and meals consumed.[25,26] Behavioral data show that although some patients with bilateral hippocampal damage have intact sensory-specific satiety (changes in food liking and desire to eat before and after eating), they have no memory for having eaten the food, which then leads to

eating multiple meals consecutively.[26] Moreover, a study examining the role of memory of a recent eating episode (i.e., last meal eaten 3 hours prior) on eating behaviors (number of snack biscuits eaten) in female adults found that women who had thought about their last meal prior to the snack ate significantly fewer biscuits, relative to those who had not been told to recall what they had last eaten.[27,28] Despite the apparent role of the hippocampus in eating, satiety, and overeating, few studies have examined the impact of childhood overweight/obesity on this neural structure.

Obesity is associated with inflammation, gliosis, and reductions in grey matter in the hippocampus.[4,8,29,30] Research among both children and adults shows differences in hippocampal structure among individuals with obesity relative to healthy weight.[4,5,8,31-35] Findings from one prior study in a sample of 6 to 9-year-old Mexican children found that children with overweight/obesity (>85%BMI), relative to healthy weight (<85%BMI), had significantly reduced left hippocampal volume.[8] In addition, prior research has shown that individuals with obesity, relative to healthy weight, show differences in hippocampal function. Adults with obesity, relatively to healthy weight, show increased activation in the hippocampus in response to high-calorie food pictures and food odors.[4,17,36-38] Similar results were found in adolescents such that higher waist circumference was significantly positively correlated to hippocampal activity in response to high-calorie food images.[39] Prior to study 1 in this staple dissertation,[40] no studies had examined the association between weight and hippocampal functioning in children. Further, the comparison of both functional and structural differences in children with obesity and healthy weight had not yet been explored.

Study 1 addressed this gap in the literature by examining hippocampal structure and functional differences among 8 to 12-year-old children living in the U.S. with obesity and

healthy weight. To our knowledge, ours was the first study to compare both hippocampal volume and its functional response to pleasant tastes in children. In addition, novel to this study, we examined whether responsivity in the hippocampus was associated with eating behaviors,[41] to establish whether neural responses translate to observable eating behaviors. A more in depth understanding of the neural underpinnings of obesity in youth could help prevent the perpetuation of obesity into adulthood through more tailored weight loss interventions promoting hippocampal health (i.e., dietary interventions). We believe that our findings contribute to the current knowledge base regarding the neural underpinnings of obesity in youth (i.e., changes in hippocampal structure and functioning).

As shown above, neural risk factors are important but understudied as concerns weight gain and obesity, especially in childhood. To date, most studies have compared the structural and functional brain differences between children with obesity, relative to healthy weight.[8,40,42-46] Yet, few studies have examined the effects of weight on the brain across the weight range.[47,48] One large cohort study including 325 children and adolescents (~70% of the sample healthy weight, 5th–84th%BMI) showed that a higher BMI was associated with reduced global grey matter volume, which was specifically found in the occipital, parietal, and temporal lobes.[48] Another study among 120 children and adolescents with BMIs in the underweight (3% sample, <5%BMI), normal weight (67% sample, 5th–84th%BMI), overweight (12% sample, 85th–95th%BMI), and obesity (17% sample, >95%BMI) categories showed that a higher BMI percentile was associated with smaller frontal and limbic brain regions including the hippocampus, parahippocampus, amygdala, cingulate, and cerebellum.[47] Due to the limited number of studies available thus far, additional research on the effects of weight among youth

across the weight range is warranted, in particular during crucial neurodevelopmental periods such as adolescence.

Adolescence is a time of significant brain maturation and reorganization in several regions including regions within the limbic system such as the hippocampus.[49,50] In fact, adolescence is associated with higher levels of neurogenesis in the hippocampus, and disruption of neurogenesis during this critical period could lead to long-lasting changes into adulthood.[49] To date, only one prior study examined the effects of excess weight on the hippocampus in adolescents.[51] Inconsistent with prior research in children and adults, this study found that adolescents with excess weight, relative to healthy weight, had increased grey matter volume in the right hippocampus.[51] It is possible that the association between weight and hippocampal volume is different during this developmental period. Yet, it should be noted that this study was relatively small and included only 52 adolescents, with unequal groups (69% had overweight or obesity relative to 31% with healthy weight).

Accordingly, **Study 2** sought to better understand the effects of weight on the hippocampus during adolescence by examining the associations between hippocampal volume and body weight in a large national sample of adolescents (N=102) using the Pediatric Imaging, Neurocognition, and Genetics database (http://pingstudy.ucsd.edu). In addition, prior rodent research suggests that obesity related factors (i.e., a western diet; high-fat, high sugar foods) can lead to damage in hippocampal tissue. In animals, signs of hippocampal pathology (e.g., damage to the blood brain barrier and mRNA expression) can be detected after only ten days on a western diet.[52] The "vicious cycle of obesity and cognitive decline" theory suggests that a western diet impacts the hippocampus through a breakdown of the blood brain barrier.[19] Yet, it was unclear whether the observed damage to hippocampal tissue could be seen as a result of

weight gain and obesity alone, independent of diet, due to inflammation and gliosis.[4,8,29,30] Moreover, although research in humans suggests that obesity negatively impacts hippocampal volume from an early age, prior to our study, the effects of weight on hippocampal tissue biology had yet to be examined. Thus, study 2 examined the potential negative association between weight and hippocampal tissue biology to further inform possible mechanisms of volumetric changes.

Finally, it is important to examine whether differences in hippocampal structure and functioning can be seen prior to weight gain, thus acting as a risk factor for obesity. Research in adults shows evidence for neural predisposition for weight gain. One study examined structural brain differences between adults at high risk (HR; BMI between 20–30 kg/m^2, one or more first degree relative(s) with a reported history of obesity, a history of past weight fluctuations (±10 lbs or more), and not actively trying to lose weight) or low risk (LR; BMI between 17–25 kg/m^2, no first-degree relatives with a history of obesity, never overweight, no past weight fluctuations (±10 lbs or more), and no high levels of physical activity (>3h/week of planned physical activity)) for obesity to determine if structural brain differences precede weight gain and obesity (i.e., potential mechanism predicting obesity risk).[53] HR adults, relative to LR, had reduced total grey matter volume as well as reduced grey matter volume in the orbital frontal cortex (OFC), insula, and cerebellum. Another study conducted among 83 young women (age = 18.4 ± 2.8; BMI range = 17.3–38.9; 78.3% white Caucasian) found a trend (p=0.06) suggesting that reductions in grey matter volume in the prefrontal cortex were associated with an increase in slope BMI from baseline to 1-year follow-up, controlling for initial BMI.[54] In summary, existing data in adults suggests that there may be neural risk factors for weight gain.

Prior to our study, only one research study had directly compared HR and LR groups to examine differences in neural responding to food cues in reward brain regions using functional MRI (fMRI) in female youth with healthy weight.[55] In this study, HR adolescents (adolescents with two overweight/obese parents, BMI≥27), relative to LR (adolescents with two healthy weight parents, BMI<25), showed greater response in reward brain regions (e.g., striatum) and somatosensory brain regions (i.e., opercular regions) in response to food cues (i.e., receipt and anticipated receipt of palatable food). However, the authors did not explore if there were any differences in brain structure between risk groups. Additionally, this study was conducted with adolescents.[51,56-58] As discussed above, adolescence is a critical brain developmental period,[49,50] associated with increases in grey and white matter volume in several regions including the hippocampus. Therefore, examining differences in hippocampal structure during childhood, prior to age-related brain changes, using a similar paradigm (i.e., HR and LR children with healthy weight) is needed.

Behavioral research in children has shown that obesity and obesity-related factors (i.e., increased adipose tissue) are associated with poorer performance on hippocampal-dependent memory tasks. In a study conducted with 126 children (7-9 years old) who were overweight/obese (>85%BMI) or healthy weight (<85%BMI), the amount of abdominal adipose tissue was a significant negative predictor of performance on a hippocampal-dependent relational memory task.[59] Yet, this has not yet been examined in HR and LR children with healthy weight to determine whether differences in hippocampal-dependent memory performance can be seen prior to obesity.

Thus, **Study 3** examined differences in hippocampal structure and hippocampal-dependent memory task performance among children with healthy weight at HR (two

overweight/obese parents) and at LR (two healthy weight parents). Using mediation models, we also explored whether the association between hippocampal structure and function, and eating behaviors[40] was moderated by risk group.

Therefore, studies 1, 2, and 3 in this dissertation defense first examined the effects of weight and obesity on hippocampal structure and function (study 1 and 2) before exploring whether differences in hippocampal structure and function can be seen in children with healthy weight dependent upon risk factors for obesity in youth (study 3). This dissertation defense will present the completed and published 1st and 2nd studies, the introduction, results and methods of the 3rd study currently in preparation for publication, and an integrated discussion of all three studies.

CHAPTER 2:

Study 1

The content within this section, titled "Chapter 2: Study 1," reflects material from a paper that has been published in the *International Journal of Obesity*. The proper citation is as follows:

Mestre, Z. L., Bischoff-Grethe, A., Eichen, D. M., Wierenga, C. E., Strong, D., & Boutelle, K. N. (2017). Hippocampal atrophy and altered brain responses to pleasant tastes among obese compared with healthy weight children. *International Journal of Obesity*, *41*(10), 1496-1502.

Abstract

The hippocampus is a key structure implicated in food motivation and intake. Research has shown that the hippocampus is vulnerable to the consumption of a western diet (i.e., high saturated fat and simple carbohydrates). Studies of patients with obesity (OB), compared with healthy weight (HW), show changes in hippocampal volume and response to food cues. Moreover, evidence suggests that OB children, relative to HW, have greater hippocampal response to taste. However, no study has examined the association of hippocampal volume with taste functioning in children. We hypothesized that OB children, relative to HW, would show a significant reduction in hippocampal volume and that decreased volume would be significantly associated with greater activation to taste. Finally, we explored whether hippocampal activation would be associated with measures on eating and eating habits. Twenty-five 8–12-year-old children (i.e., 13 HW, 12 OB) completed a magnetic resonance imaging scan while participating in a taste paradigm (i.e., 1 ml of 10% sucrose or ionic water delivered pseudorandomly every 20 s). Children with OB, relative to HW, showed reduced left hippocampal volume (t=1.994, P=0.03, 95% confidence interval (CI)=–40.23, 755.42), and greater response to taste in three clusters within the left hippocampus (z=3.3, P=0.001, 95% CI=–0.241, –0.041; z=3.3, P=0.001, 95% CI=–0.2711, –0.0469; z=2.7, P=0.007, 95% CI=–0.6032, –0.0268). Activation within the hippocampus was associated with eating in the absence of hunger (EAH%; t=2.408, P=0.025, 95% CI= 1.751708, 23.94109) and two subscales on a measure of eating behaviors (Food responsiveness, t=2.572, P=0.017, 95% CI= 0.9565195, 9.043440; Food enjoyment, t=2.298, P=0.032, 95% CI=0.2256749, 4.531298). As hypothesized, OB children, relative to HW, had significantly reduced hippocampal volume, and greater hippocampal activation to taste. Moreover, hippocampal activation was associated with measures

of eating. These results contribute to research on the relationship between OB, overeating and cognitive impairment.

Introduction

Approximately one-third of children in the United States are either overweight or obese.[60] Childhood obesity (OB) is associated with poorer health outcomes and is highly correlated with adult obesity.[61-63] Research suggests that obesity is associated with brain changes which may lead to hyperphagia and cognitive impairment.[4] In particular, increased weight is associated with damage in memory regions (i.e., hippocampus) and faster rates of cerebral atrophy, as well as increased dementia risk in elderly adults.[5-8] Due to the high prevalence of obesity in the United States, further research is needed to establish whether these observed changes can be seen in early childhood, to understand mechanisms which may impact critical neural developmental periods.

Overeating is a major contributor to obesity.[14] Although the hypothalamus and hindbrain are identified as key neural structures in eating,[18,64,65] other less studied higher order brain regions, such as the hippocampus, are also considered crucial to the initiation and termination of eating.[18,19] The hippocampus plays an important role in food intake regulation by detecting learned signals which are paired with eating and the consequences of eating.[18,20-22] The hippocampus receives input from brain regions involved in the perception of internal cues, taste, reward (e.g., hypothalamus, thalamus, amygdala), as well as metabolic and neurochemical signals known to be associated with energy intake and weight regulation (e.g., Ghrelin, CCK, Insulin).[20,23] Animal research shows that damage or inactivation of this area impairs perception

and processing of interoceptive signals of energy states, increases food-seeking behaviors and food intake, and decreases the postprandial intermeal interval (i.e., amount of time from the end of one meal to the beginning of the next).[22-24] Moreover, in humans, the hippocampus is implicated in eating behaviors and body weight regulation. In amnestic patients with bilateral hippocampal damage, difficulties in identifying hunger states and consumption of several meals consecutively are reported.[20,25,26]

Due to its role in eating, the hippocampus has become a region of interest in the study of obesity. Obesity is associated with inflammation, gliosis and reductions in grey matter in the hippocampus,[4,8,29,30] which have been associated with memory and hippocampal-dependent cognitive impairments.[59,66,67] These changes in hippocampal grey matter volume are hypothesized to be related to the consumption of western diets (i.e., High saturated fat foods).[19,23,68,69] In animals, signs of hippocampal pathology (e.g., damage to the blood brain barrier (BBB), mRNA expression) were detected after only ten days on a western diet.[52] Moreover, in adults, a western diet, independent of normal aging, was associated with a smaller left hippocampal volume,[70] while in children ages eight to ten years, OB children showed reduced hippocampal volume, relative to healthy-weight (HW).[8] A "vicious cycle of obesity and cognitive decline" was proposed such that a high-fat, high-sugar diet impacts hippocampal structure and function through a breakdown of the blood brain barrie.[19] Damage in this area can then result in an inability to inhibit the activation of memories related to food and the rewarding consequences of eating, and increased food intake due to difficulties in detecting hunger and satiety signals.[19-21,23]

An emerging body of research in humans is beginning to demonstrate functional activation differences in the hippocampus among individuals who are OB and HW. Changes in hippocampal functioning have been detected early in the lifespan as higher waist circumference

was associated with greater hippocampal activation in adolescents in response to high calorie food images.[39] Additionally, abnormal activity in the hippocampus in response to a satiating meal does not appear to return to its previous functionality after weight reduction.[71] Interestingly, no study has examined both functional and structural differences between OB and HW individuals. Considering that structural changes to the hippocampus are associated with changes in function, more research is needed on whether both structure and functional changes are detected. This is especially important in youth, as the hippocampus develops through mid-adolescence.[72]

This study aimed to fill the gap in the literature by evaluating hippocampal differences among 8-12-year-old OB and HW children. To our knowledge, this is the first study to compare both hippocampal volume with its functional response to pleasant tastes in youth. We expect that OB children will show reduced hippocampal volume compared to HW children. Additionally, we expect that OB children, relative to HW, will display significantly greater activation in response to taste in the hippocampus. Novel to this study, we predict that hippocampal volume will be associated with activation in the hippocampus in response to taste. We will also exam whether responsivity in the hippocampus was associated with eating behaviors. These findings could contribute to the current knowledge base regarding the neural underpinnings of obesity and food cue reactivity and resulting cognitive impairments.

Methods

Subjects. Twelve OB children and 13 age and gender matched HW children (8-12 years old) were recruited from the community and participated in this study. A subset of this sample was analyzed in a prior publication which focused on responses to appetitive tastes in the insula

and amygdala in OB compared to HW children following a satiating meal [42]. The functional analyses for this manuscript included the same sample as in a prior publication [42], but specifically focused on hippocampal functioning, while the structural analyses included two additional children that were not in the original study. Children were recruited who were either OB (>95%BMI) or HW (<85% BMI)), right-handed, fluent in English, and liked cheese pizza (needed for the Eating in the Absence of Hunger (EAH) task). Exclusion criteria included any diagnosis of an eating disorder (diagnosed by Child Eating Disorder Examination [chEDE])[73] or other significant psychiatric disorder (Mini International Neuropsychiatric Interview for Children and Adolescents [MINI-KID]).[74] In addition, children could not have any other medical/neurologic concerns or conditions contraindicative to MRI (e.g., traumatic brain injuries, surgical metallic implants and claustrophobia). Child height in centimeters and weight in kilograms were measured twice using a portable Schorr height board (Schorr Inc, Olney, MD) and Tanita Digital Scale (model WB-110A). Using the average of the 2 values for height and weight, Body Mass Index (BMI;kg/m^2) was calculated and translated to BMI for age percentile score using the CDC growth charts.[75] This study conformed to the Institutional Review Board regulations of the University of California, San Diego. Written informed assent and consent was acquired from the children and their parents respectively. This study represents a secondary data analyses of primary aims previously published.[42]

Experimental Design. During the first study visit, the MINI-KID[74] and chEDE-C[73] were used to rule out any significant psychiatric or eating disorders in children. Parents completed the Child Eating Behavior Questionnaire (CEBQ-PR)[76,77] and children participated in the EAH[78] paradigm, which measures the percent of daily caloric needs consumed of snack foods when sated in a free access session (EAH%).[78,79] Prior to the scan, children participated in a mock-scan

to acclimate to the noises and experience of the MRI scanner. During a second visit, following a standardized breakfast (i.e., one bagel with cream cheese, one banana, orange juice), children completed a 1-hour functional magnetic resonance imaging (fMRI) scan during which structural scans, in addition to a taste paradigm previously described elsewhere,[80] were performed.

MRI/fMRI. Imaging data were collected with a 3T Signa Excite scanner (GE Medical Systems). FMRI was collected with gradient-recalled echoplanar imaging (EPI) (TR=2000 ms, TE=30 ms, flip angle=80°, 64 x 64 matrix, ASSET factor=2, 40 2.6-mm ascending interleaved axial slices with a 0.4-mm gap, 200 volumes).[42] The first four volumes of each run were discarded so as to discount T1 saturation. EPI-based field maps were acquired for correcting susceptibility-induced geometric distortions.[42] A high resolution T1-weighted image (SPGR, TI=600 ms, TE=min full, flip angle=8°, 256 x 192 matrix, 170 1.2 mm contiguous slices) was obtained for subsequent spatial normalization and later used for structural analyses.

Definition of Anatomical Regions of Interest. Our region of interest (ROI) (i.e., bilateral hippocampus) was chosen based on literature showing the importance of this region in feeding behaviors and body weight regulation. A single bilateral ROI for the hippocampus was derived from the Harvard-Oxford Atlas.[81]

Statistical Analysis. All children (i.e., 13 HW, 12 OB) were included in the structural analyses. The Freesurfer version 5.3.0 image analysis suite (http://surfer.nmr.mgh.harvard.edu/) was used for the volumetric segmentation of subcortical gray and white matter regions. Individual cortical and subcortical region volumes for each subject were normalized to the subject's total brain volume estimated by the Freesurfer segmentation process. The Freesurfer segmentation files for all subjects were visually inspected for quality assurance. No segmentation file required hand-editing. This method has been previously described in detail.[82-84] The

individual brain volume segments for each group (i.e., HW and OB) were then averaged over all subjects for each group and compared using linear regression models in R.

Analysis of Functional NeuroImages (AFNI) software[85] and R statistical packages (http://www.r-project.org)[86] were used to preprocess and analyze functional images, as described in our publication.[42] Briefly, EPI images were motion-corrected and aligned to high-resolution anatomical images. AFNI's 3dToutcount was used to generate outliers and volumes with 10% of voxels marked as outliers were not used in subsequent analyses. Based on motion exclusion criteria (i.e., greater than 20% of the volumes being censored and/or over 3mm of movement), two children in the OB group were excluded from the fMRI analyses. Ten OB children (50% female; BMI>95th% for age; age 10.09 ± 1.00 years) and 13 HW children (38.4% female; BMI<85th%; age 10.38 ± 1.26 years) were included in functional analyses.

A linear mixed effects (LMEs) analysis in R was performed for each voxel within the left and right hippocampus. Two separate models were constructed in which subject was treated as a random effect. In one model, diagnosis (OB, HW) was treated as the between subjects' factor and condition (sucrose, water) as the within subjects' factor. As no group by condition interaction was observed, we reduced the model to include group (OB, HW) and condition (i.e., combining the sucrose and water conditions) as the between subjects' factors. Small volume correction was determined with Monte-Carlo simulations (via AFNI's 3dClustSim) to guard against false positives. To correct for multiple comparisons, a thirteen-voxel cluster-size threshold and a twelve-voxel cluster-size threshold was used in the left and right hippocampus respectively.

Correlational analysis. Huber robust regression models were performed in R to examine potential correlations between the mean percent signal change in each significant cluster within

the left hippocampus and left hippocampal grey matter volume in all children (i.e., N=23). Potential correlations between measures of eating variables and significant clusters in the functional analyses were also examined.

Results

Participant Demographics

There were no significant differences in age ($t = 0.68$, $p = 0.51$), gender ($\chi^2 = 0.03$, $p = 0.87$) or race ($\chi^2 = 0.12$, $p = 0.73$) between OB or HW children (Table 1). OB children had significantly higher BMIs relative to HW (BMI, $t = 7.77$, $p < 0.0001$; BMI %, $t = 5.13$, $p < 0.0001$). OB children, relative to HW, also scored significantly higher on six measures of the CEBQ-PR (Table 2.1), and tended to have higher EAH ($p < 0.07$). No differences in age, gender or race were found in the functional MRI subsample (i.e., 13 HW, 10 OB).

Structural analyses

In OB children, linear regression models showed a significant reduction in left hippocampal volume relative to HW children ($t = 1.994$, $p = 0.03$) (Figure 2.1). No significant group differences were found in right hippocampal volume ($p = 0.34$).

Functional imaging results

LME models revealed a significant main effect of group in the left hippocampus in three clusters. Post hoc analyses showed that OB children, compared to HW, had significantly greater response to taste (sucrose and water) within the tail (dorsal) (Cluster 1: 24,-33,-8, $z = 3.3$, $p = 0.001$), body (Cluster 2: -31,-21,-15, $z = 3.3$, $p = 0.001$) and head (ventral) (Cluster 3: -20,-8,-24,

z = 3.3, p = 0.007) of the left hippocampus (Figure 2.2). No interaction of group by condition or main effect of condition was found. No significant effects were seen in the right hippocampus.

Correlations between functional activation and grey matter volume

Huber robust regressions in R, including all children, assessed associations between hippocampal volume and strength of left hippocampal activation in response to taste in each of the three significant clusters. A trend for significance was found in the dorsal left hippocampus (37 voxels) such that stronger activation to taste tended to be associated with reduced left hippocampal volume (t = -1.56, p = 0.07). A significant association was found in the body of the left hippocampus (24 voxels) such that stronger activation to taste was significantly associated with reduced left hippocampal volume (t = -2.22, p = 0.02). No association was found between activation in the ventral left hippocampus (23 voxels) and left hippocampal volume (t = -1.40, p = 0.18) (Figure 2.3).

Correlations between functional activation and eating variables

Huber robust regressions were performed in R to assess the association between total EAH% and strength of left hippocampal activation in response to taste in all significant clusters. Results showed a significant association in the ventral left hippocampus (23 voxels) such that stronger activation to taste was significantly associated with greater total calories consumed during EAH (t = 2.408, p = 0.025) (Figure 2.4). No significant associations were found in the two other clusters within the left hippocampus.

The association between parent report of the child's food responsiveness, enjoyment of food and satiety responsiveness and strength of left hippocampal activation in response to taste in each of the three significant clusters was also assessed. Results showed a significant association in the dorsal left hippocampus (37 voxels) with the food responsiveness (t = 2.572, p= 0.017)

and enjoyment of food ($t = 2.298$, $p = 0.032$) (Figure 2.4). No other associations were found between the other two clusters and the subscales on the CEBQ-PR.

Discussion

This is the first study to demonstrate differences in hippocampal structure and function between OB and HW children. Prior studies have focused on regions associated with food reward and motivation such as the amygdala, insula, and ventral striatum.[4] The hippocampus has been shown to be important in feeding behaviors and weight regulation through its role in the perception and processing of hunger and satiety signals, as well as in its role in initiating or terminating eating using learned interoceptive satiety cues.[18-21] In this study, OB children, relative to HW, showed significantly reduced left hippocampal volume as well as a hypersensitivity to taste cues in three clusters within in left hippocampus. Activation to taste in the left hippocampus was negatively associated with left hippocampal volume. This could suggest a hypersensitivity to food taste in OB children, relative to HW, as evidenced by increased scores on behavioral measures of food sensitivity (i.e., CEBQ-PR food responsiveness subscale) or an over-compensation in this region due to a reduction in volume. Importantly, this study demonstrated that functional activation to taste in the hippocampus was associated with eating in the lab, food responsiveness and enjoyment of food in children. In sum, our data show that OB children, relative to HW, had lower hippocampal volume and greater activation in the left hippocampus, and that activation in this region is associated with eating when satiated, responsiveness to food, and greater enjoyment of food.

Our study adds to a small body of literature showing a left hippocampal volume reduction in OB children, compared to HW. This finding is consistent with one other study which examined the association between obesity in children and reductions in hippocampal volume.[8] These observed changes in the hippocampus could be due to the impact of sustained consumption of a western diet on the blood brain barrier (BBB).[19] The maintenance of a western diet may lead to damage of the BBB including a reduction in expression of the proteins that make up the BBB and an increase in BBB permeability.[87] The BBB in the hippocampal formation area is especially prone to damage associated with the western diet.[87,88] This increased vulnerability is thought to be a result of the high nutrient demands and pronounced cellular plasticity of the hippocampus.[19,89] As a result, hippocampal functioning is particularly susceptible to damage by western diets. It should be noted that other processes (i.e., inflammation and hormonal imbalance) have also been implicated in the relationship between obesity and hippocampal damage.[4,29] Moreover, our data are cross-sectional, and it is impossible to determine whether changes in structure precede or are a result of children becoming obese from a sustained western diet. Future studies should include a dietary recall variable to assess the effect of the consumption of a western diet. In addition, prior research has shown that the left hippocampus may be more prone to neurodegeneration.[70] Greater vulnerability of the left hippocampus, relative to the right, is consistent with our results and may be explained by the functional lateralization of this region. The left hippocampus is more involved in context-dependent episodic/autobiographical memory.[90] Since eating is a social experience, involving more contextual cues, the left hippocampus may be more implicated in food cue signaling and satiety, making it more vulnerable to the effects of diet.

Our results also showed that OB children had higher responsivity to taste in the dorsal, body, and ventral portions of the left hippocampus compared to HW children. This is in line with a prior study in adolescents which showed that waist circumference was significantly positively associated with activation in the left hippocampus in response to high-calorie food pictures.[39] Although the dorsal and ventral portions of the hippocampus differ in terms of functioning,[91] no significant difference in activation were found among our three clusters. Yet, this study demonstrates that altered response to food tastes in the hippocampus using a taste paradigm can be seen as early as 8 years old. Considering that the hippocampus is involved in memory, place preference and hunger and satiety detection, these results could help explain why OB children tend to overeat as they may be less able to perceive and process satiety signals.

Hippocampal activation in response to food taste was also associated with reduced hippocampal volume in OB and HW children. In particular, our study showed associations between left hippocampal volume and activation to taste, which is consistent with the "vicious cycle of obesity and cognitive decline".[19] Damage to the integrity of the BBB, and resulting increased BBB impermeability, due to consumption of the Western diet[19,87] could lead to a heightened vulnerability of the hippocampus to toxins or illnesses and thus to alterations in hippocampal functioning. In addition, animal research has shown that long term consumption of a western diet may lead to neuroinflammation in the hippocampus,[92] reduced hippocampal levels of brain-derived neurotrophic factor (BDNF; i.e., protein promoting neurogenesis, synaptic transmission, and memory performance),[93] and impairments in long-term potentiation in the hippocampus.[94] Therefore, it is possible that a greater consumption of the western diet could lead to damage to the hippocampus and heightened response in the hippocampus to food taste as a

compensatory mechanism. Future longitudinal studies should explore this further using dietary recall.

Moreover, this study found associations between structure and activation in the hippocampus in one out of the three significant clusters in the left hippocampus. It is possible that more clusters would show significance in older individuals who have been obese for longer.[4] It is also possible that certain individuals at risk for obesity could have a predisposition for hippocampal dysfunction, making them susceptible to overeating and further dysfunction in this brain region.

Importantly, this study demonstrates that response to taste in the hippocampus is associated with eating behavior in OB and HW children. Greater response to taste within the ventral left hippocampus was positively associated with the total amount of calories consumed when sated. In addition, greater response to taste within the dorsal left hippocampus was positively associated with parent report of child's food responsiveness and enjoyment of food. Taken together, these results demonstrate that activation to taste in the hippocampus is associated with eating, reward and responsiveness to food in this sample.

As far as we are aware, this is the first study to specifically examine the response to taste in the hippocampus in children as young as 8 years old. It is crucial to study the impact of obesity on the brain as childhood is an important stage in neural development. The hippocampus has been shown to continue developing into mid adolescence.[72] Therefore, damage done during developmental years could have long lasting effects and could predispose individuals to a lifetime of overeating. Our own review found that OB children, compared to HW, have poorer cognitive functioning exhibited by deficits in the areas of executive functioning, attention, visuo-spatial and motor skills, learning and memory, language and academic achievement.[95] These

observed deficits in executive dysfunction, motor skill, and academic achievement have been related to obesity-related behaviors (e.g., increased disinhibited eating and sedentary activity). It is possible that these changes perpetuate overeating or worsen if these children remain in a state of obesity. The hippocampus is one of two regions known for neurogenesis, which further supports the need for early intervention to promote potential recovery of function in this region.[72] Thus, these findings show the importance to develop interventions to promote healthy eating and reduce food cue reactivity, and highlights the need to intervene earlier than 8 years of age.

A strength of our study is the use of a sample of young children which provides a better understanding of the development of underlying mechanisms and of the early neural changes associated with obesity. Our study adds to this growing body of literature by showing that these changes can be detected earlier than previously reported.[46] In addition, this study examined both structural and functional changes in the hippocampus, and the relationship between the two, in a pediatric sample. Although one study demonstrated similar differences among OB and HW children in hippocampal structure,[8] none to date has examined differences among OB and HW children's hippocampal functioning and none have demonstrated the relationship between activation in the hippocampus and eating behavior, food responsiveness and enjoyment of food. As in all studies, there are weaknesses that need to be considered. Our sample size was relatively small and had high inter-subject variability. Additionally, this study is cross-sectional, limiting causal implications. Another limitation was the lack of a neurocognitive measure to assess for overall cognitive ability, which prevented the exploration of the association between hippocampal volume and function, and general cognitive impairment.

However, these results raise questions for further research regarding the relationship between obesity and hippocampal functioning in youth. It is unclear whether changes in

hippocampal volume and structure precede weight gain in youth or are a result of obese status. It is also unclear if changes in hippocampal volume and structure would persist over time or whether these observed changes are reversible. Future studies should implement a longitudinal design to examine whether a state of childhood obesity leads to alterations in hippocampal response and overall volume, and whether these changes then perpetuate into adulthood. In addition, interventions to promote a healthy diet (i.e., minimizing western diets) in children across the weight spectrum could potentially prevent hippocampal damage and dysfunction.

In conclusion, this study suggests that OB children, compared to HW, have reduced left hippocampal volume, alterations in activation to food taste in this region, and a relationship between structure and function in the hippocampus. Importantly, alterations in hippocampal activation are associated with overeating when sated, as well as food responsiveness and enjoyment of food in children, identifying the hippocampus as a key structure involved in obesity and eating behavior in youth.

Dissertation Author's Acknowledgements

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Figures

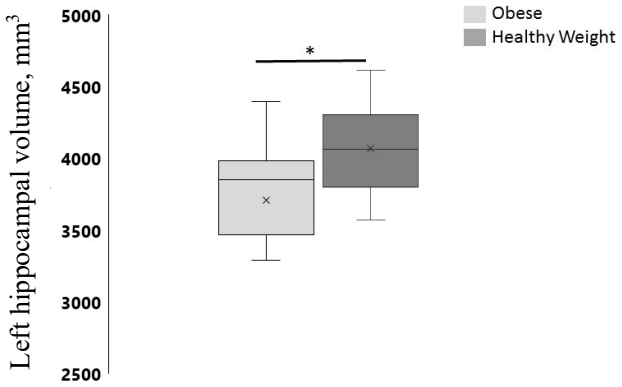

Figure 2.1. *Comparison of left hippocampal volume between obese (OB) and healthy weight (HW) children. OB children had significantly lower left hippocampal volume compared to HW children (t = 1.994, p = 0.03). Data are means of group left hippocampal volume. * p < 0.05*

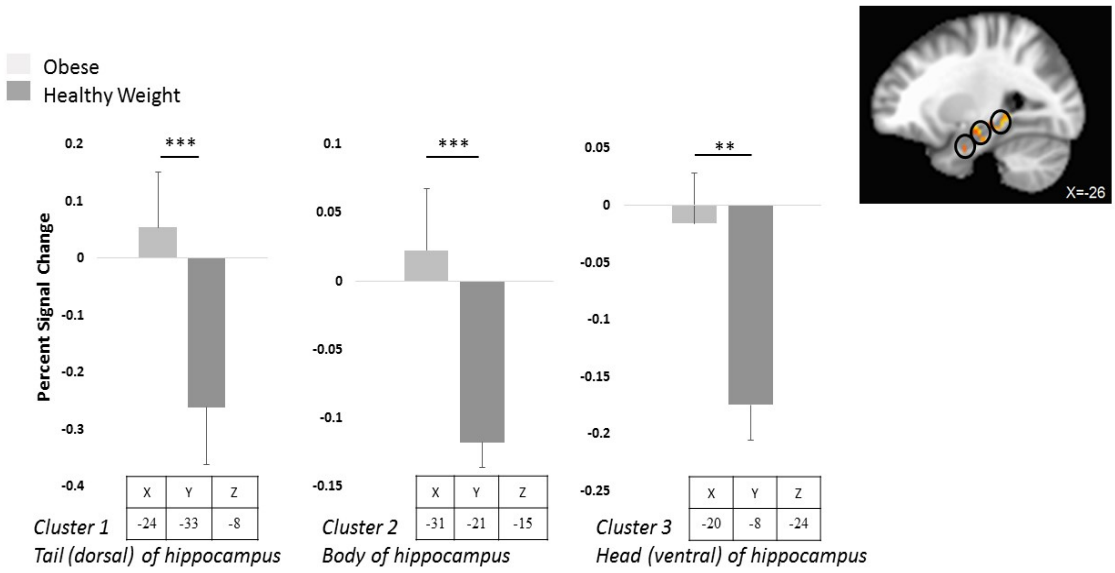

Figure 2.2. *Brain activation by taste (i.e., water and sucrose combined) in obese (OB) and healthy weight (HW) children. In OB children, relative to HW, brain activation by taste was significantly higher in the tail (dorsal), body and head (ventral) portions of the left hippocampus. p values derived from Huber robust regressions and r values derived from Pearson product-moment correlations. *p < 0.01, **p < 0.001*

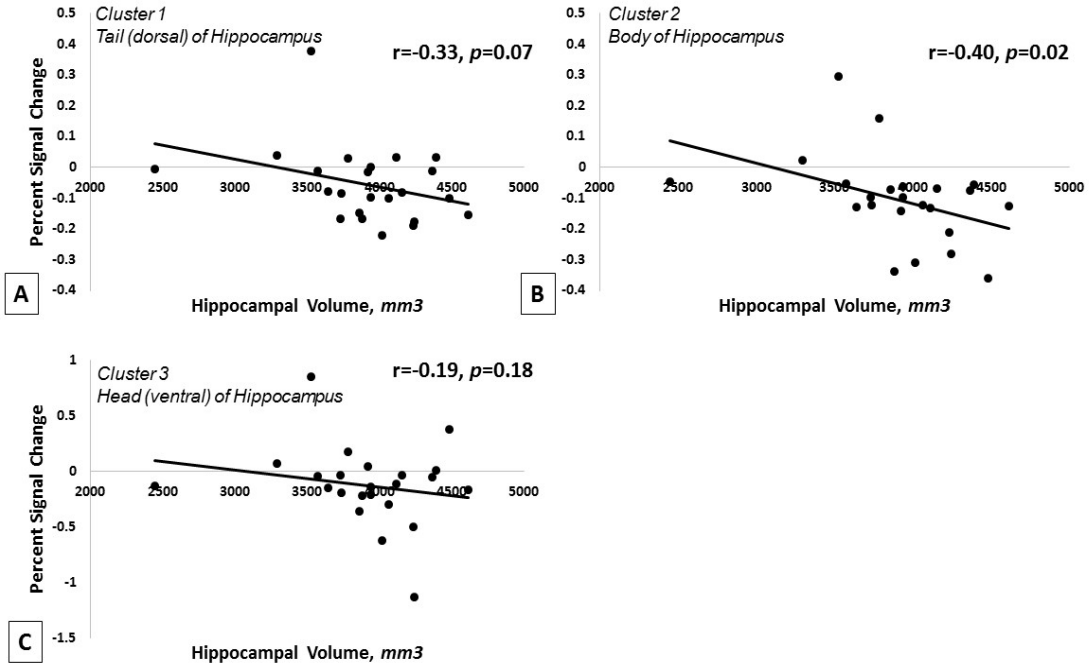

Figure 2.3. Association between A) left hippocampal activation in the tail (dorsal) of the hippocampus and left hippocampal grey matter volume, B) left hippocampal activation in the body of the hippocampus and left hippocampal grey matter volume, and C) left hippocampal activation in the head (ventral) of the hippocampus and left hippocampal grey matter volume, across all participants included in fMRI analyses (OB and HW). Results show a negative trend with lower activation in the dorsal hippocampus associated with greater left hippocampal volume, and a significant association with lower activation in the body of the hippocampus associated with significantly greater left hippocampal volume. No association was found in the ventral hippocampus. p values derived from Huber robust regressions and r values derived from Pearson product-moment correlation

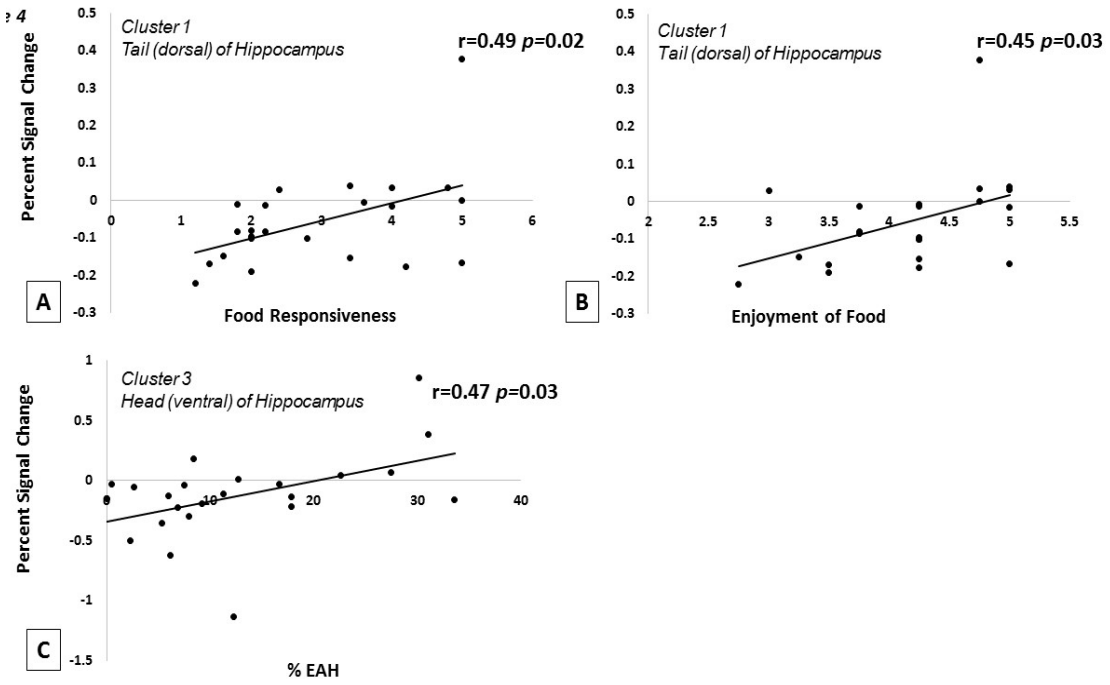

Figure 2.4. Association between A) left hippocampal activation in the tail (dorsal) of the hippocampus and food responsiveness, B) left hippocampal activation in the dorsal hippocampus and food enjoyment, and C) left hippocampal activation in the head (ventral) of the hippocampus and % EAH, across all children included in fMRI analyses (OB and HW). Results show significant positive associations with greater activation in the dorsal left hippocampus associated with food responsiveness and enjoyment, and greater activation in the ventral left hippocampus associated greater % EAH. p values derived from Huber robust regressions and r values derived from Pearson product-moment correlations

Tables

Table 2.1. *Values are means and standard deviations from the mean. No significant differences were found between healthy weight and obese children on age, gender,or race. As expected, obese children, relative to healthy weight, had a significantly higher BMI ($p < 0.001$). Obese children, relative to healthy weight, also had significantly greater scores on six measures of the Child Eating Behavior Questionnaire Parent-Report (CEBQ-PR) (i.e., Food Responsiveness, $p < 0.001$; Emotional Over Eating, $p < 0.05$; Enjoyment of Food, $p < 0.01$; Satiety Responsiveness, $p < 0.01$; Slowness of Eating, $p < 0.05$; Food Fussiness, $p = 0.05$), and tended to score higher on EAH ($p < 0.07$). P values derived from T-test. *p < 0.05, **p<0.01, ***p < 0.001*

Subject Demographics	**Healthy Weight (N=13)**	**Obese (N=12)**
N, *female*	*5 (38.4%)*	*5 (42%)*
Age, *years*	*10.38 ± 1.26*	*10.08 ± 1.00*
BMI, *kg/m²*	*17.71 ± 1.90*	*26.10 ± 3.23***
Ethnicity		
N, *White*	*8 (62%)*	*6 (50%)*
N, *Black*	*5 (38%)*	*5 (42%)*
N, *Asian*	*0*	*1 (8%)*
EAH	*9.63 ± 2.96*	*17.10 ± 2.50*
Child Eating Behavior Questionnaire (Parent-Report)		
Food Responsiveness	*2.20 ± 0.23*	*3.92 ± 0.34****
Emotional Over Eating	*1.60 ± 0.17*	*2.60 ± 0.41**
Enjoyment of Food	*3.79 ± 0.13*	*4.58 ± 0.20***
Desire to Drink	*2.36 ± 0.14*	*2.90 ± 0.39*
Satiety Responsiveness	*2.74 ± 0.15*	*1.94 ± 0.19***
Slowness of Eating	*2.62 ± 0.13*	*1.86 ± 0.24**
Emotional Under Eating	*2.83 ± 0.26*	*2.93 ± 0.28*
Food Fussiness	*2.40 ± 0.18*	*3.22 ± 0.33**

CHAPTER 3:

Study 2

The content within this section, titled "Chapter 3: Study 2," reflects material from a paper that has been published in the *Obesity* journal. The proper citation is as follows:

Mestre, Z., Bischoff-Grethe, A., Wierenga, C. E., Jernigan, T., Eichen, D. M,, Chang, L., Ernst, T., & Boutelle, K. (2020). Associations between body weight, hippocampal volume, and tissue signal intensity in 12-to 18-year-olds. *Obesity*, *28*(7), 1325-1331.

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Abstract

The hippocampus is a key structure in feeding behaviors and weight regulation. Obesity may lead to disruptions in hippocampal structure. In animals, obesity related factors (e.g., high fat/sugar foods) are associated with hippocampal insult (e.g., alterations in the blood brain barrier). In humans, individuals with obesity, relative to healthy-weight, have smaller hippocampal volumes. Few studies have examined the association between body weight and the hippocampus during adolescence, a critical brain development period. This study examined hippocampal volume and tissue signal intensity in adolescents across the weight spectrum. Structural magnetic resonance imaging and anthropomorphic data were available for 102 12-18-year-old adolescents [52% Female; 15.02±1.84 years; standardized BMI (BMIz) scores using the CDC growth charts: 0.54±1.17] from the Pediatric Imaging, Neurocognition, and Genetics database (PING; http://ping.chd.ucsd.edu). Linear regression models controlling for age, sex, genetic ancestry, scanner, and household income examined the relationship between BMIz, hippocampal volume, and T2-weighted hippocampal signal intensity. BMIz was negatively associated with T2-weighted hippocampal signal intensity in the left ($t=-3.05$, $p=0.003$; $r = -0.21$) and right ($t=-2.50$, $p=0.01$; $r = -0.36$) hippocampi. BMIz was not significantly associated with hippocampal volume. BMIz is associated with hippocampal tissue characteristics during adolescence, which could impact later brain development.

Introduction

Approximately one third of children and adolescents in the United States have overweight or obesity.[96] This is concerning considering the comorbidities of obesity, which

include cardiovascular disease, Type II diabetes, osteoarthritis, cancer, and overall poor quality of life.[3] Furthermore, obesity in childhood tracks well into adulthood, as children with obesity are 6-7 times more likely to become adults with obesity, relative to children with healthy weight.[9] Brain risk factors are an important but understudied element related to weight gain and obesity, especially in childhood. To date, most studies have compared neural measures between children with obesity and those with healthy weight.[4,41-43] Yet, few studies have examined neural measures in youth across the weight spectrum, and even fewer during adolescence.[51,56-58] Adolescence is a critical period of brain development, and changes in brain development during this period often persist into adulthood.[49] Thus, understanding the association between weight and brain structures during adolescence could inform obesity prevention and treatment.

The hippocampus is a key structure involved in feeding behaviors and weight regulation.[18,20] Animal research shows that damage to or inactivation of the hippocampus impairs perception and processing of interoceptive signals of energy states, increases food-seeking behaviors and food intake, and decreases the postprandial inter-meal interval.[22,24] In humans, patients with bilateral hippocampal damage have difficulty identifying hunger and satiety cues, and consume several meals consecutively if allowed.[97] Thus, disruptions to the integrity of the hippocampus could lead to excessive eating, weight gain and ultimately a state of obesity.

Obesity is associated with inflammation and reductions in grey matter in the hippocampus.[4,29] Both adults and children with obesity show smaller hippocampal volumes, relative to those with healthy weight,[4,34,41] suggesting that smaller hippocampi in relation to greater body weight might have been present early in life. In addition to the smaller than normal hippocampal volumes, prior animal research has shown that obesity related factors (i.e., a western diet; high-fat, high sugar foods) can lead to disruptions in the hippocampal tissue (i.e.,

inflammation and gliosis).[98,99] In fact, a western diet impacts the hippocampus in animals after only ten days[52] through a breakdown of the blood brain barrier and reduced levels of brain-derived neurotrophic factor (BDNF), a protein involved in neurogenesis, synaptic transmission, and memory performance.[19] However, it is unclear whether such alterations to hippocampal tissue are a direct result of weight gain and obesity, independent of diet, due to inflammation.[4,29,98] Moreover, although research in humans suggests that obesity negatively impacts hippocampal volume from an early age, to date the association between weight and hippocampal tissue morphometry has not yet been examined.

Differences in brain tissue can be assessed by examining T2-weighted signal intensity within a specific region of interest.[100,101] Most of the research to date has focused on the effects of obesity and diet on hypothalamic inflammation (i.e., gliosis).[101-103] In human adults, gliosis is associated with increased T2-weighted signal intensity.[104,105] Notably, adults with obesity, relative to those with healthy-weight, had increased T2-weighted signal intensity in the hypothalamus, indicative of gliosis.[101] Likewise, in children with obesity, increased T2-weighted signal intensity (suggesting hypothalamic gliosis) was positively associated with adiposity.[106] Moreover, histological studies specifically examining hippocampal tissue have shown that different types of hippocampal tissue alterations can lead to either increased T2-weighted signal intensity (i.e., hippocampal sclerosis and gliosis) or decreased T2-weighted signal intensity (i.e., increased vacuolation) within this region.[107,108] However, although obesity related factors (i.e., a western diet; high-fat, high sugar foods) are associated with alterations in the hippocampal tissue (i.e., inflammation and gliosis),[98,99] no study to date has examined changes in T2-weighted signal intensity within the hippocampus in relation to weight or BMI

in children or adults. Possible associations may demonstrate that T2 signal intensity in the hippocampus might be useful for monitoring future interventions.

To date, the majority of prior research in children has examined differences in hippocampal volume between children with obesity and those with healthy weight,[8,41] yet very little is known about hippocampal health in children and adolescents across the weight range. One study of 120 children and adolescents with BMIs in the underweight (3% sample, <5%BMI), healthy weight (67% sample, 5th–84th%BMI), overweight (12% sample, 85th–95th%BMI), and obesity (17% sample, >95%BMI) categories showed that a higher BMI percentile was associated with smaller frontal and limbic brain regions, including the hippocampus, parahippocampus, amygdala, cingulate, and cerebellum.[47] Due to the limited number of studies available thus far, additional research on the effects of weight among youth across the weight range is warranted, in particular during crucial neurodevelopmental periods such as adolescence.

Four studies have examined the association between weight or factors related to obesity and hippocampal structure in adolescents.[51,56-58] However, three of the four studies focused their analyses on insulin resistance,[57] metabolic syndrome,[58] and Type 2 diabetes and their effects on the hippocampus,[56] rather than body weight alone. Although the fourth study focused on the effects of excess weight on the hippocampus,[51] it included only 52 adolescents, with unequal groups (69% had overweight or obesity relative to 31% with healthy weight). Adolescence is a time of significant brain maturation and reorganization in several regions including the limbic system (e.g., the hippocampus).[49,50] Indeed, adolescence is associated with higher levels of neurogenesis in the hippocampus, and disruptions of neurogenesis during this critical period

could lead to long-lasting changes into adulthood.[49] Thus, a better understanding of the association between weight and brain development during adolescence is imperative.

The current study aimed to examine whether differences in hippocampal volume and T2-weighted signal intensity (i.e., alterations in tissue properties) can be seen in a diverse sample of adolescents across the weight range. We hypothesized that a greater standardized body mass index (BMIz) would be negatively associated with total hippocampal volume. In addition, prior research showed that hippocampal gliosis can lead to increased T2-weighted signal intensity within the hippocampus.[107,108] Since obesity related factors (i.e., high fat diet) were associated with hippocampal inflammation,[98,99] we hypothesized that BMIz would be positively associated with T2-weighted signal intensity in the hippocampus. Based on prior studies showing laterality of findings,[8,41] we chose to examine the associations between BMIz, and volume and T2-weighted signal intensity in the left and right hippocampus separately.

Methods

Participants

Participants were part of the Pediatric Imaging, Neurocognition, and Genetics (PING) study. As described previously,[109] PING participants were recruited through local postings and outreach activities in the greater metropolitan areas of Baltimore, Boston, Honolulu, Los Angeles, New Haven, New York, Sacramento, and San Diego. Inclusion criteria for the entire PING sample included being between the ages of 3 and 20 years and fluent in English. Exclusion criteria included neurological disorders, history of head trauma, preterm birth <36 weeks, diagnosis of an autism spectrum disorder, bipolar disorder, schizophrenia, mental retardation,

pregnancy, daily illicit drug use by the mother for more than one trimester, and contraindications for magnetic resonance imaging (MRI).

This study utilized a subset of the PING sample for whom height and weight information was available. Three sites were able to provide this information: Honolulu (N=206), New Haven (N=38), and New York (N=41). Neuroimaging data from participants collected from the three sites were acquired from the PING website (http://ping.chd.ucsd.edu) and matched to the height and weight information. Given our goal to study adolescents and to maximize the sample size, we decided to include only children ages 12-18 in our sample. The final PING adolescent sample for this study included 102 12-18-year-old adolescents (53% (N=54) female; 15.07 ± 1.84 years; BMI: 23.16 ± 5.32 kg/m^2; BMIz: 0.54 ± 1.17). *(See Table 3.1 for full demographic information).* In our study sample of 102 adolescents, 4 had BMIs in the underweight category (4% sample, <5%BMI), 66 had BMIs in the healthy weight category (65% sample, 5th–84th%BMI), 13 had BMIs in the overweight category (13% sample, 85th–95th%BMI), and N=19 had BMIs in the obese category (19% sample, >95%BMI), which matches the proportion of children with overweight and obesity in the United States.[96]

Measures

Anthropometrics. BMI was calculated (kg/m^2) for each participant using the height and weight information provided by the three sites and translated to standardized BMI (BMIz) scores using the CDC growth charts.[75]

Genetic Ancestry Factor. Ancestry and admixture proportions were calculated using a supervised clustering approach implemented in the ADMIXTURE software.[110] Each participant had 6 genetic ancestry factors (GAF; European, African, Native American, East Asian, Central Asian, and Oceanic) which were used in our models as covariates.

Image acquisition and processing. All sites used a standardized multiple-contrast structural MRI protocol which included a 3-dimensional (3D) T1-weighted (TE = 3.5 ms, TR = 8.1 ms, TI = 640 ms, flip angle = 8°, receiver bandwidth = ± 31.25 kHz, FOV = 24 cm, freq = 256, phase = 192, slice thickness = 1.2 mm) and 3D T2-weighted volume (TE = 69.3 ms, TR = 1500 ms, echo train = 40, FOV = 24 cm, freq = 256, phase = 192, slice thickness = 1.2 mm). Additionally, two axial 2D DTI scans (30 directions, b-value = 1000, TE = 83 ms, TR = 13600 ms, freq = 96, phase = 96, slice thickness = 2.5 mm) were acquired. Imaging data from all three sites were collected on 3T Siemens Tim Trio scanners.

As described previously,[109] cortical and subcortical volumes of regions of interest (ROIs; bilateral hippocampi in this study) were obtained using a modified processing stream developed for PING using FreeSurfer, and which included additional software modifications developed at UCSD Multimodal Imaging Laboratory (MMIL). Subcortical ROIs were then labeled using an automated, atlas-based, volumetric segmentation procedure (volumes in mm^3). Although a 3D T2-weighted volume scan was part of the standardized structural MRI protocol, not all sites were able to collect this sequence. Therefore, the T2-weighted intensities were calculated from the average of the diffusion-weighted b = 0 images (averaged if multiple b = 0 images) and normalized by a scaling factor calculated as the slope of the relationship (across all brain voxels) between mean diffusivity and b=0 values.[109] The T2-weighted intensities were not normalized with whole brain intensity or with cerebral spinal fluid (CSF) intensity due to concerns that the normalization may be heavily influenced by partial volume artifacts in the estimate of CSF T2. The T1-weighted volumetrics for bilateral hippocampi were extracted from the data set for analyses.

Imaging Data Quality Control. *Raw Image Quality Control*. Following established protocols,[109] each recruiting site uploaded DICOM images for each scan session using a secure web-based application. All uploaded data were then automatically checked for completeness and protocol compliance, and trained technicians reviewed images for motion artifacts, excessive distortion, operator error, or scanner malfunction. T1-weighted images were examined slice-by-slice for excessive motion (e.g., stark ribbon or criss-cross artifacts within the parenchyma and ghosting artifacts outside the head) and each volume was rated as either acceptable or recommended for rescan. Diffusion images were also examined slice-by-slice for signs of artifacts or poor image quality. Volumes with five or more slices showing significant slice-to-slice motion, motion artifacts, or whole-slice dropout were rejected (i.e., recommended for rescan). Overall quality control ratings of good, average, or unacceptable were assigned to each dataset and only data rated good or average were used. Quality information was recorded into the quality control utility within 24 hours from time of upload to allow re-scanning of subjects if possible.

Processed Image Quality Control. Following established protocols,[109] all processed images (i.e., subcortical volumetric segmentations, cortical areal parcellations, and white and pial surface reconstructions) were examined for all participants. Quality control movies were made in Matlab for each subject to help with data examination. White matter texture consistency and underestimation of temporal regions were examined using movies showing coronal views in sequence and a related horizontal sequence. Pial and dural overestimation along parietal regions and signs of excessive head motion were examined using a movie showing sagittal views.

Statistical Analyses

Linear regressions were run in R studio (http://www.rstudio.com/; Version 1.0.136) to examine the relationship between BMIz, and hippocampal signal intensity and volume, controlling for age, sex, scanner serial number, GAF, and household income. To maintain consistency with prior PING studies, we opted to retain all covariates in our models, regardless of statistical significance. We ran one linear model, to assess for potential associations between BMI z-scores and all covariates included in our models. We found no significant associations between BMI z-scores and any covariates. We also assessed for potential differences in BMI z-scores and demographic measures (i.e., parental income and genetic ancestry factors) between the three different scanning sites using their device serial numbers. In order to test the specificity of our results to the hippocampus, we also examined the relationship between BMIz and bilateral amygdala and nucleus accumbens volumes. In models examining hippocampal volume, covariates also included total intracranial volume to normalize the hippocampal volumes. The final models examining hippocampal volume included a total of 90 subjects (i.e., 12 subjects were removed due to the following: N=2 missing structural T1-weighted MRI data, N =1 missing scanner device serial number, N =4 missing household income information, N = 5 missing GAF information). The final models examining hippocampal T2-weighted signal intensity included a total of 83 subjects (i.e., 19 subjects removed due to: N=9 missing T2-weighted intensity data, N =1 missing scanner device serial number, N =4 missing household income information, N = 5 missing GAF information). To examine the potential effects of outliers, models were run with and without four outliers (< -2 BMIz). Since our results did not significantly change whether these outliers were included or not, we kept them in our models and reported on the whole sample. Effect sizes were also calculated using Pearson correlations to examine the strength of each association.

Results

Sample descriptive statistics

ANOVAs were run to assess for potential differences in BMI z-scores and demographic measures (i.e., parental income and genetic ancestry factors) between the three different scanning sites. We found no differences in BMI z-scores or parental income between sites. There were significant differences between genetic ancestry factors (GAF) such that there were significantly more East Asian ($F = 4.16$, $p = 0.01$), Central Asian ($F = 2.76$, $p < 0.05$) and Oceania GAF ($F = 4.21$, $p = 0.01$), and significantly less African GAF ($F = 5.89$, $p < 0.01$) in the second scanner site relative to the other two sites. Therefore, we included the device serial number as one of our covariates in every model.

Hippocampal volume

Linear models including all covariates (i.e., age, sex, site, genetic ancestry factors, parental income, and total intracranial volume) showed that BMIz was not significantly associated with left ($t = -0.94$, $p = 0.35$) or right ($t = -0.57$, $p = 0.57$) hippocampal volume, although the relationship was in the predicted direction (Figure 3.1). Total intracranial volume was significantly associated with left ($t = 4.32$, $p<0.001$) and right ($t = 4.32$, $p<0.001$) hippocampal volume.

Amygdala and nucleus accumbens volumes

Linear models including all covariates (i.e., age, sex, site, genetic ancestry factors, parental income, and total intracranial volume) showed that BMIz was not significantly associated with left ($t = -1.15$, $p = 0.25$) or right ($t = -0.50$, $p = 0.62$) amygdala volume or left ($t = -0.83$, $p = 0.41$) or right ($t = -1.99$, $p = 0.05$) nucleus accumbens volume (Figure 3.2).

T2-weighted signal intensity

Linear models including all covariates (i.e., age, sex, site, genetic ancestry factors, and parental income) showed that BMIz was negatively associated with left ($t = -3.26$, $p = 0.002$) and right ($t = -2.57$, $p = 0.01$) hippocampal signal intensity (Figure 3.3). These associations remained significant after removing four potential outliers from the models (i.e., left ($t = -2.63$, $p = 0.01$) and right ($t = -3.59$, $p = 0.001$). Effect sizes were $r = -0.2$ in the left hippocampus $r = -0.36$ in the right hippocampus.

Discussion

This study examined the association between whole body weight and hippocampal volume and tissue signal intensity in a diverse sample of adolescents across the weight range. Although prior research in children showed that a higher BMIz was associated with smaller hippocampal volume,[8,41] this study found only a trend for this relationship. However, this study is the first to examine the relationship between body weight and T2-weighted signal intensity within the hippocampus to assess for potential alterations in hippocampal tissue properties (e.g., gliosis). In this study, higher BMIz values were associated with lower bilateral hippocampal T2-weighted signal intensities. Most of the prior literature that examined the relationship between weight and T2-weighted signal intensity focused on the hypothalamus and showed a positive association between BMI and T2-weighted signal intensity.[101] Thus, it is possible that this association manifests itself differently in the hippocampus. Overall, these results suggest that in adolescents, greater body weight is associated with altered hippocampal tissue integrity, but not altered volumes.

Our results did not show that heavier body weight was associated with smaller hippocampal volume. A prior study in adolescents also found no differences in hippocampal grey matter volumes in adolescents with excess weight relative to healthy weight.[51] It is possible that being overweight in adolescence does not negatively impact hippocampal volume as seen in younger children. Alternatively, the association between excess body weight and hippocampal volume may happen earlier in life, while weight-related alterations in hippocampal tissue integrity is more apparent during adolescence. In fact, adolescence is associated with significant brain development, with increases in hippocampal volume.[111] Therefore, it is possible that the negative association between total body weight and hippocampal volume is compensated by the higher levels of neurogenesis in the hippocampus[49] during this time period. Future research should examine the association between total body weight and hippocampal structure over time using a longitudinal design to examine if changes in hippocampal volume vary at different ages during development in these children. In addition, our results revealed a trend between body weight and right nucleus accumbens volume. Based on prior research showing that children with obesity show hyperactivation to food cues in this region,[107,108] future research should further examine the potential association between total body weight and nucleus accumbens structure.

Contrary to our prediction and prior findings,[107,108] our results found that greater weight was associated with lower T2-weighted signal intensity within the hippocampi. However, our findings may be explained by a prior study that showed abnormalities in tissue composition (i.e., tissue viscosity; accumulation of macromolecules and lipid in the tissue) were associated with decreased T2-signal intensity.[112] In that study, the authors postulated that an accumulation of macromolecules and lipids would lead to an increase in tissue viscosity and a shortening of T2 relaxation times, which would appear as a decreased signal intensity on T2-weighted

images. Therefore, our results could indicate that adolescents with greater body weight also had more accumulation of lipids in the hippocampal tissue, resulting in an increase in tissue viscosity and decreases in T2-weighted signal intensity. The greater lipid accumulations could have resulted from their consumption of a high-fat diet, which has been associated with alterations in hippocampal tissue in animals (e.g., breakdown of blood brain barrier and a reduction in levels of BDNF).[87,113] Unfortunately, this study did not include dietary information, so we were unable to explore this hypothesis. Future research should include dietary intake assessments to examine whether a high fat diet (i.e., western diet; high saturated fats and sugar) also might contribute to the inverse relationship between body weight and T2-weighted signal intensity in the hippocampal regions. In addition, hypertension and associated microhemorrhages are associated with decreases in T2-weighted signal intensity.[114] Therefore, it is possible that teenagers with a higher BMI-z score also have higher blood pressure which could result in micro lesions and differences in T2-weighted signal. Future research should include blood pressure measurements to assess for their possible relationships with T2-weighted signal intensities in adolescents.

Overall, this study suggests a negative association between body weight and hippocampal tissue property in adolescents. Negative influences on the hippocampus during adolescence, which is a critical time period for brain development, could have long-lasting effects and might predispose these adolescents to a lifetime of overeating and overweight. However, the hippocampus is known for its capability for neurogenesis.[72] Thus, additional research to determine possible mechanisms related to the negative association between body weight on the hippocampus in youth could help to guide interventions to promote hippocampal health.

This study has a number of strengths. First, this study included a diverse sample of adolescents from various backgrounds and environments (i.e., Honolulu, New Haven, and New York), which increases the generalizability of the study findings. In addition, all data included in the study were analyzed by the same team at UCSD using an automated and robust processing stream, limiting the possibility for variability in data processing and human error. However, this study also has some limitations. Due to the cross-sectional nature of this study, we were unable to examine causal implications for the reported associations. The sample size in our study was smaller than originally planned due to missing imaging data or other relevant information in some of the participants. In addition, although all models included variables that accounted for the different scanners, it is still possible that unmeasured variables associated with the scanner could impact these outcomes. Future studies should strive to use data collected under similar, if not exactly the same, conditions (i.e., same site and same scanner). Further, although we sought to account for the effects of pubertal development by only including children between the ages of 12-18, we do not have a formal measure of puberty and therefore could not truly account for this confound. Future studies should include a formal measure of pubertal development (e.g., pubertal hormones) which can be used as a covariate in models examining brain development. Finally, due to the lack of dietary information, we were unable to examine whether a high fat diet also might have contributed to the lower signal intensity in those with higher body weight. It is crucial that future studies examine the effects of diet on hippocampal structure and tissue integrity.

In conclusion, this is the first study to show that greater body weight in adolescents is associated with lower T2-weighted signal intensity, suggesting lipid accumulations in the tissue. In addition, although our results did not show a significant correlation between heavier body

weight and smaller hippocampal volume, the expected inverse correlation was observed. Our results provide important information which will improve our understanding of the association between weight and neural structures and will contribute to prevention programs during a critical brain development period (i.e., evaluate outcomes on brain images after interventions on weight control to improve hippocampal health). Future research should further examine the underlying mechanisms for the hippocampal tissue integrity in overweight children.

Dissertation Author's Acknowledgements

I, Zoe Mestre (primary investigator and author of this material), would like to thank all coauthors – Drs. Bischoff-Grethe, Wierenga, Jernigan, Eichen, Chang, Ernst, and Boutelle – for their contributions to this work. I also would like to thank the journal of *Obesity* for accepting this work for publication.

Figures

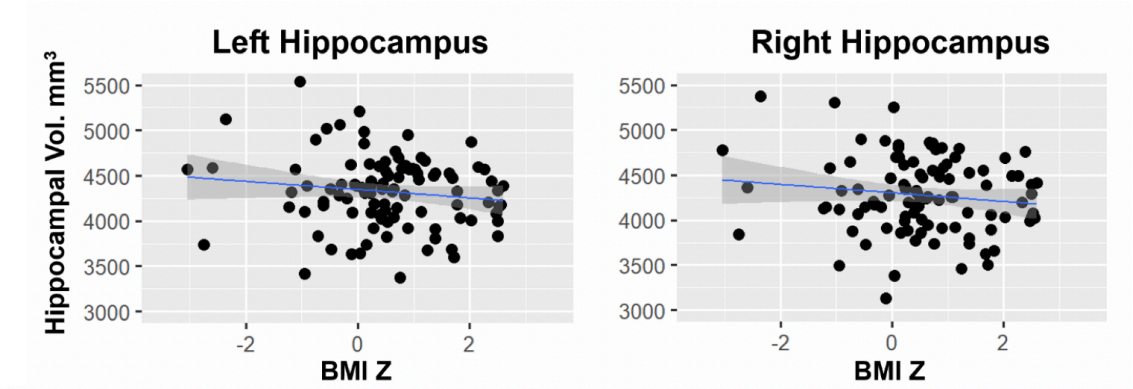

Figure 3.1. *Linear regressions between BMIz and hippocampal volume (mm^3). No significant relationship was found between BMIz and left (t = -0.94, p = 0.35) or right (t = -0.57, p = 0.57) hippocampal volumes after controlling for age, sex, scanner device number, GAF, household income*

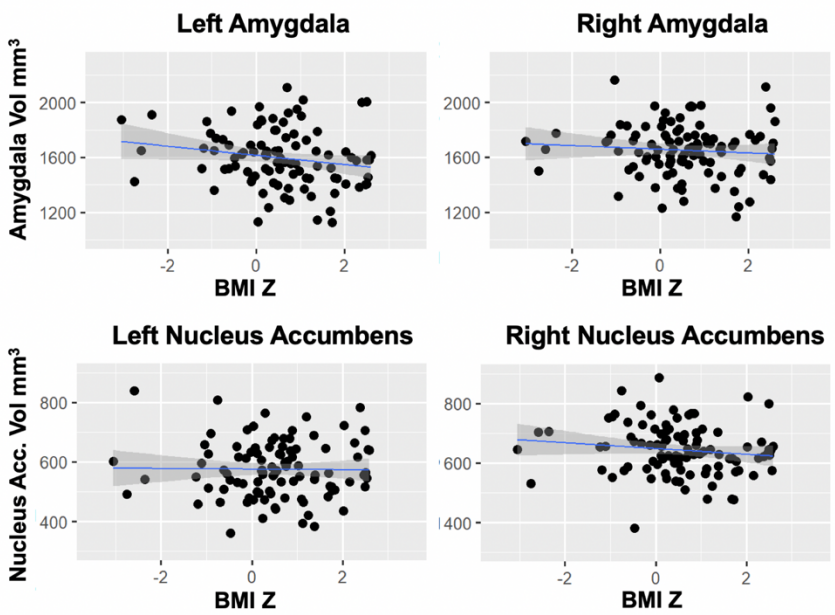

Figure 3.2. *Linear regressions between BMIz and amygdala and nucleus accumbens volumes (mm3). No significant relationships were found between BMIz and left (t = -1.15, p = 0.25) or right (t = -0.50, p = 0.62) amygdala volume or left (t = -0.83, p = 0.41) or right (t = -1.99, p = 0.05) nucleus accumbens volume after controlling for age, sex, scanner device number, GAF, household income*

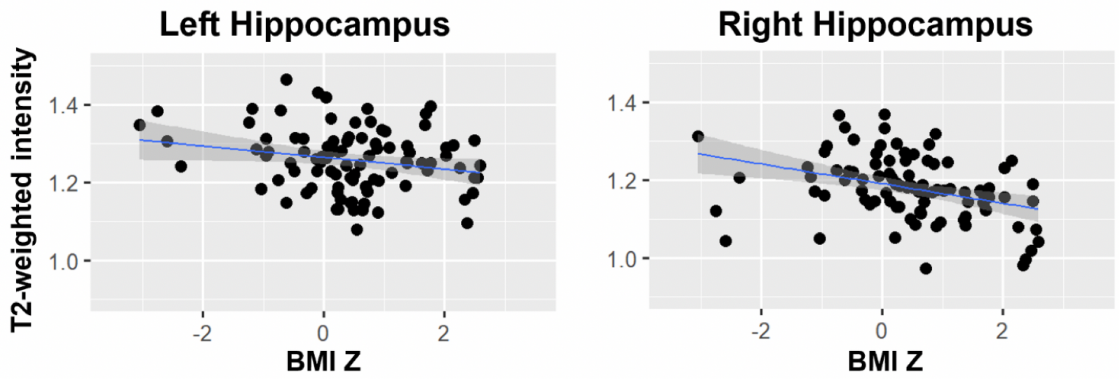

Figure 3.3. *Linear regressions between BMIz and hippocampal T2-weighted signal intensity. Significant relationships were found between BMIz and left (t=-3.26, p=0.002) and right (t=-2.57, p=0.01) hippocampal T2-weighted signal intensity after controlling for age, sex, scanner device number, GAF, household income*

Tables

Table 3.1. *Child demographics*

	Mean ± Std deviation
Age, years	15.07 ± 1.84
Sex, Female	53% (N=54)
BMI, kg/m^2	23.16 ± 5.32
BMIz	0.54 ± 1.17
Ethnicity	
White	50%
Pacific Islander	10%
Asian	27%
Black	9%
Indian American	4%
Genetic Ancestry Factor	
European	0.57 ± 0.38
African	0.12 ± 0.26
Indian American	0.01 ± 0.05
East Asian	0.27 ± 0.36
Oceania	0.01 ± 0.03
Central Asia	0.02 ± 0.11

CHAPTER 4:

Study 3

The content within this section, titled "Chapter 4: Study 3," presents the introduction, methods and results for the third study in this staple dissertation. This study in currently in preparation for publication.

Mestre, Z., Bischoff-Grethe, A., Wierenga, C. E., Strong, D., & Boutelle, K. N. (i*n preparation)*. Differences in hippocampal structure and hippocampal-dependent memory in children at high or low risk for obesity.

Abstract

The hippocampus is a key structure implicated in weight regulation due to its involvement in food motivation and intake. Children with obesity (OB), compared to healthy-weight (HW) peers, have significantly reduced hippocampal volume as well as significantly poorer performances on hippocampal-dependent memory. HW adults at high-risk for future OB, relative to those at low-risk, also show reduced total grey matter volume in regions including the hippocampus. No study has examined hippocampal structure in HW children at risk for OB. This study examined differences in hippocampal structure and hippocampal-dependent memory between HW children at high-risk for OB (HR; two parents with overweight or OB) and those at low-risk for OB (LR; two parents with HW). Eighty-two HW 8-11-year-old children (41HR, 41LR) completed magnetic resonance imaging (MRI), two subscales of the Child Memory Scale (CMS) (i.e., dot locations and word pairs), and behavioral eating measures and questionnaires (i.e., absence of hunger paradigm, parent-report child Reward-Based Eating (RED) questionnaire, and parent-report Child Eating Behavior Questionnaire (CEBQ)). Voxel-wise differences in hippocampal volume between groups were examined using FSL-FIRST and voxel-based-morphometry protocols. Differences in neurocognitive performance between groups were examined by comparing the scaled scores from both CMS subscales. Finally, we explored associations between hippocampal volume and memory, and eating behaviors. False Discovery Rate (FDR) correction was used for the main hypotheses to account for multiple comparisons. Robust Huber linear regressions showed that HR children, relative to LR, had significantly smaller left hippocampal volumes ($t = -2.11$, $p = 0.04$) after controlling for covariates (i.e., age, sex, ethnicity, BMIz, Child Epidemiology Depression Scale (CESD) score, and Intracranial volume (ICV)). This finding fell to a trend after FDR correction ($p = 0.08$). Results did not show

group differences in localized hippocampal morphology after controlling for covariates. On the CMS word pairs task, HR children, relative to LR, performance significantly worse on immediate total recall ($t = -2.74$, $p = 0.008$) and long delay recall ($t = -2.40$, $p = 0.019$) subscales after controlling for all covariates. These findings held true after FDR correction ($p = 0.04$ and $p = 0.048$ respectively). Results did not show any significant group differences on the CMS dot location task. Finally, exploratory analyses showed a trend between right hippocampal volume and scores on the RED ($t = 1.90$, $p = 0.07$), and a trend between right hippocampal volume and scores on the CEBQ enjoyment of food subscale ($t = -1.77$, $p = 0.08$). Moreover, results showed significant interactions between scores on the CEBQ enjoyment of food subscale and left hippocampal volume ($t = 2.64$, $p = 0.009$) and right hippocampal volume ($t = 2.11$, $p = 0.038$). This study showed that hippocampal structure and hippocampal-dependent memory differences could be seen in HW children at HR for OB, relative to LR. These findings suggest that differences in brain structure and cognition are independent of weight gain and OB and may be related to genetics and environmental factors.

Introduction

As of 2016, 16.6% of children in the United States had overweight (OW), and 18.5% had obesity (OB).[115,116] Critically, children with overweight/obesity are at increased risk for serious health complications, including cardiovascular disease, Type II diabetes, osteoarthritis, cancer, and overall poorer quality of life.[3] Children with OB are also 6 to 7 times more likely to become adults with OB.[9] Moreover, according to the CDC, the annual medical cost of OB in the US is a staggering $147 billion. OB in childhood alone is estimated to cost $14 billion in direct health

expenses.[117] Research to uncover potential mechanisms in the development of OB in children is crucial to further understand the underlying factors and develop new preventative interventions.

Overeating (i.e., an imbalance between energy intake and expenditure) is a well-known factor associated with a state of OB.[1,2,10,11,14] Overeating may also indirectly lead to overweight/OB due to disruptions in hippocampal structure and functioning.[18,20] Large increases in adipose mass due to prolonged periods of overeating have been associated with adipose tissue dysfunction and metabolic inflammation, as well as increased circulating adipokines and free fatty acids.[118,119] In turn, these circulating pro-inflammatory adipokines increase blood-brain barrier permeability[120] which then allows the entry of free fatty acids, shown to directly alter hippocampal function,[121] and lead to further hippocampal injury and subsequent atrophy.[119] Research in both adults and children has shown differences in hippocampal structure in individuals with OB relative to HW.[4,5,8,31-35] Children and adolescents with a higher body mass index (BMI), relative to those with healthy BMIs, have reduced brain volume in frontal and limbic regions,[47] as well as decreased hippocampal volume.[40,58] Our prior research in children and adolescents across the weight range has also shown that a higher standardized BMI score (BMIz) was associated with lower bilateral hippocampal T2-weighted signal intensities, suggesting potential alterations in hippocampal tissue properties (e.g., gliosis), as well as a trend for smaller hippocampal volume.[122] Given the alterations in hippocampal tissue (e.g., breakdown of the blood-brain barrier, increased lipids in the hippocampal tissue)[122] with OB, an important question is whether these changes occur in HW individuals, independently of weight gain/OB.

The hippocampus is an important brain structure in feeding behaviors and weight regulation through its role in the perception and processing of hunger and satiety signals, and in initiating and terminating eating in response to the perception of hunger and satiety.[18-21] Amnestic

adults with bilateral hippocampal damage show difficulties in identifying hunger and satiety and consume several meals consecutively if allowed.[25] To date, three studies have shown that children with OW/OB show differences in hippocampal structure and functioning relative to HW.[8,40,42] Very few studies have examined differences in hippocampal structure and function in children at-risk for OB.[47,48] These studies showed that in samples of children across the weight range, BMIz scores were associated with smaller grey matter volumes in frontal and limbic regions. [47,48] However, none of these studies have examined the temporal relationship between OW/OB and hippocampal changes.

In adults, there is some evidence for neural predisposition for weight gain. One study examined structural brain differences between adults at high risk (HR; BMI between 20–30 kg/m^2, one or more first degree relative(s) with a reported history of OB, a history of past weight fluctuations (±10 lbs. or more), and not actively trying to lose weight) or low risk (LR; BMI between 17–25 kg/m^2, no first-degree relatives with a history of OB, never overweight, no past weight fluctuations (±10 lbs. or more), and high levels of physical activity (>3h/week of planned physical activity)) for OB to determine if structural brain differences precede weight gain and OB (i.e., potential mechanism predicting OB risk).[53] HR adults, relative to LR, had reduced total grey matter volume as well as reduced grey matter volume in the orbital frontal cortex (OFC), insula, and cerebellum. Another study conducted among 83 young women (age = 18.4 ± 2.8; BMI range = 17.3–38.9; 78.3% white Caucasian) found a trend ($p=0.06$) suggesting that reductions in grey matter volume in the prefrontal cortex were associated with an increase in slope BMI from baseline to 1-year follow-up, controlling for initial BMI.[54] Although data in adults suggests that there may be neural risk factors for weight gain, only one study to date has examined this in youth. In that particular study, HR adolescents (adolescents with two

overweight/obese parents, BMI≥27), relative to LR (adolescents with two HW parents, BMI<25), showed a greater response in reward brain regions (e.g., striatum) and somatosensory brain regions (i.e., opercular regions) in response to food cues (i.e., receipt and anticipated receipt of milkshake).[55] However, the authors did not explore if there were any differences in brain structure, such as the hippocampus, between risk groups. Additionally, this study was conducted with adolescents. Adolescence is a critical brain developmental period,[49,50] associated with increases in grey and white matter volume in several regions including the hippocampus. Therefore, examining differences in hippocampal structure during childhood, prior to adolescence, using a similar paradigm (i.e., HR and LR children with HW) would help us determine if changes in hippocampal volume are in fact associated with obesity risk.

Finally, behavioral research in children has shown that OB and OB-related factors (i.e., a greater amount of adipose tissue) are associated with poorer performances on hippocampal-dependent memory tasks. In a study conducted among 126 children (7-9 years old) who were overweight/OB (>85%BMI) or HW (<85%BMI), the amount of abdominal adipose tissue was a significant negative predictor of performance on a hippocampal-dependent relational memory task.[59] However, we do not know which precedes which. Due to the role of the hippocampus in memory, eating, satiety, and overeating, a greater understanding of differences in the hippocampus in children at risk for OB will contribute to an understanding of the neurobiological risk factors for excessive weight gain and OB and could provide important information to design prevention programs for youth (i.e., interventions to increase hippocampal health).

This study aims to compare hippocampal volume and hippocampal-dependent memory in HR children (i.e., two OW/OB parents), relative to LR children (i.e., two HW parents). We predict that hippocampal structure (i.e., reductions in hippocampal grey matter volume) and

performance on hippocampal-dependent memory tasks will be significantly different between HR and LR children. We predict that HR children, relative to LR, will have smaller overall hippocampal grey matter. We also predict that HR children, relative to LR, will show localized morphometric reductions in the ventral hippocampus.[123] We also expect that HR children, relative to LR, will have poorer performance on hippocampal-dependent word-pair and visuospatial memory tasks. This study will also examine whether there are associations between hippocampal structure, memory functioning, and eating behaviors and whether these associations are moderated by risk group (i.e., HR and LR). Results from this study could help determine whether differences in brain structure and cognition are independent of weight gain and OB and may be more related to factors such as genetics or environmental factors (i.e., in-utero environment, western diet).

Methods

Participants.

As part of a larger study, children and their parents were recruited from the community using study flyers, listservs, Research Match, and Craigslist, Facebook ads, and through advertisements to local providers in family and internal medicine clinics. After parents completed an initial phone screen, eligible families (one parent and child) were invited to the UCSD Center for Healthy Eating and Activity Research (CHEAR). Following parental consent and child assent, the child and parent completed the initial interview-based screening for the study. If eligible (see inclusion/exclusion criteria), the child and parent were invited to attend two more visits for the study, during which the child and their parent completed behavioral

assessments, questionnaires, a mock scan, and an fMRI scan. Inclusionary criteria included: HW child (5-75% BMI for age) age 8-11 years old, right-handed (participating child), either two biological parents who were overweight/obese (HR) or no biological parents that were overweight/obese (LR), fluent in English for speaking, reading, and writing (participating parent and child), and liking of cheese pizza and chocolate milkshake (participating child; necessary for the procedures included in the parent R01). Exclusionary criteria included: Current or past eating disorder diagnosis (participating child); first degree relative with Anorexia Nervosa or Bulimia Nervosa, presence of significant psychiatric disorder (participating child), acute suicidality and/or self-injurious behaviors, MRI contraindications (e.g., braces, metallic foreign object or device in the body, piercings that cannot be removed, tattooed permanent makeup containing metal, use of Bigen permanent hair dye, severe claustrophobia), Type I or Type II Diabetes, cognitive impairment or disability, inability to lie still on back for at least 30 minutes, vision problems uncorrectable with lenses, food allergies related to cheese pizza, chocolate milkshake, or the snack foods used in the study, menarche in female participants at time of enrollment, mid-pubertal or higher range on the Pubertal Development Scale[124] (participating child), and medications that might interfere or affect neural response to the fMRI task (in the parent study).

One hundred children were recruited for this study (i.e., 45 HR, 55 LR), and 82 were included in the final study (i.e., 41 HR, 41 LR). An a-priori power analysis using G-Power showed that a total sample size (HR and LR) of N=81 would be needed to achieve a medium effect size of $f = .50$ (power of $1\text{-}\beta = .90$, and two-tailed $\alpha = .05$) with ≤ 7 predictors in our linear regression models. Since our models never exceeded 7 predictors (i.e., 1 independent variable of interest and 6 covariates), our analyses were deemed well powered with a sample size of N=82.

Measures.

Anthropometry. Height was measured to the nearest 0.1 cm using a portable Schorr height board (Schorr Inc, Olney, MD) in duplicate, and the average of the 2 values was used for analysis. Bodyweight in kilograms was measured to the nearest 0.1 kg in duplicate on a Tanita Digital Scale (model WB-110A), and the average of the 2 values was used for analysis. Height and weight were converted to body mass index (BMI= [kg/m2]). For children, BMI was then translated to standardized BMI scores (BMIz) using the CDC growth charts.[75]

Clinical interviews. The Eating Disorder Examination,[125] adapted for children (ChEDE),[126] is the gold-standard semi-structured interview for assessing disordered eating cognitions and behaviors. The ChEDE has demonstrated strong interrater reliability and discriminant validity for eating episodes in youth.[73] The ChEDE was used to exclude children if they met diagnostic criteria for Anorexia or Bulimia Nervosa. The Mini International Neuropsychiatric Interview for Children and Adolescents (MINI-KID)[74] was administered to assess psychological difficulties and psychiatric disorders. The MINI-KID is a reliable and valid assessment measure with strong specificity of psychiatric diagnoses in youth.[74] Children were excluded from participation in the present study if they met criteria for any of the following disorders: depression, generalized anxiety, obsessive-compulsive disorder, ADHD, bipolar disorder, substance use, or psychosis.

Memory assessment. The Children's Memory Scale (CMS)[127] is a comprehensive learning and memory assessment for children and adolescents aged 5 to 16 years old. It assesses declarative learning and hippocampal-dependent memory tasks in three domains (i.e., Auditory/Verbal, Visual/Nonverbal, and Attention/Concentration (working memory)). Hippocampal dependent memory for this study was assessed using two subtests (i.e., dot locations and word pairs)[127-130] based on the hippocampus's role in word-pair and visuospatial

memory.[131] In the dot locations subtest, children were assessed on their ability to learn and recall an array of nine randomly positioned dots in immediate and delayed conditions. Performance learning and recalling the array in an immediate recall condition was measured by the "Immediate Total Score" subscale. Children's ability to recall the array of dots following a delay was measured by the Delayed Recall subscale. On the word pairs subtest, children were assessed on their ability to learn and retrieve an orally presented list comprising semantically unrelated word pairs in immediate and delayed conditions. Performance learning and recalling the word-pairs list in an immediate condition was measured by the "Immediate Total Score" subscale. Children's ability to recognize and recall the word-pairs list following a delay was measured by the Delayed Recall and Delayed Recognition subscales.

Eating behaviors. Eating in the absence of hunger (EAH) is a lab-based measurement of how much children eat when physiologically satiated. The assessment measure of EAH was developed by Birch and colleagues[78,132] and has been associated with longitudinal weight gain.[132] To assess EAH, each child participated in a standard ad libitum pizza dinner and self-report post-meal satiety using a cartoon representation of three levels of fullness.[133] Ten minutes following the completion of the meal, the child was left alone for a "free access session" in a room with containers holding generous pre-weighted portions of eight snack foods (e.g. Cheese-its, Doritos, Cheetos, Skittles, M&Ms) as well as toys and games. After the 10 minutes of free access, the amounts of remaining food items were weighed, and total calories consumed by each child was calculated by food and in total and translated to the percent of calorie needs consumed using age-specific formulas for calculating energy requirements.[134] Eating behaviors were further assessed using including the Child Eating Behavior Questionnaire,[76] and the Reward-Based Eating questionnaire[135] (secondary aim 1).

MRI Collection. MRI images were acquired on a 3.0 T GE MR750 scanner equipped with quantum gradients providing echo planar capability, located at the Keck Center for fMRI on the UCSD campus. Each 90-minute session included a three-plane localizer scan. Meant to ensure appropriate head positioning and whole-brain coverage, and structural MRI. Whole brain, sagittally acquired (0.8 mm slice thickness, FOV=256x240 mm) T1-weighted (MPRAGE PROMO, TE=2.3 ms, flip angle=8°, matrix=320, 2x in-plane acceleration, scan time=7:50) and T2-weighted (3D CUBE, TE=60 ms, variable flip angle, matrix=320, 2x in-plane acceleration, scan time=6:51) sequences were acquired for volumetric analyses of white matter, gray matter, and CSF, alignment and morphometry.

MRI Analysis. One hundred 8-11-year-old children (i.e., 45 HR, 55 LR) completed the MRI session. Structural images (i.e., T1- and T2-weighted images) were visually inspected for quality assurance, and images were rated as either: unusable, poor, fair, good, or excellent. Participants with data rated as unusable or poor were excluded from analyses (i.e., 4 HR, 7 LR). Also, based on recommendations to have equal sample sizes to prevent bias in neuroimaging analyses (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM/UserGuide), an additional 8 LR children with structural data rated as fair or good were excluded from analyses which resulted in the final sample of 41 HR, and 41 LR with fair to excellent neuroimaging data.

The Human Connectome Project (HCP; humanconnectome.org) pipeline with FreeSurfer (FS; http://surfer.nmr.mgh.harvard.edu) version 6.0 was used for data processing. Both T1w and T2w images were used as input to help reduce noise artifact from dura and blood vessels and to improve pial surface reconstruction.[136] This project used the first two structural HCP pipelines (PreFreeSurfer and FreeSurfer) which align the T1w and T2w images and reconstruct the cortical surface (surface-based analysis) and subcortical segmentation (volume-based analysis).

Subcortical regions are then automatically labeled.[84] We obtained estimated total intracranial volume (eTIV) from the parcellation statistics output. We then used the MNI aligned images to execute FSL-FIRST[137] (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST), a robust and reliable method for subcortical segmentation, to extract hippocampal volumes. All FSL-FIRST extracted hippocampal volumes for each subject were visually inspected and deemed acceptable. FSL-FIRST extracted hippocampal volumes for each subject were then averaged over all subjects for each group (i.e., HR and LR). All children were included in the volumetric analysis. Also, to identify smaller, voxel-wise differences in hippocampal volume between HR and LR children, we used voxel-based-morphometry (VBM). This was performed using FSL tools[138] and the FSL-VBM protocol[139] (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM), an optimized VBM protocol.[140] Structural T1 images were first brain-extracted and gray matter-segmented before being registered to the MNI 152 standard space using non-linear registration.[141] Due to poor brain-extraction of 2 subjects (i.e., 1 HR, 1LR), those data sets were excluded at this time for the VBM analysis. The remainder of the resulting images (i.e., 40HR, 40LR) were then averaged and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template. All native gray matter volume images were then non-linearly normalized to the study-specific template and "modulated" to compensate for local contraction/enlargement due to the non-linear component of the spatial transformation. All modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. VBM analysis and a voxel-level general linear model (GLM) using FSL-Randomise[142] (i.e., permutation-based method (5,000 permutations)), correcting for multiple comparisons across space, was then used to further examine for localized group differences in hippocampal gray matter volume. The VBM analysis was first restricted to the hippocampus search region of interest (ROI) defined by the Harvard-

Oxford atlas. The hippocampus ROI was applied to the gray matter image from the study-specific template and groups were compared, adjusting for age, BMIz, sex, and depression scores. Threshold-Free Cluster Enhancement was used as a method for finding clusters in the data[143] with thresholds set at $p < 0.05$, corrected.

Correlational analysis. Two Huber robust regression[144] models were performed in R to examine potential group differences (i.e., HR versus LR) in hippocampal volume. In addition, five Huber robust regression[144] models performed in R examined potential group differences in performance on hippocampal-dependent memory and visuospatial tasks. False Discovery Rate (FDR) correction for multiple comparisons was run for models within each hypothesis. Exploratory Huber robust regression models were also used to examine the independent relationship between hippocampal volume and neurocognitive performance (on the CMS dot locations and word pairs task)[127] and eating behavior (EAH,[78,132] CEBQ enjoyment of food and food responsiveness subscales,[76] and reward-based eating).[135] Finally, to evaluate whether the strength of the relationship between structure, neurocognitive performance, and eating behaviors differs across risk groups (i.e., moderation effect) we added two-way interactions between group and indices of structure and function. All models included demographics planned covariates (i.e., age, gender, BMIz, and Ethnicity) and study condition (i.e., HR or LR). All models including subcortical volumes also included each subject's total brain volume (i.e., Intracranial volume (ICV)) estimated by Freesurfer to normalize subcortical volumes. Finally, we also included Child Epidemiology Depression Scale (CESD) scores as a covariate in all models after CESD scores were found to significantly differ by group and due to the known association between depression, hippocampal volume,[145] and cognition.[146]

Results

Participant Demographics

The final study sample included 41 HR children (48.8% female, age = 9.12 ± 0.87 years, BMIz = 0.17 ± 0.32) and 41 LR children (51.2% female, age = 9.51 ± 1.12 years, BMIz = -0.19 ± 0.56). The LR group, relative to HR, had significantly lower BMIz scores ($p < 0.001$) and significantly greater intracranial volumes ($p = 0.03$) which may be due to this group tending to be older ($p = 0.08$). In addition, LR children, relative to HR, had significantly higher scores on the CESD ($p < 0.05$). See Table 4.1 for complete demographic information.

Hippocampal volume

FSL-FIRST

Robust Huber linear models examining hippocampal volumes showed that HR children, relative to LR, had significantly smaller left hippocampal volumes (t = -2.11, $p = 0.04$) after controlling for all covariates (i.e., age, sex, ethnicity, BMIz, CESD score, and ICV) (Figure 4.1). This finding fell to a trend after FDR correction ($p = 0.08$). There were no significant group differences in right hippocampal volumes.

Voxel Based Morphometry (VBM)

VBM results restricted to the hippocampal ROI did not show group differences in localized hippocampal morphology after controlling for covariates. Boys, relative to girls, had significantly greater volume in the left hippocampal region (x = 60, y = 53, z = 26; $p = 0.003$) (Figure 4.2).

Hippocampal-dependent memory performance

Robust Huber linear models were used to examine group differences in hippocampal-dependent memory (i.e., CMS dot locations and word pairs task). Results showed that on the CMS word pairs task, HR children, relative to LR, performed significantly lower on immediate total recall ($t = -2.74$, $p = 0.008$) and long delay recall ($t = -2.40$, $p = 0.019$) (Figure 4.3) subscales after controlling for all covariates. These findings held true after FDR correction ($p = 0.04$ and $p = 0.048$ respectively). There were no significant group differences on the CMS Delayed Recognition subscale ($p = 0.35$). There were also no significant group differences on the CMS dot location immediate total recall subscale ($p = 0.39$), or on the delayed recall subscale ($p = 0.95$).

Exploratory analyses

Independent relationship between hippocampal volume, hippocampal-dependent memory and eating behaviors

After controlling for covariates, results showed a trend between right hippocampal volume and scores on the RED ($t = 1.90$, $p = 0.07$), and a trend between right hippocampal volume and scores on the CEBQ enjoyment of food subscale ($t = -1.77$, $p = 0.08$) (Figure 4.4). No associations were found between right hippocampal volume and EAH total calories. No associations were found between left hippocampal volume and any of the measures of eating behaviors. No significant associations were found between any of the dot locations and word pairs subscales on the CMS and eating behaviors (i.e., EAH total calories, RED score, and CEBQ enjoyment of food and food responsivity subscales).

Moderating effect of group on the association between hippocampal volume, hippocampal-dependent memory and eating behaviors

Results from the Robust Huber linear models with an added interaction term between the group variable (i.e., HR and LR) and subscales on the CMS and hippocampal volumes did not show any moderating effects of group between any of the subscales on the CMS and eating behaviors. However, after controlling for covariates, results showed significant interactions between scores on the CEBQ enjoyment of food subscale and left hippocampal volume ($t = 2.64$, $p = 0.009$) and right hippocampal volume ($t = 2.11$, $p = 0.038$) by group (Figure 4.5).

Discussion

This is the first study to demonstrate differences in hippocampal volume and hippocampal-dependent memory in HW children at HR or LR for OB. Because the hippocampus is an important structure in feeding behaviors and weight,[18-21] examining differences in this region in children with HW could help us gain a better understanding of the neurological underpinnings of childhood OB. In this study, HR children, relative to LR, had significantly smaller left hippocampal volumes. Although this finding became a trend after FDR correction, this suggests that differences in hippocampal structure could occur in HW children, independently of weight gain or OB. This is also the first study to show that children at HR for OB, relative to LR, have lower performances on a measure of hippocampal-dependent memory. Finally, exploratory analyses showed that hippocampal volume was associated with enjoyment of food and reward based eating. Moreover, we found significant interactions between bilateral hippocampi volumes and enjoyment of food depending on risk group. Thus, HR children, relative to LR children, may already be showing patterns similar to children with OB.

Our study adds to a small body of literature showing that structural brain differences can be seen in children with HW. Prior studies in adults have shown that individuals at HR for OB, relative to LR, have reduced total grey matter volume as well as reduced grey matter volume in the orbital frontal cortex (OFC), insula, and cerebellum.[53] However, no study to date had examined structural brain differences in HR and LR children using a similar paradigm, and no study had focused on hippocampal structure. Prior research suggests that the children of women with OB may be at increased risk of cognitive problems and psychiatric problems (e.g., attention deficit hyperactivity, eating disorders and psychotic disorders).[147] Additionally, one conference presentation found that higher maternal pre-pregnancy BMI was associated with smaller total hippocampus volume in their offspring, suggesting that exposure to maternal OB adversely impacts hippocampus volume in children.[148] In one rodent study, offspring of mothers with OB, relative to those born to mothers with HW, showed poorer spatial memory performances.[149] Thus, differences in hippocampal structure between HR and LR HW children could be related to differences in in-utero conditions. Alternatively, these differences could be diet-related (i.e., blood-brain barrier (BBB) breakdown due to consumption of western diet) since animals fed a high fat western diet, relative to a low-fat diet, exhibit impairments in learning and hippocampal-dependent memory tasks (i.e., more spatial working memory errors on tasks requiring spatial cue learning) prior to weight gain.[150,151] Future studies should try to parse apart the contribution of genetics and environmental factors in the observed differences in hippocampal structure in HR children, relative to LR. Nevertheless, these findings are significant as reductions in grey matter volume have been associated with increases in slope BMI from baseline to 1-year follow-up, controlling for initial BMI.[54] Therefore, children at HR for OB, relative to LR, may be at an increased risk to develop overweight due to reduced brain volume in the hippocampus.[18-21]

VBM did not show localized differences in hippocampal morphometry between HR and LR children. However, results did show that boys, relative to girls, have significantly greater volume in the left hippocampal region. Although our groups were well balanced in terms of covariates, the strong effect of gender could be overshadowing the effect of group on hippocampal morphology. Alternatively, our sample size may be too small to detect small morphological differences between groups. Therefore, future studies should examine this further in larger samples, and if possible, stratify their analyses by gender.

Our neurocognitive results were consistent with prior findings showing that in children, OB and OB-related factors (i.e., a greater amount of adipose tissue) are associated with poorer performances on hippocampal-dependent memory tasks.[59] However, our study is the first to show that these differences can be seen in HW children. One prior study in HW children and adolescents did not find any relationship between increased BMI and neuropsychological function.[152] This may further highlight the fact that differences in cognition may not be a result of weight gain, but perhaps be a risk factor for future weight gain. Prior studies have shown that lower intellectual/cognitive function could be a risk factor for subsequent overweight or OB.[153-155] In addition, bilateral hippocampal damage in adult patients has been associated with difficulties in identifying hunger and satiety and consumption of several meals consecutively if allowed.[25] Also, a study examining the role of memory of a recent eating episode (i.e., last meal eaten 3 hours prior) on eating behaviors (number of snack biscuits eaten) in neurologically intact female adults, found that women who had thought about their last meal prior to the snack ate significantly fewer biscuits, relative to those who had not been told to recall what they had last eaten.[27,28] Therefore, future longitudinal studies should examine if differences in hippocampal-dependent memory in HW children are predictive of weight gain over time.

Finally, our exploratory analyses showed trends between hippocampal structure and eating behaviors, and that these associations were moderated by risk group (HR and LR). This is in line with a previous study by our group which showed that in children with OB and HW, greater hippocampal response to taste was positively associated with eating and parent report of child's food responsiveness and enjoyment of food.[40] In addition, the previous study showed that stronger activation to taste was significantly associated with reduced left hippocampal volume. Therefore, it is possible that in children with OB, reduced hippocampal volume and increased response to food taste can lead to increased food responsiveness and greater risk for overeating. This may help explain our current results which showed that right hippocampal volume tended to be associated with measures of child enjoyment of food and reward based eating. Moreover, our finding that the association between bilateral hippocampi volumes and enjoyment of food is moderated by risk group may be due to the fact that only HR children are already showing patterns similar to children with OB. Future studies should examine if brain structure differences and resulting hypersensitivity to food translates to future weight gain/OB.

This study has a number of strengths. First, this study included a fairly large sample of children (N=82) in the context of a neuroimaging study, which increases the generalizability of the study findings. Our study is also the first to use a HR/LR paradigm in HW children to examine differences in hippocampal structuring and functioning depending on risk group. In addition, all data included in the study had minimal motion artifact and was analyzed using well known and validated processing streams (HCP pipeline), which increases the reliability of our findings. Furthermore, children were screened for pubertal development, and only pre-pubescent children were included in the study. This increases our confidence that our findings are not confounded by the effects of puberty on brain development. As in all studies, there are

weaknesses that need to be considered. Our study is cross-sectional, limiting causal implications. While we tried to evenly balance our groups, we still saw significant differences in depression scores and mean BMIz between the HR and LR children. Since both of these variables are known to affect brain structure, greater efforts should be made to ensure that future groups are well balanced in these regards. Finally, due to the lack of dietary and genetic information, we were unable to examine whether diet or genetics might have contributed to the observed differences in hippocampal volume and functioning. Future studies should examine this.

In conclusion, this study shows that children at HR for OB, relative to LR, tend to have lower hippocampal volume, have significantly lower performances on hippocampal-dependent tasks, and that the association between bilateral hippocampal volume and child enjoyment of food is moderated by risk group. These findings suggest that differences in hippocampal volume and hippocampal-dependent memory can be seen in HW children and may not be a result of weight gain or OB as previously thought. As suggested above, it is possible that these observed differences are more so related to genetics or environmental factors (i.e., in-utero environment, western diet). Future studies should further investigate the potential underlying mechanisms of hippocampal differences in HW children. Importantly, this study further identifies the hippocampus as a key structure involved in eating behavior in youth and underlines the importance of studying this region.

Dissertation Author's Acknowledgements

I, Zoe Mestre (primary investigator and author of this material), would like to thank all coauthors – Drs. Bischoff-Grethe, Wierenga, Strong, and Boutelle – for their contributions to this work.

Figures

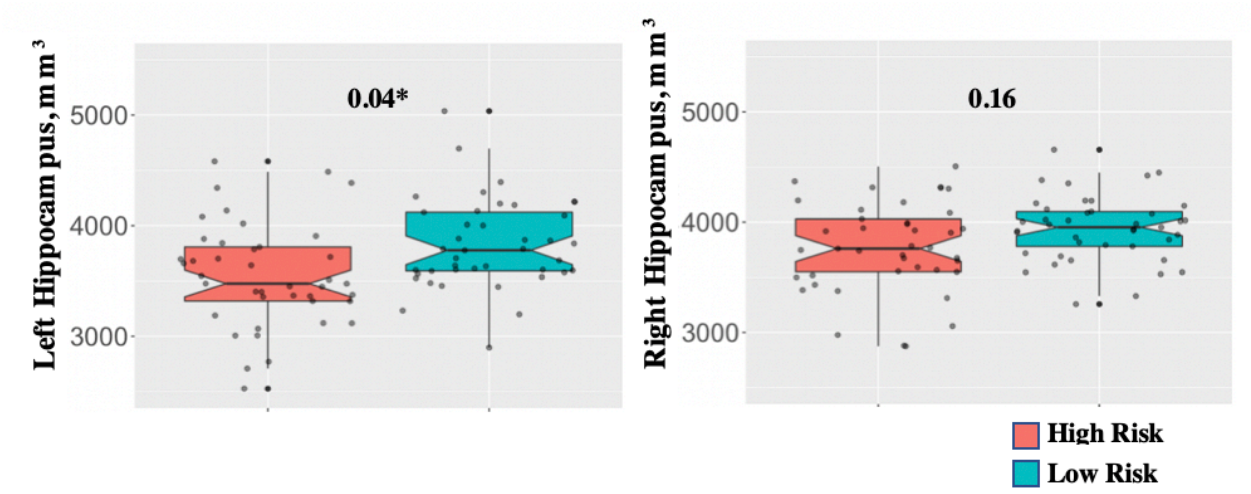

Figure 4.1. *Robust Huber linear models showed that HR children, relative to LR, had significantly smaller left hippocampal volumes (t = -2.11, p = 0.04) after controlling for all covariates (i.e., age, sex, ethnicity, BMIz, CESD score, and ICV) (Figure 3). This finding fell to a trend after FDR correction (p = 0.08). There were no significant group differences in right hippocampal volumes*

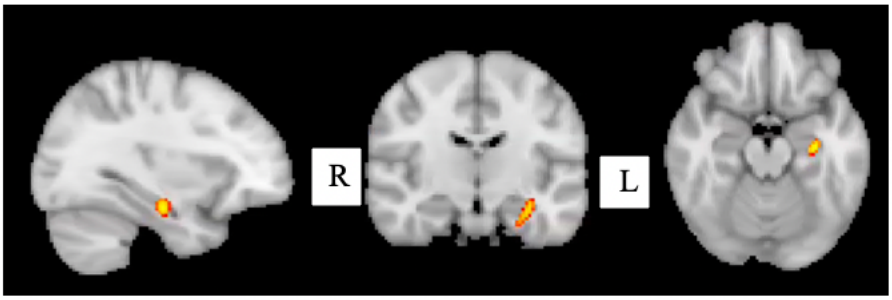

Figure 4.2. *After controlling for covariates, VBM results restricted to the hippocampal ROI showed, boys, relative to girls, had significantly greater volume in the left hippocampal region (x = 60, y = 54, z = 26; p = 0.014)*

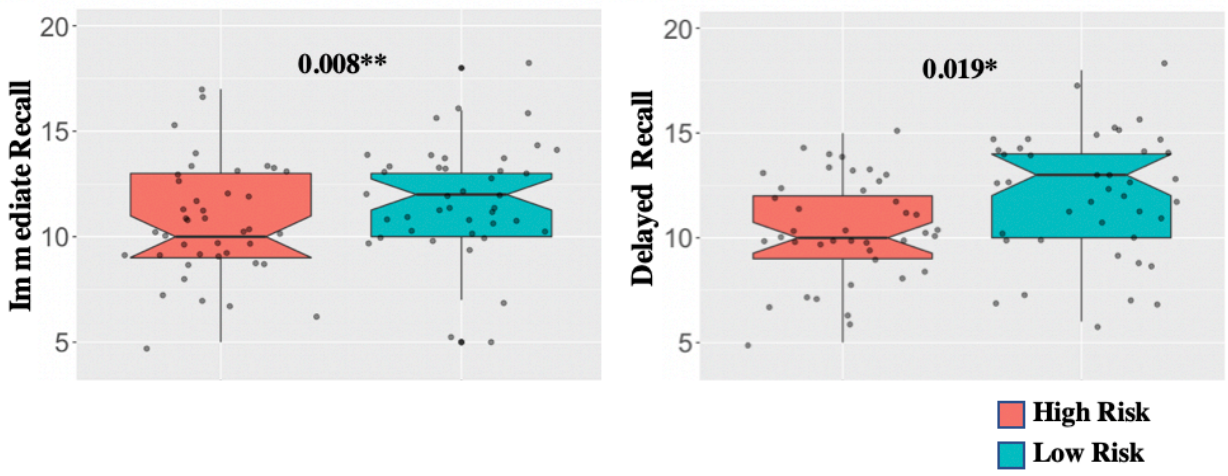

Figure 4.3. *Robust Huber linear models showed that on the Child Memory Scale (CMS) word pairs task, HR children, relative to LR, performance significantly lower on immediate total recall (t = -2.74, p = 0.008) and long delay recall (t = -2.40, p = 0.019) (Figure 4) subscales after controlling for all covariates. These findings held true after FDR correction (p = 0.04 and p = 0.048 respectively)*

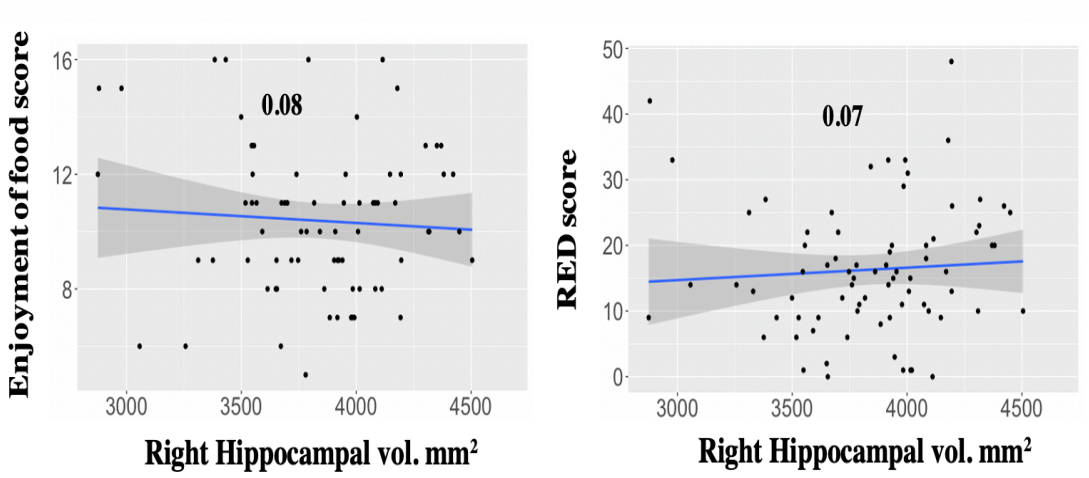

Figure 4.4. *Robust Huber linear models, after controlling for covariates, showed that right hippocampal volume tended to be negatively associated with scores on the CEBQ enjoyment of food subscale (t = -1.77, p = 0.08), and tended to be positively associated with scores on the RED (t = 1.90, p = 0.07)*

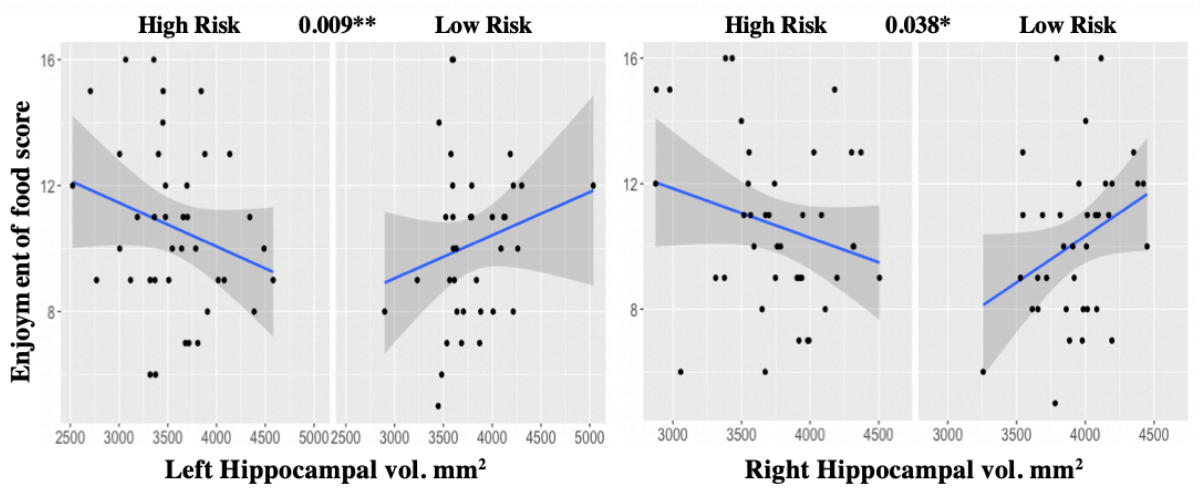

Figure 4.5. *Robust Huber linear models showed significant interactions between scores on the CEBQ enjoyment of food subscale and left hippocampal volume (t = 2.64, p = 0.009) and right hippocampal volume (t = 2.11, p = 0.038) after controlling for covariates.*

Tables

Table 4.1. *Child demographics*

	Mean ± Std deviation		
	High Risk	**Low Risk**	**P-values**
Age, years	9.12 ± 0.87	9.51 ± 1.12	0.08
Gender, Female	48.8% (N=20)	51.2% (N=21)	0.84
BMIz	0.17 ± 0.32	-0.19 ± 0.56	<0.001***
Pubertal Development Scale score	3.02 ± 0.91	2.93 ± 0.98	0.64
Ethnicity			
Non Hispanic White	58.5% (N=24)	70.7% (N=29)	0.50
Hispanic	12.2% (N=5)	9.8% (N=4)	
Other	29.3% (N=12)	19.5% (N=8)	
Intracranial Volume, mm3	1543410 ± 122884	1610520 ± 150609	0.03*
Child Epidemiology Depression Scale	11.7 ± 6.07	14.98 ± 8.34	<0.05*
Eating Absence Hunger, total calories	267.53 ± 125.70	302.62 ± 116.76	0.20
Reward Based Eating Drive Scale	16.53 ± 11.58	15.26 ± 7.99	0.62
Parent report Child Eating Behavior			
Food Responsiveness	5.63 ± 4.03	6.04 ± 2.38	0.63
Enjoyment of Food	10.69 ± 2.89	9.67 ± 2.45	0.15

CHAPTER 5:

Integrated Summary

With the continued rise in the rate of childhood obesity in the United States,[2] it is crucial to conduct more research to identify potential risk and maintenance factors for this disease. Although there is some research to suggest that obesity is associated with neural changes related to hyperphagia and cognitive impairment,[4] few studies have examined structural and functional brain differences in children with obesity, and even fewer have examined this across the weight range or even before weight gain in children with HW. Thus, the three studies that were proposed and completed in this staple dissertation project first explored the effects of weight and obesity on hippocampal structure and function in children with OB relative to HW (*Study 1*), then examined whether these differences could be observed in adolescents across the weight range (*Study 2*), and finally examined whether differences in hippocampal structure and function could be seen in children with HW dependent upon risk factors for obesity (*Study 3*).

Study 1 examined hippocampal differences among 8-12-year-old children with OB and HW. This was the first study to compare both hippocampal volume and its functional response to pleasant tastes in children. This study also examined whether responsivity in the hippocampus was associated with eating behaviors. *Study 1* results showed that children with OB, relative to HW, had significantly reduced left hippocampal volume. This finding was consistent with one prior study in children with OB,[8] and was thought to possibly be due to the impact of sustained consumption of a western diet on the blood-brain barrier (BBB) in the hippocampal region.[19] *Study 1* also found a hypersensitivity to taste cues in three clusters within in left hippocampus (the dorsal, body and ventral portions of the left hippocampus compared to HW children). Thus, altered response to food tastes in the hippocampus using a taste paradigm could be seen as early

as 8 years old, which could help explain why children with OB tend to overeat (i.e., less able to perceive and process satiety signals). Also, this study found that functional activation to taste in the left hippocampus was positively associated with children's eating in the lab (i.e., the total amount of calories consumed when sated), as well as parent report of child's food responsiveness and enjoyment of food. These findings suggest that activation to taste in the hippocampus is associated with eating, reward, and responsiveness to food in this sample. Thus, findings from *Study 1* contribute to the current knowledge base regarding the neural underpinnings of obesity and food cue reactivity and resulting cognitive impairments.

Based on *Study 1* findings showing that differences in hippocampal structure and function can occur early in the disease process, *Study 2* examined whether differences in hippocampal volume could be seen across the weight range in a diverse sample of adolescents. We also examined potential differences in T2-weighted signal intensity (i.e., alterations in tissue properties) since obesity-related factors (i.e., high fat diet) have been associated with hippocampal inflammation,[98,99] and hippocampal gliosis has been shown to increase T2-weighted signal intensity within this region.[107,108] Although findings from *Study 1* showed that a higher BMIz was associated with smaller hippocampal volume,[8,41] *Study 2* findings showed only a trend for this relationship. As discussed in *Study 2,* it is possible that the association between excess body weight and hippocampal volume occurs earlier in life and that this negative association is compensated for by the higher levels of neurogenesis in the hippocampus[49] during adolescence. This should be further explored using a longitudinal design to examine if changes in hippocampal volume vary at different ages during development in these children. However, *Study 2* findings did show that higher BMIz values were associated with lower bilateral hippocampal T2-weighted signal intensities. As discussed above, prior research has shown that

abnormalities in tissue composition (i.e., tissue viscosity; accumulation of macromolecules and lipid in the tissue) were associated with decreased T2-signal intensity.[112] Therefore, results from *Study 2* could indicate that adolescents with greater body weight have a greater accumulation of lipids in the hippocampal tissue, resulting in an increase in tissue viscosity and decreases in T2-weighted signal intensity. These results could also indicate teenagers with a higher BMI-z score also have higher blood pressure which could result in micro lesions and differences in the T2-weighted signal.[114] In sum, *Study 2* showed that in a sample of adolescents across the weight range, greater body weight was associated with lower T2-weighted signal intensity, suggesting lipid accumulations in the tissue.

Finally, S*tudy 3* sought to shed light on the nature or nurture question regarding body weight and differences in hippocampal structure and functioning. Therefore, *Study 3* examined whether differences in hippocampal structure and functioning could be seen in children with HW at risk for obesity. Specifically, *Study 3* compared hippocampal volume and hippocampal-dependent memory in HR children (i.e., two overweight/obese parents), relative to LR children (i.e., two healthy weight parents). Based on findings from *Study 1*, we believed that HR children, relative to LR, would have smaller overall hippocampal grey matter volume. Also, based on prior research in children with overweight,[59] we expected that HR children, relative to LR, would have poorer performance on hippocampal-dependent word-pair and visuospatial memory tasks. Finally, *Study 3* explored potential associations between hippocampal structure, memory functioning, and eating behaviors and whether these associations are moderated by risk group (i.e., HR and LR). Consistent with *Study 1,* findings from *Study 3* showed that HR children, relative to LR, had significantly smaller left hippocampal volumes. Although we did not see group differences in localized hippocampal morphology, results from VBM did show that boys,

relative to girls, had significantly greater volume in the left hippocampal region. From a cognitive perspective, *Study 3* showed that HR children, relative to LR, had significantly poorer performance on a hippocampal-dependent word memory task. Therefore, these findings may indicate that differences in hippocampal volume and cognition can be found in HW children and may be due to genetics or environmental factors (i.e., in-utero environment, western diet) rather than being the results of weight gain/OB. Finally, results from the moderator models showed that the association between eating behaviors and bilateral hippocampal volume differed by risk group. Thus, HR children, relative to LR children, may already be showing patterns similar to children with OB.

Collectively, the findings from the three studies in this staple dissertation project show that differences in hippocampal structure and functioning may present in HW children, persist in childhood once obesity has occurred, and present differently during adolescents when there are higher levels of neurogenesis in the hippocampus. More importantly, the present findings highlight that preventative interventions for childhood obesity should start in HW children at high risk for OB, and should focus on child hippocampal health, possibly as early as in-utero, using dietary, exercise, and cognitive interventions.

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