ACUTE AND MATERNAL EFFECTS OF A HIGH FAT DIET: FOCUS ON THE HIPPOCAMPUS

by

EMILY ROSE TRUNNELL

(Under the Direction of Gaylen L. Edwards)

ABSTRACT

Obesity is one of the leading drivers of healthcare costs associated with preventable chronic disease. On average, U.S. adults gain on average 0.4 to 1 kg per year and it is likely that much of this annual weight gain is an additive result of short-term dietary excess. A significant factor driving short-term weight gain is overconsumption of foods high in fat. Even prior to weight gain, high dietary fat consumption can induce metabolic and physiological changes that are predictive of future weight gain and disease. Animal models used to study the effects of dietary manipulations have paid particular attention to the impact of diet on the brain; however the impact of acute fat intake on the brain has been less well studied. The following chapters provide a rationale for studying the impact of high fat diet on the hippocampus, a brain structure important for learning and memory, and supply novel evidence that acute high fat diet consumption alters hippocampal gene transcription as well as behavior in rats. Finally, we show that exposure to a high fat diet prior to weaning impacts the hippocampal gene expression in mouse offspring in a sex- and time-specific manner.

INDEX WORDS: Diet, Obesity, Hippocampus, Learning and Memory, Epigenetics

ACUTE AND MATERNAL EFFECTS OF A HIGH FAT DIET: FOCUS ON THE HIPPOCAMPUS

by

EMILY ROSE TRUNNELL

B.S., The University of Georgia, 2010

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

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2016

ACUTE AND MATERNAL EFFECTS OF A HIGH FAT DIET: FOCUS ON THE HIPPOCAMPUS

by

EMILY ROSE TRUNNELL

Major Professor:	Gaylen L. Edwards
Committee:	Richard B. Meagher
	John J. Wagner
	Nikolay M. Filipov
	Jesse R. Schank

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2016

DEDICATION

I dedicate this body of work to those who gave the ultimate sacrifice. Over 200 animals, mice and rats, were euthanized in the direct collection of primary research presented in the following chapters. Countless more provided the wealth of data from which our experiments were conceived, improved, and interpreted. May the quality of our work respect and be worthy of their legacy.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. Gaylen Edwards for his support and guidance during the final years of my dissertation work. He took me on as a graduate student during what was a difficult time for the both of us, and has done so much to broaden my knowledge and scope as a neuroscientist. I would like to thank my previous mentor, the late Dr. Clifton Baile, for his generous, although at times confusingly unwavering, confidence in me and for pushing me to pursue and accept opportunities I otherwise would not have. I have really missed his enthusiastic support. I would like to thank Dr. Richard Meagher, a graduate committee member and a friend, who provided me a raft when the seas were rocky, and with whom I've enjoyed exploring the new and impossibly intricate world of the brain. I would like to thank the members of my graduate committee, Drs. John Wagner, Nikolay Filipov, and Jesse Schank for their time and effort in shaping this work. I would like to thank my colleagues Drs. Suresh Ambati, Jeong Yeh Yang, and Colette Miller as well as Diane Hartzell, Kimberly Freeman, Natalie Hohos, and Ping Yu for their assistance, knowledge, troubleshooting help, and general camaraderie.

Thank you to my funding sources, most especially the ARCS Foundation Atlanta Chapter. Thanks to mentees, to family and friends. Thank you to my loving husband Mathieu for his steadfast support, and for going it alone in Savannah while I remained in Athens during my final research year. Thank you all for the love and encouragement.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION: "TEN TRICKS TO REVERSE HOLIDAY WEIGHT GAIN – FAST!"

Obesity is one of the leading drivers of healthcare costs associated with preventable chronic disease, totaling up to $210 billion annually in the United States alone (Cawley and Meyerhoefer, 2012). According to the most recent 2012 report, more than one-third of U.S. adults are obese (Ogden et al., 2014). For many, weight gain is a slow process: U.S. adults gain on average 0.4 to 1 kg (0.9 to 2.2 lbs.) per year (Lewis et al., 2000). Interestingly, it is often reported in the lay press that vacations and holidays are times of significant weight gain. For example, users of an online poll reported weight gain between 5 and 10 pounds over the course of a 7-day cruise (Cruisereviews, 2008). A quick Internet search will recover thousands of articles touting tips and tricks for preventing weight gain on vacation, during the summer, or over the holiday season. In peer-reviewed literature, the data paint a less alarming, but still important, picture. A study of 94 college students at the University of Oklahoma reported weight gain between 0.2 and one kg over Thanksgiving Holidays (Hull et al., 2006) and a summary of holiday weight gain studies concluded that holiday weight gain (November to January) is highly variable and amounts to less than 1 kg per year on average (Schoeller, 2014). Over a vacation period of one to three weeks, 122 adults gained on average only 0.32 kg, but importantly they maintained this gain for six weeks following the vacation's end (Cooper

and Tokar, 2016). Considering these figures, it is likely that much of the annual weight gain observed in U. S. adults is an additive result of holidays and vacations.

A significant factor driving short-term weight gain is overconsumption of foods high in fat. However, even prior to weight gain, high dietary fat consumption can induce metabolic and physiological changes that are predictive of future weight gain and disease. In humans, five days of high fat diet (HFD) consumption caused increased fasting serum endotoxin, a marker associated with certain types of obesity (Moreno-Navarrete et al., 2012), in otherwise healthy adult men (Anderson et al., 2015). At seven days, HFD consumption impaired glucose tolerance, a precursor for diabetes mellitus and cardiovascular disease (Barr et al., 2007), and altered signaling pathways in peripheral blood mononuclear immune cells in a similar cohort (Wan et al., 2014a, Wan et al., 2014b).

Dietary fats aid in the absorption of fat-soluble vitamins and are an important component of a healthy diet. In fact, it has been proposed that increased consumption of fat aided in evolution of the larger and more complex human brain by allowing for the allocation of a greater percentage of our daily energy budget to brain metabolism and by supplying adequate levels of fatty acids critical to brain development (Leonard et al., 2010). However in current calorie-abundant societies, high intake of fat, particularly saturated fat, is considered a major contributor to the development of obesity and its associated comorbidities (Bray and Popkin, 1998). Animal models used to study the effects of dietary manipulations have paid particular attention to the impact of diet on the brain. Over the long term, high dietary fat consumption is associated with increased risk

of dementia and neurodegenerative disease (Farooqui et al., 2007, Morris and Tangney, 2014). The impact of acute HFD intake on the brain has been less well studied.

We took a molecular approach to study the effects of diet on the brain, particularly asking whether or not the manipulation produces changes in gene expression. While increases or decreases in gene transcript levels are not absolute predictors of changes in functional protein levels, mRNA dynamics provide important insight into the rapid effects of a stimulus prior to the onset of phenotypic changes. Fatty acids are known to regulate members of the PPAR and NFκB families, as well as SREBP1 (Jump and Clarke, 1999), transcription factors with vital roles in the CNS (Sampath and Ntambi, 2004). In addition, recent advances in the field of epigenetics provide exciting new insights into how nutrition and metabolism may influence physiology and disease. Chapter 2 will provide a rationale for studying the impact of high fat diet on gene expression and chromatin modifications in the hippocampus, a brain structure important for learning and memory.

Unlike areas of the brain such as the hypothalamus, which regulates food intake to meet metabolically driven needs, the hippocampus drives food intake partly by encoding positive or negative associations with food experiences. We are particularly interested in the functional distinction of the hippocampal formation along its longitudinal axis. While both the dorsal and the ventral hippocampus are important for memory encoding, they are thought to be responsible for different aspects of memories. The dorsal hippocampus is associated with contextual and situational memory and projects to areas of the brain involved in cognitive processing such as the frontal cortex, nucleus accumbens, and substantia nigra (Fanselow and Dong, 2010). The ventral hippocampus is thought to aid

in the contribution of emotional salience to certain memories and tasks and to be more associated with memories that are associated with visceral responses and is highly interconnected with diencephalic brain structures involved in autonomic and endocrine regulation such as the amygdala, hypothalamus, also the nucleus accumbens, and to the bed nucleus of the stria terminalis (Moser and Moser, 1998, Fanselow and Dong, 2010). Both the dorsal and the ventral hippocampus connect indirectly to the ventral tegmental area (VTA), a critical component of the reward pathway Luo et al, 2011). When we experience something pleasurable, such as a sweet, creamy bite of rich cheesecake, neurons from the VTA release dopamine into areas of the brain involved in decision-making, such as the prefrontal cortex. The hippocampus participates in cognitive control of food selection by reminding us that intake of a particular food item was either satisfying or dissatisfying in the past. Connections between the hippocampus and reward pathways promote attribution of a positive or negative salience to our context and experiences (Luo et al, 2011).

In the following chapters, we will supply novel evidence that acute high fat diet consumption alters gene transcription differently in opposite functional poles of the hippocampus as well as changes behavior in rats. In addition, we show that hippocampal gene expression in male and female mouse offspring exposed to a high fat diet in utero and prior to weaning is dependent on sex and age and that these expression profiles can be modulated by maternal administration of an immunomodulatory glycan.

References

Anderson AS, Haynie KR, McMillan RP, Osterberg KL, Boutagy NE, Frisard MI, Davy BM, Davy KP, Hulver MW (2015) Early skeletal muscle adaptations to short-term high-fat diet in humans before changes in insulin sensitivity. Obesity (Silver Spring) 23:720-724.

Barr EL, Zimmet PZ, Welborn TA, Jolley D, Magliano DJ, Dunstan DW, Cameron AJ, Dwyer T, Taylor HR, Tonkin AM, Wong TY, McNeil J, Shaw JE (2007) Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). Circulation 116:151-157.

Bray GA, Popkin BM (1998) Dietary fat intake does affect obesity! Am J Clin Nutr 68:1157-1173.

Cawley J, Meyerhoefer C (2012) The medical care costs of obesity: an instrumental variables approach. J Health Econ 31:219-230.

Cooper JA, Tokar T (2016) A prospective study on vacation weight gain in adults. Physiol Behav 156:43-47.

Cruisereviews (2008) What is the average weight gain on a 7-day cruise? El Segundo, CA: Internet Brands, Inc.

Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65:7-19.

Farooqui AA, Horrocks LA, Farooqui T (2007) Modulation of inflammation in brain: a matter of fat. J Neurochem 101:577-599.

Hull HR, Radley D, Dinger MK, Fields DA (2006) The effect of the Thanksgiving holiday on weight gain. Nutr J 5:29.

Jump DB, Clarke SD (1999) Regulation of gene expression by dietary fat. Annu Rev Nutr 19:63-90.

Leonard WR, Snodgrass JJ, Robertson ML (2010) Evolutionary Perspectives on Fat Ingestion and Metabolism in Humans. In: Fat Detection: Taste, Texture, and Post Ingestive Effects (Montmayeur, J. P. and le Coutre, J., eds) Boca Raton (FL).

Lewis CE, Jacobs DR, Jr., McCreath H, Kiefe CI, Schreiner PJ, Smith DE, Williams OD (2000) Weight gain continues in the 1990s: 10-year trends in weight and overweight from the CARDIA study. Coronary Artery Risk Development in Young Adults. Am J Epidemiol 151:1172-1181.

Luo AH, Tahsili-Fahadan P, Wise RA, Lupica CR, Aston-Jones G (2011) Linking context with reward: a functional circuit from hippocampal CA3 to ventral tegmental area. Science 333:353-356.

Moreno-Navarrete JM, Ortega F, Serino M, Luche E, Waget A, Pardo G, Salvador J, Ricart W, Fruhbeck G, Burcelin R, Fernandez-Real JM (2012) Circulating lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. Int J Obes (Lond) 36:1442-1449.

Morris MC, Tangney CC (2014) Dietary fat composition and dementia risk. Neurobiol Aging 35 Suppl 2:S59-64.

Moser MB, Moser EI (1998) Functional differentiation in the hippocampus. Hippocampus 8:608-619.

Ogden CL, Carroll MD, Kit BK, Flegal KM (2014) Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 311:806-814.

Sampath H, Ntambi JM (2004) Polyunsaturated fatty acid regulation of gene expression. Nutr Rev 62:333-339.

Schoeller DA (2014) The effect of holiday weight gain on body weight. Physiol Behav 134:66-69.

Wan Z, Durrer C, Mah D, Simtchouk S, Little JP (2014a) One-week high-fat diet leads to reduced toll-like receptor 2 expression and function in young healthy men. Nutr Res 34:1045-1051.

Wan Z, Durrer C, Mah D, Simtchouk S, Robinson E, Little JP (2014b) Reduction of AMPK activity and altered MAPKs signalling in peripheral blood mononuclear cells in response to acute glucose ingestion following a short-term high fat diet in young healthy men. Metabolism 63:1209-1216.

CHAPTER 2

LITERATURE REVIEW: DO OBESITY AND DIET REPROGRAM THE HIPPOCAMPUS?

Trunnell E. R. and R. B. Meagher. To be submitted to *Medical Hypotheses.*

Abstract

Obesity may be viewed as disease state in which both the body and mind have been inappropriately programmed. This article builds from the premise that diminished activities in the hippocampus contribute to hyperphagia and obesity. The hippocampus appears to control the memory of and the emotional response to a meal and is well connected to other brain structures known to have a direct influence on hunger and satiety. Preliminary analysis of research in this area suggested the working hypothesis that *"obesity and diet reinforce aberrant eating behavior in part by the reprogramming of hippocampal functions."* A synthesis of data from brain imaging, physiological, molecular, genetic, epigenetic, and behavioral studies in humans and rodent models was used herein to examine this hypothesis. Initial experimental evidence suggests that neurons in the hippocampus have been reprogrammed to produce states of altered gene expression. We only have a hint at the extent and quality of these changes. This review highlights current literature supporting this hypothesis and future translational applications.

2.1. Introduction

There has been an epidemic increase in global rates of obesity over the last 30 years. For example, in America in 2011-2012 more than a third of adult Americans were classified as obese (BMI ≥ 30) as compared to only 14% in 1980 (Ogden et al., 2014). Current efforts to prevent or reduce obesity by diet, exercise, education, and drug therapies are failing to provide effective long-term solutions to this epidemic, suggesting we have much to learn about the disease. Without denying the importance of genetics,

purely genetic models relying solely on genetic polymorphisms in the human population do not appear to account for the current occurrence of the disease in such a large genetically diverse population. There is also a growing awareness that the influence of alterations in the central nervous system (CNS) on eating behavior and metabolism further complicate the obesity problem. The field of epigenetics encompasses somatically and in some cases meiotically inherited modifications to chromatin structure. Epigenetic changes allow for the expression or repression of certain gene transcripts, providing mechanisms by which environment, such as diet or physical activity, may impact phenotype. Epigenetic models also accommodate some of the influences of CNS-controlled behavior and age on reprogramming gene expression. In addition, the weak penetrance of some epigenome-induced phenotypes may account for the seemingly stochastic display of obesity-related disease symptoms over time.

Although many parts of the brain and various peripheral organs are known to influence obesity (e.g., the hypothalamus, adipose tissues) and obesity-related health risk, the hippocampus has recently emerged as critical to problems of weight control. Before considering the potential roles that epigenome-induced pathologies of the hippocampus may play, we will present evidence that aberrant hippocampal functions lead to eating disorders and most often to obesity. **Figure 2.1** summarizes the influence of hippocampal epigenetic reprogramming on the physiological management of food intake.

2.2. Obesity and diet result in physical and functional alterations in the hippocampus

2.2.1 Ingestive behavior in human subjects with hippocampal damage

Situated in the brain's temporal lobe, the hippocampus is a brain structure long recognized for its role in learning and memory. A critical function of the hippocampus is to form episodic memories, recording the "what, when, and where" of experiences and events. What does this mean in the context of food intake? Evidence shows that pharmacological disruption of hippocampal function affects the timing and size of subsequent meals. In one study, following bilateral excitotoxic lesioning of the hippocampus, rats showed a marked increase in meal frequency and a decrease in meal size (Clifton et al., 1998). In a more recent study, infusion of the $GABA_A$ agonist muscimol into the hippocampus increased meal frequency and increased the size and duration of the next meal (Henderson et al., 2013). These studies show that normal hippocampal activity is required to maintain meal patterning in rodents, specifically to delay consumption of the next meal. Data from the historical patient H. M. and other individuals suffering from temporal lobe damage reveal aberrant meal patterning related to hippocampal dysfunction in humans as well. These subjects report altered perceptions of hunger and satiety, cannot remember having just eaten, and will eat another meal right away if offered (Hebben et al., 1985; Higgs, 2008). In H. M.'s case, his hunger levels did not differ whether he reported them before or after a meal, as they do in control subjects, and his reported hunger ratings never approached satiety even when collected after he had consumed nearly two dinners (Hebben et al., 1985). Similar results have been reported from other amnesic patients with comparable brain damage who were able to consume large quantities of food without reported changes in hunger and satiety (Higgs, 2008). It is clear that hippocampal-based learning and memory regarding past and recent

eating experiences play into our decisions about what and when to eat next, as well as how much, and so provide insight into the etiology of overeating and obesity.

2.2.2 Human brain imaging

Human brain imaging studies provide interesting evidence regarding how the brain is activated in response to information about food as well as the effects of obesity on the human hippocampus. 1. After viewing high calorie food images, functional magnetic resonance imaging (fMRI) revealed that human subjects with largest waist measurements showed the most significant activation of the hippocampus (Wallner-Liebmann et al., 2010). 2. Human subjects who are told to simply imagine a food craving show increases in hippocampal blood-oxygen-level dependent signal relative to hippocampal activity when they are told to imagine a less appealing food. This hippocampal response was similar to that observed in cravings for addictive drugs such as cocaine, making the obvious implication that overeating may be linked to addiction (Pelchat et al., 2004). In addition, a number of studies also suggest that obesity physically damages or causes the deterioration of the hippocampus: 3. Waist to hip ratio, a positive measure of obesity, negatively and significantly correlates with hippocampal volume (Jagust et al., 2005). 4. Morphometric measurements show that when obese, overweight, and normal-weight individuals are compared, increasing BMI negatively correlates with total grey matter (GM) volume and white matter (WM) volume in the brain ($p= 0.001$) and this negative linear correlation is particularly strong for the hippocampus ($r = -0.31$) and orbital frontal cortex ($r = -0.31$) (Raji et al., 2010). In other words, these two studies suggest that the hippocampus is reduced in size in most obese individuals. 5. Even studies on a more defined group, individuals with Type II Diabetes

Mellitus, show that those with high levels of visceral adipose tissue and high BMI have a significant decrease in hippocampal volume relative to those with normal levels of visceral adipose tissue and lower BMI (Anan et al., 2010). 6. When MRI analysis of brain GM density is compared between the brains of overweight/obese subjects and those of normal BMI there is a negative correlation between BMI and hippocampal GM density and a positive correlation of BMI with serum levels of neuron-specific enolase (NSE) (Mueller et al., 2012), an enzyme normally present in neuronal cell cytoplasm. When found in serum, NSE is a marker used as a proxy for neuronal cell death (Chaves et al., 2010; Guzel et al., 2008). Therefore, because reduced hippocampal GM density is significantly associated with increased serum NSE levels, these data suggest that loss of GM density resulted from neuronal cell death.

These MRI studies leave open the question of what mechanisms reduce hippocampal size and contribute to cell death. In the case of the diabetic patients, insulin resistance is an obvious point of focus. A 2015 paper by Kang and colleagues showed that HFD-fed, insulin resistant mice had increased hippocampal cell death as confirmed by TUNEL staining (Kang et al., 2015). In humans, peripheral insulin resistance has been directly linked to reduced hippocampal volume (Burns et al., 2007) and cognitive dysfunction (Watson and Craft, 2004) in Alzheimer's disease (AD) patients. While the major function of insulin in the periphery is to facilitate the uptake of glucose, glucose utilization in the CNS is independent of insulin action. However, insulin receptors are present throughout the brain and at a particularly high density in the hippocampus (Dore et al., 1997), where insulin is thought to act as a growth factor and enhance synaptic plasticity (Banks et al., 2012), as well as modulate learning and memory processes

(McNay et al., 2010). Diet-induced obesity produced by consumption of a high-fat diet (HFD) impairs insulin signaling in the hippocampus (McNay et al., 2010), and so could contribute to the hippocampal pathologies observed in obese individuals.

2.2.3 Hippocampal dysfunction in obese or HFD-fed rodents

Rodent studies addressing the topic of diet-induced obesity and consumption of HFD or high-energy diet (HED, defined as high fat/high sugar) provide further evidence of hippocampal dysfunction associated with obesity. In tests designed to study hippocampal-dependent memory, such as the Morris water maze, the radial arm maze, novel object recognition test, spontaneous or variable-interval delayed alternation tasks, and contextual conditioning, rodents fed HFDs or HEDs perform worse than their chow or low-fat-fed counterparts (Darling et al., 2013; Davidson et al., 2012; Greenwood and Winocur, 1990; Kanoski and Davidson, 2010; Kanoski et al., 2007; Kanoski et al., 2010; McNay et al., 2010; Molteni et al., 2002; Reichelt et al., 2015; Ross et al., 2012; Stranahan et al., 2008; Valladolid-Acebes et al., 2013; Valladolid-Acebes et al., 2011). Several of these studies point to specific diet- or obesity-induced molecular perturbations to explain the observed deficits, such as a leptin signaling deficiency (Valladolid-Acebes et al., 2013), insulin resistance (McNay et al., 2010; Stranahan et al., 2008), reduced dendritic spine density and reduced long-term potentiation (LTP) (Molteni et al., 2002; Stranahan et al., 2008), detrimental effects on the blood-brain barrier in the vicinity of the hippocampus (Davidson et al., 2012; Kanoski et al., 2010), and abnormally high levels of lipid in the plasma and liver (Darling et al., 2013; Ross et al., 2012). In addition, some studies report reductions in molecules positively associated with LTP and neuronal plasticity such as brain-derived neurotrophic factor (BDNF) (Kanoski et al., 2007;

Stranahan et al., 2008) and reelin (Reichelt et al., 2015) in the hippocampus following HFD or HED consumption. Leptin, a hormone released by adipose tissue and present in excess in obese individuals, is known for its classical role modulating hunger and satiety through hypothalamic circuits. In the hippocampus, leptin can bi-directionally alter the strength of excitatory synaptic transmission (Harvey, 2007), influencing learning and memory processes. Chronic administration of leptin to adult mice increases hippocampal neurogenesis in a dose-dependent manner (Garza et al., 2008). Much like insulin resistance, leptin resistance occurs over time in the state of obesity and likely plays a role in obesity-associated learning and memory deficits. If insulin and leptin both serve to maintain cell proliferation and activity in the hippocampus, impairment of their signaling could, in part, explain hippocampal atrophy observed in obese patients.

2.2.4 Obesity: an inflammatory state

It has been well documented that obesity and metabolic disorders represent states of chronic inflammation (Hotamisligil, 2006; Odegaard and Chawla, 2013). HFD consumption (Boitard et al., 2014; Pistell et al., 2010; Sobesky et al., 2014) or diabetes in mice (Dinel et al., 2011; Erion et al., 2014) results in performance impairments on spatial memory (Dinel et al., 2011; Erion et al., 2014; Pistell et al., 2010) and memory consolidation tasks (Boitard et al., 2014), as well as in fear conditioning experiments (Sobesky et al., 2014) and these impairments have been directly linked to increases in pro- and anti-inflammatory cytokines such as IL-1 (Boitard et al., 2014; Dinel et al., 2011; Erion et al., 2014; Sobesky et al., 2014), IL-6 (Dinel et al., 2011; Pistell et al., 2010), TNFα (Dinel et al., 2011; Pistell et al., 2010), and MCP-7 (Pistell et al., 2010) in the hippocampus. Several reports also reveal enhanced microglial activation in the

hippocampus following HFD consumption (Erion et al., 2014; Pistell et al., 2010; Tucsek et al., 2013). Results from these studies suggest that HFDs, HEDs, and obesity modify, and most often diminish, hippocampal function. In a recent systematic review, Bartholdy and colleagues have brought to light an apparent deficit in maintenance of behavioral inhibition in obese individuals performing the stop signal task, especially when the task was specific to food (Bartholdy et al., 2016). Though the hippocampus has not been directly implicated in performance on this task, it is functionally connected to regions of the brain that have, such as the subthalamic nucleus, prefrontal cortex, and insular cortex (Bartholdy et al., 2016; Cenquizca and Swanson, 2007; Frank, 2006; Lambert et al., 2012; Robbins, 2007).

2.2.5 Hippocampal connectivity

Within the brain the hippocampus is well poised for being responsive to and influencing food intake. The hippocampal formation lies in the brain's temporal lobe and includes the dentate gyrus; the CA3, CA2, and CA1 fields; and the subiculum. The hippocampus is intimately connected via neuronal processes with other areas of the brain involved in food intake, such as the hypothalamus, bed nucleus of the stria terminalis, amygdala, ventral striatum, lateral septum, and the olfactory and insular cortices (well reviewed in (Berthoud, 2002)). Considering the hippocampus' role in memory formation, one might view the hippocampus as an integrator of information, assimilating physiological cues about energy status from a number of brain structures and correlating this information with consciously recognized context and time to form a "memory" of a food-related event. Conditioned responses to these cues are then fed back down the effector pathway with resulting output from regions such as the hypothalamus. In addition, the

hippocampus contains receptors for numerous feeding-related hormones and adipose tissue-derived signaling molecules such as leptin (Garza et al., 2008), insulin (McNay et al., 2010), and ghrelin (Diano et al., 2006). Hence, both the structural interconnectivity and receptor biology of the hippocampus further support its role in ingestive behavior and the assertion that it should be considered a major player in the study of obesity (Davidson et al., 2012; Kanoski and Davidson, 2011 ; Parent et al., 2014; Pasinetti and Eberstein, 2008).

2.3.Responsiveness of the hippocampal epigenome

2.3.1 Epigenetics of learning and memory

Not only is the hippocampus involved in regulating food intake, it has been central to the study of epigenetics in the brain. Modification of the epigenome occurs at a level above the basic genetic code and involves secondary chemical modifications such as DNA methylation; histone methylation, acetylation, and phosphorylation; nucleosome repositioning, and small RNA gene silencing. Environmental inputs and experiences are believed to modify the epigenome to allow the expression or repression of certain genes, adding a layer of complexity to the central dogma of molecular genetics. Many recent publications in the field of epigenetics have focused on the role of these modifications in hippocampal learning and memory. Sir Francis Crick first proposed that secondary modification of biomolecules was the mechanism for memory formation in a letter to *Nature* in 1984 (Crick, 1984) and more than 30 years later, we are beginning to realize the depth of his hypothesis (Meagher, 2014). In particular, he emphasized the contrasting roles of rapid molecular turnover inherent in secondary modifications in synaptic

plasticity and maintenance of these modifications to retain long-term memories. For some informative reviews on the epigenetics of hippocampal-based learning and memory, see those by Zovkic, Meagher, Lipsky, Day and Sweatt, and Woldemichael (Day and Sweatt, 2011a; Lipsky, 2013; Meagher, 2014; Woldemichael et al., 2014; Zovkic et al., 2013a). The turnover cycles for 5′-modification of DNA cytosine and nucleosomal histone side chain acetylation and methylation are summarized in **Figure 2.2**. There are a few factors that make it desirable to consider the importance of molecular turnover in a discussion of reprogramming the hippocampal epigenome. First, many of the major factors catalyzing the formation and removal of chromatin modifications are highly expressed in the hippocampus, often higher than in other regions of the brain. This includes several histone lysine acetyltransferases (KATs) (Bousiges et al., 2010), the majority of histone deacetylases (HDACs) (Broide et al., 2007; de Ruijter et al., 2003), three DNA methyltransferases (DNMTs) (Kadriu et al., 2012), and the three ten eleven translocase methylcytosine dioxygenases (TETs) that catalyze the conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and to other more oxidized forms (Rudenko et al., 2013; Zhang et al., 2013). Second, 5hmC, the first intermediate in the removal of 5mC is present at a higher concentration in the hippocampus than in most other brain regions (Cadet and Wagner, 2014; Lister et al., 2013; Szulwach et al., 2011) suggesting higher rates of turnover for modified DNA cytosine residues in this region.

2.3.2 Epigenetic consequences of stress and environment

Third, there is mounting evidence that environmental stimuli may be recorded as chromatin modifications in the hippocampus. Stressors such as social defeat or physical

restraint activate the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis and contribute to behavioral alterations, such as enhancing social avoidance and anhedonia and changing eating patterns, increasing or decreasing food intake depending on the paradigm (Beery and Kaufer, 2015; Maniam and Morris, 2012). Rats exposed to a social defeat stress exhibited increased depressive-like behavior and had different hippocampal histone acetylation profiles when compared to their unstressed peers. Just 30 minutes following social defeat stress, nucleosomal histone H3 became hyperacetylated in the hippocampus, an effect that increased during the 24 hours following stress and then returned to baseline levels after 72 hours (Hollis et al., 2010). Histone acetylation is typically associated with an open chromatin formation and enhanced gene transcription (Allfrey et al., 1964; Hebbes et al., 1988), suggesting that social defeat stress ramped up gene expression in the hippocampus within 30 minutes. No effects of stress were seen in the amygdala or prefrontal cortex (PFC) in this time frame, emphasizing the rapid responsiveness of the hippocampal epigenome. DNA methylation typically occurs in CG islands within a gene and is associated with transcriptional silencing (Deaton and Bird, 2011), while demethylation via oxidation to 5hmC is typically associated with open chromatin and transcriptional potentiation (Guo et al., 2011). Psychosocial stress has been shown to differentially affect cytosine 5′ methylation of the *BDNF* gene in functional poles of the hippocampus, increasing methylation in the dorsal CA1 (with a concurrent reduction in *BDNF* mRNA) and decreasing methylation in the ventral CA3 (Roth et al., 2011). Restraint stress increased levels of 5hmC in the glucocorticoid receptor (*NR3C1*) gene in the hippocampus (Li et al., 2015). While this particular paper did not correlate this change with gene expression,

previous studies show increased *NR3C1* transcript levels in mice exposed to a single acute stress (Gray et al., 2014). Enriching the living environments of rodents by the addition of tunnels, running wheels, or differently colored or textured toys to their home cages (Kuzumaki et al., 2011), or by exposure to these stimuli during defined sessions (Irier et al., 2014), can also affect the hippocampal epigenome, altering histone lysine methylation at *BDNF* promoters (and increasing *BDNF* mRNA levels) (Kuzumaki et al., 2011) and preventing aging-induced DNA hydroxymethylation in genes important in axon guidance, learning, and memory (Irier et al., 2014). Clearly, a variety of environmental stimuli significantly impact the hippocampal epigenome. To summarize, there is strong evidence that hippocampal function is essential for normal eating behavior and that epigenetics exerts control over hippocampal learning and memory formation.

2.4. Obesity and diet reinforce aberrant eating behavior by epigenetic reprogramming of hippocampal functions

2.4.1 Caloric restriction and acute overnutrition

The current literature addressing how epigenetic controls in the hippocampus might oppose or support obesity come from studies of obese animals, altered diet, exercise, and neurological dysfunction and lend support to our working hypothesis: "*obesity and diet reinforce aberrant eating behavior by physically damaging and/or by the reprogramming of hippocampal function*." Epigenetic events following the contrary treatment of caloric restriction have revealed that dietary manipulation does indeed result in hippocampal chromatin modification. In one example, Chouliaras and colleagues fed male mice a calorie-restricted diet resulting in a 50% reduction in calories for 1 or 2 years

(Chouliaras et al., 2012). In the control group, levels of 5-hydroxymethylcytosine (5hmC) increased in the hippocampus with aging, but this increase in 5hmC was significantly suppressed by caloric restriction. 5hmC is a relatively stable intermediate in the cycle of DNA demethylation and remethylation (**Figure 2.2a**) (Wu and Zhang, 2014). Supporting a role for 5hmC in the regulation of gene expression is evidence that 5hmC is positively correlated with transcription and chromatin accessibility and is most enriched in gene bodies and associated enhancers of the most highly expressed genes or genes "poised for on demand gene regulation" (Irier et al., 2014; Lister et al., 2013). Age-associated increases in 5hmC have been specifically linked to the expression of genes implicated in neurodegenerative disorders (Sherwani and Khan, 2015; Song et al., 2011), suggesting that disruption of normal cytosine turnover **(Figure 2.2a**) might contribute to these pathologies. These findings agree with other evidence of the beneficial effects of caloric restriction on aging-associated diseases (Graff et al., 2013), but also suggest an epigenetic mechanism, whereby caloric restriction may prevent the rise of 5hmC in genes associated with neurodegenerative disorders, hence preventing their appropriate regulation. A second example involves sirtuin 1 (SIRT1), an NAD-dependent protein deacetylases, whose activities include histone deacetylation. Caloric restriction induced the expression of SIRT1 in the hippocampi of mice (Graff et al., 2013). Though no specific genes were implicated as SIRT1 targets in this experiment, increased expression of SIRT1 is generally correlated with gene silencing (**Figure 2.2b**).

Increased caloric load can also affect levels of chromatin modifying enzymes. In a recent study we showed that after just 72 hours of consuming a HFD (45% fat by kcal), transcript levels of the histone deacetylase HDAC4 were increased in the ventral

hippocampus of male rats relative to rats fed a low fat diet (10% fat by kcal) *ad libitum* (Gan et al., 2015). HDAC4, another enzyme involved in gene silencing, is colocalized with neurons containing neurotransmitters such as orexin, serotonin, oxytocin, and vasopressin; is known to play a role in synaptic plasticity; and has been investigated for its role in neurodegenerative disease (Mielcarek et al., 2015). Though regulation of HDAC4 is thought to occur via altered protein shuttling between the cytoplasm and the nucleus of the cell, the level of protein itself can be reduced by caspase-3, a protease upregulated by lipopolysaccharide (LPS) and essential to apoptosis (Bray et al., 2002; Guyenet et al., 2013; Mielcarek et al., 2015). LPS is increased in the gut (Cani et al., 2007) and serum (Laugerette et al., 2011) following HFD consumption and high plasma levels are associated with obesity (Moreno-Navarette et al., 2012). This introduces the possibility that HFD- or obesity-induced LPS could be upregulating caspase-3 in the ventral hippocampus, degrading HDAC4 protein and leading to a compensatory increase in HDAC4 transcription to restore protein levels.

2.4.2 Maternal contributions

A large portion of the relevant current literature on obesity and epigenetic reprogramming of the hippocampus focuses on the second generation and early life effects of dietary manipulations. Mice fed a HFD from the time of weaning until 6 months of age show decreases in base excision repair (BER, **Figure 2.2a**) resulting from DNA damage in the hippocampus, which was further enhanced if they were born to a dam with a folate-deficient diet (Langie et al., 2013). In the hippocampus, there was associated increased DNA cytosine methylation, decreased 5hmC/5mC ratio, and changes to the methylation status and expression of genes involved in the BER pathway

in these offspring (Langie et al., 2013). Other variations in the micronutrient profile of the maternal diet alter chromatin structure in the hippocampi of offspring: 1. A low choline maternal diet resulted in hypomethylation of CG islands near the promoter region of genes involved in angiogenesis (Mehedint et al., 2010), 2. A methyl donor-deficient maternal diet was associated with changes in methylation status at a variety of CG sites in the neuronatin gene *NNAT* (Konycheva et al., 2011), 3. Vitamin B_{12} deficiency and omega-3 supplementation both resulted in global DNA hypermethylation in the hippocampus (Sable et al., 2015), and 4. A maternal diet high in fat-soluble vitamins (A, D, E, and K) increased DNA methylation in the gene for dopamine receptor 1 (Sanchez-Hernandez et al., 2015), all as measured in the offspring. These findings may reflect the fact that many epigenetic enzymes rely on micronutrients as cofactors (Helin and Dhanak, 2013; Kaelin and McKnight, 2013; Vanhees et al., 2014), but nonetheless highlight the interconnectedness of nutrition and hippocampal epigenetic programming during development. Later in this work, we will explore how a maternal high fat diet during pregnancy and prior to weaning impacts gene expression in the dorsal and ventral hippocampi of male and female offspring.

2.4.3 Exercise

While it cannot simply be thought of as the "opposite" of obesogenic diet consumption, aerobic exercise counters many of the negative health outcomes of obesity and a sedentary lifestyle (Bishop-Bailey, 2013). A few exceptional studies relevant to our working hypothesis suggest that aerobic exercise enhances hippocampal neuronal plasticity and learning and memory performance in laboratory animals via epigenetic reprogramming. 1. Gomez-Pinilla and colleagues found that free access to a running

wheel reduced DNA cytosine methylation (**Figure 2.2a**) of the *BDNF* promoter and increased BDNF mRNA and protein in the hippocampus (Gomez-Pinilla et al., 2011a). This study also reported exercise effects on molecules known to regulate the transcription of *BDNF*: voluntary running elevated the levels of the active, phosphorylated form of methyl-CG-binding protein 2 (MECP2) and reduced levels of histone deacetylase 5 (HDAC5) mRNA and protein. In addition, exercise increased acetylation of histone 3 and elevated the phosphorylated forms of calcium/calmodulin-dependent protein kinase II (CAMK2) and cAMP response element binding protein (CREB), both of which are known to be involved in chromatin modifications such as histone deacetylation and histone phosphorylation (Awad et al., 2015; Sanchez-Molina et al., 2014). 2. One week of exercise significantly increases the hippocampal expression of proteins essential to learning and memory such as BDNF, inducing DNA cytosine demethylation at one of the four *BDNF* promoters and increasing *BDNF* transcript levels in the hippocampus (Gomez-Pinilla et al., 2011b). Treating rats with inhibitors of DNMT-catalyzed formation of DNA cytosine methylation (**Figure 2.2**), such as 5-aza-2-deoxycytidine, also results in increases in *BDNF* activity (Sales et al., 2011), further pinpointing a mechanism behind exercise-induced BDNF increase. Hence, it appears that exercise may modify hippocampal activity via epigenetic mechanisms acting on an important neurotrophic factor, BDNF. Expression of the gene for synapsin (*SYNI*), a protein important for neurotransmitter release and synaptic plasticity, is known to be under the control of promoter DNA methylation (Paonessa et al., 2013), and is increased in the hippocampus following one week of exercise (Vaynman et al., 2004), but confirmatory evidence demonstrating that exercise de-represses *SYNI* by DNA demethylation is yet to

be published. 3. The ratio of histone acetylation activity to histone deacetylation activity (KAT/HDAC, **Figure 2.2b**) correlates with increased neuronal gene expression. When 3-month-old rats are given a single exercise session of 20 minutes on a motorized running wheel there is a 2-fold increase in hippocampal histone 4 lysine acetylation activity (KAT) and a 5-fold decrease in histone deacetylase (HDAC) activity as assayed immediately or 1 hour after exercise (Elsner et al., 2011). The KAT/HDAC ratio increased 7- to 10-fold. No change from the control was measured by 18 hours after exercise, nor was there a change in KAT or HDAC levels when measured after two weeks of chronic exercise (20 minutes/day). The physiological response to an acute exercise regimen in rodents may be due to an increase in systemic stress induced by forced running (Brown et al., 2007). Exercise stimulates extremely rapid changes in hippocampal histone acetylation, but there is a rapid return to normal levels. The data suggest that the resulting histone acetylation has a very short half-life (Meagher, 2014).

4. The methylation levels of hippocampal nucleosomal histone 3 lysine 9 methylation (H3K9Me) are controlled by a balance between methyltransferase and demethylase activities **(Figure 2.2c**). H3K9Me levels are positively associated with neuronal gene silencing and reduced memory performance (Day and Sweatt, 2011b; Zovkic et al., 2013b). A single exercise session of 20 minutes or a chronic exercise protocol (2 weeks, 20 minutes daily) produces a significant 2- to 4-fold reduction in hippocampal H3K9Me levels in young 3-month-old rats 1 hour and 18 hours after exercise (Elsner et al., 2013). The results were quite different for 18-month-old rats, where a single exercise session produced increases in H3K9Me and a chronic exercise protocol had no effect. Histone lysine methylation rapidly responds to exercise across the

lifespan, but the direction in which it is modulated by exercise depends on age. Alterations in H3K9Me levels have been linked to cellular metabolic memory in vascular smooth muscle cells (Villeneuve et al., 2008) and cardiomyocytes (Yu et al., 2012), as well as synaptogenesis and neurobehavioral phenotypes in mice (Subbanna and Basavarajappa, 2014). These results have not yet been directly connected to expected exercise-induced increases and decreases in neuronal gene expression in young and old rats, respectively.

2.4.4 Ingestive behavior in Alzheimer's patients and Alzheimer's disease-models

Appetite disturbances are core symptoms observed in neurodegenerative disorders such as Alzheimer's disease (AD) patients and AD model mice. Loss of memory and physical damage to and/or deterioration of the frontal cortex and hippocampus are among the first changes detected in the AD brain. Considering the data presented earlier, it is easy to imagine the connection between hippocampal dysfunction and appetite disturbances. While the cause-and-effect molecular relationships for this occurrence in AD patients are not clear, aberrant changes to chromatin structure are clearly implicated. Controlled and quantified data from AD model mice lend the strongest support to the argument that AD contributes to eating and weight control disorders and that an early obese phenotype is due to epigenetic modification in the hippocampus. Over expression of wild type APP (amyloid precursor protein), and particularly the double missense mutant forms of APP_{Swe} ($APP_{K595N,\ M596L}$), are correlated with AD in humans and transgenic model mice. APP and APP_{Swe} are both processed into secreted Aβ amyloid plaque that accumulates in the AD brain, a signature symptom of AD. However, APP is also a transcription factor that associates with the KAT5 Tip60 (KAT, **Figure 2.2b**) to

promote histone acetylation in the brain. If fed *ad libitum,* various transgenic AD-model mice that overexpress the human amyloid precursor protein APP_{Swe} tend toward early obesity, with or without other AD-inducing genetic defects (Adebakin et al., 2012; Good and Hale, 2007; Good et al., 2007; Holcomb et al., 1998; Holcomb et al., 1999; Knight et al., 2013; Knight et al., 2012; Kohjima et al., 2010; Mody et al., 2011; Naumann et al., 2010; Oddo et al., 2003a; Oddo et al., 2003b). In a study directly addressing obesity, the 3xTg mice carrying the APP_{Swe} mutation in conjunction with loss of two other AD-associated gene functions are obese from 2 to 5 months of age, but by 12 months they are significantly underweight (Adebakin et al., 2012; Knight et al., 2013; Knight et al., 2012). It appears that these mice are obese from 2 to 5 months (e.g, 36% heavier than controls) because they eat 17% more chow, but burn less energy, having significantly lower O_2 consumption and lower CO_2 expiration than controls. By 12 months of age, however, they are consuming 30% more calories than controls, but they weigh 15% less. This later weight loss appears due the fact that they are burning more calories as evidenced by their consuming 21% more O_2 and expiring 29% more CO_2 than controls. These data imply that over expression of the transcription factor and epigenetic modulator APP_{Swe} has a substantial, age-dependent impact on metabolism, apart from its effects on cognition and the development of AD.

Evidence that overexpressed APP_{Swe} may be acting via a dominant inhibitory effect on Tip60's role as a histone acetyltransferase comes from studies with the histone deacetylase inhibitor phenylbutyric acid (PBA) (Ricobaraza et al., 2009). Sixteen-month-old mice with the APP_{Swe} mutation are extremely defective in hippocampal-based spatial memory and learning potential as evidenced by their inability to efficiently learn to solve

the Morris water maze. Remarkably, treating these mice with PBA restored their ability to solve the maze to the levels of wild type controls. There was a concomitant restoration of histone H3 and H4 acetylation level in hippocampal neurons compared to those of wild type. PBA treatment did not reduce Ab plaque formation in the hippocampus of these APP_{Swe} mice, focusing attention on the epigenetic role of APP. In a parallel study by the same group, treating APP_{Swe} mice with PBA also restored that AD-associated abnormally low levels of dendritic spines in hippocampal neurons to normal levels, an increase of 18% (Ricobaraza et al., 2012). The obvious implication here is that PBA restored hippocampal neuronal histone acetylation, neuronal gene expression, and neuronal development to APP_{Swe} mice, thereby reestablishing more normal memory performance and that this phenotype was not related to plaque formation. Interestingly, treatment with another histone deacetylase (HDAC) inhibitor, valproic acid (VA), increases GABA levels 15-45% in the rodent brain (MacDonald and Roskams, 2009) and as mentioned in Section A, increased GABA in the hippocampus can alter meal patterning in rodents (Henderson et al., 2013). In fact, human patients prescribed VA to treat epilepsy, mania associated with bipolar disorder, or migraine, experience weight gain as a side effect of the medication (Martin et al., 2009). In summary, these indirect data support our hypothesis by suggesting that epigenetic reprogramming of hippocampal neurons will alter the potential of learning and memory processes to regulate meal onset and food consumption.

2.5. Translational epigenetics

Current translational research is limited by the lack of information connecting specific epigenetic mechanisms in the hippocampus to obesity and hyperphagia. However, a consideration of how translational epigenetics might be applied to control obesity-related health risk is informative. Since the 1960s valproic acid (VA) has been used to treat brain seizures and more recently mood disorders, but it wasn't until 2001 that VA was revealed as an HDAC inhibitor (Gottlicher et al., 2001; Phiel et al., 2001). VA produces loss of hippocampal-based spatial memory and cell proliferation (Umka et al., 2010) and is associated with increased weight, hunger, and binge eating (Martin et al., 2009; Verrotti et al., 2011). These data reporting an undesired side effect, while only correlative, demonstrate that a small molecule altering chromatin structure may influence eating behavior and/or metabolism. As mentioned above, VA increases GABA levels in the rodent brain (MacDonald and Roskams, 2009), and the GABA agonist muscimol increases meal frequency, size, and duration (Henderson et al., 2013), suggesting a possible mechanism for VA-induced hyperphagia.

Enhancer of zeste homolog 2 (EZH2) is a histone lysine methyltransferase that catalyzes the formation of nucleosomal histone H3K27Me1/2/3 in the promoter region of the BDNF gene to enhance its expression (Qi et al., 2014). EZH2 regulates hippocampal learning and memory, neurogenesis, and dendrite arborization (Zhang et al., 2014). Novel small molecule drugs such as Tazemetostat (EPZ-6438), an inhibitor of EZH2, have been used to treat Hodgkin's Lymphoma and solid tumors of the brain (Knutson et al., 2014). Although EZH2 regulates hippocampal learning and memory (Zhang et al.,

2014),to our knowledge EZH2 inhibitors have not been applied to treating hippocampal dysfunction in obese individuals.

Cause-and-effect relationships will continue to be difficult to assign from studies correlating epigenetic changes with phenotypes. This problem may addressed in at least two ways. First, by applying inhibitors of chromatin modifying machinery to problems of obesity such as histone deacetylase inhibitors (e.g., trichostatin A, sodium butyrate, phenylbutyric acid), or acetylated histone mimics that inhibit bromodomain chromatin remodelers (e.g., I-BETs, benzodiazepines, phenyl isoxazoles), DNA methyltransferase inhibitors (5-azacytidine), or TET inhibitors (2-hydroxyglutarate). Second, mutational or RNA-mediated silencing of chromatin remodeling machinery has not been widely used to directly examine problems of obesity and the epigenetic response to high fat diet. For example, because butyrate and phenylbutyrate restore some aspects of hippocampal based spatial learning, these drugs may also impact the eating behavior of rodents fed *ad lib* on a HFD. These inhibitors of epigenetic function may be used to dissect neuronal epigenetic regulation in relation to problems of obesity giving rise to new therapeutics.

2.6. Discussion

Early and continuing studies support the premise that diminished activities in the hippocampus generally produce hyperphagia and obesity. It appears that on the whole, the hippocampus exerts restraint over appetite and damage to or hypofunction of the hippocampus reduces memory or causes the formation of inappropriate memories, resulting in orexigenic behavior and obesity. MRI data suggest that the hippocampus is involved in the emotional and visual responses to food that again are altered in obese or

formerly obese individuals to support overeating. Preliminary interpretations of MRI data on the response of obese individuals to images or thoughts of food also suggest the view that hyperphagia and obesity parallel addictive behaviors. In support of this tantalizing view of obesity, the most effective weight loss programs, ones which overcome the weight-loss and regain paradigm for obese individuals, appear to involve not only diet and exercise, but long-term meetings and social activities with a continuous focus on behavioral modification: programs similar those treating alcohol and drug addiction (Fuller et al., 2012; Jebb et al., 2011; Jolly et al., 2010; Jolly et al., 2011; Mitchell et al., 2013; Ogden et al., 2012; Racette et al., 2009; Rankinen and Bouchard, 2008). This and other MRI-based studies point out the power of imaging the living brain to enhance obesity research.

Independent physiological, live imaging, molecular genetic, epigenetic, and behavioral studies suggest diet and obesity may reduce hippocampal function and impact normal restraint from hyperphagia, leading to obesity. Thus, there is reasonable support for most aspects of the working hypothesis addressed herein, *"obesity and diet reinforce aberrant eating behavior by physically damaging and/or by the reprogramming of hippocampal functions*." Research that explores how obesity and diet result in the epigenetic reprogramming of the hippocampus is newly arising. The fusion of diverse arguments presented herein makes clear that interdisciplinary information from experimental science is needed to understand the role of hippocampal functions in obesity. Taking together these various research results, there is strong support for a cyclic relationship, where high fat diets and obesity cause dysfunction of the hippocampus, which in turn reinforces hyperphagia and continued obesity (**Figure 2.1**).

The way forward: Accepting a strong role for the hippocampus in obesity, there is a real need for more complete analyses of chromatin remodeling in the hippocampus as model animals respond to changes in diet, changes in weight, and exercise. Comprehensive epigenetic studies of post-mortem human hippocampal samples comparing the molecular genetic and epigenetic changes among lean and obese individuals should help pinpoint epialleles or groups of interacting epialleles with relevance to obesity. Only a few epitypes, primarily DNA methylation and three classes of PTMs, histone acetylation, methylation, and phosphorylation, have been studied in any detail in relation to epigenetic programming in the hippocampus (Zovkic et al., 2013b). However there are several dozen other histone PTMs and a few additional modifications of cytosine, adenine, and thymidine counted among the known epigenetic marks. These chromatin modifications may also be involved in neuronal reprogramming in the response to stimuli such as diet, exercise, and obesity. Nucleosome position is determined in part by histone variant composition and in part by the nucleotide composition of ten base-pair repeats in the DNA sequence with the potential to make contact with each nucleosome (Meagher, 2010; Meagher and Mussar, 2012). This relationship of DNA sequence to nucleosome position likely connects some allelic changes in DNA sequence (e.g., SNPs, small insertions and deletions (INDELs)) associated with obesity (Fall and Ingelsson, 2012; Lu and Loos, 2013) with epigenome-induced pathologies. Such DNA sequence polymorphisms are the likely cause of a subset of multigenerationally inherited epigenome-induced risks for obesity (Meagher and Mussar, 2012). Neither nucleosome position nor histone variant composition has yet been reported in any detail for particular regions of the brain or for their relationship to

obesity. Neither have there been genome-wide correlations of SNPs and INDELs associated with risk in relation to changes in the epigenome. Clearly, the epigenetic analysis of neuronal reprogramming is only at its beginning, particularly as it impacts hippocampal controls over eating behavior.

Epigenetics from its dual inception by David Nanney and Conrad Waddington has been focused on organ, tissue, and cell-type specific differences (Haig, 2004; Nanney, 1958; Waddington, 1957). In blood, genome wide analysis of the human methylome among seven types of leukocytes, showed that all pairwise comparisons differed in a remarkable 8% to 40% of their DNA cytosine methylation sites (Reinius et al., 2012). The hippocampus is comprised of cell types including neurons, glia, vascular cells and nucleated white blood cells. Each is likely to have its own epigenome for any one epitype and is as likely to have as much difference as that among classes of leukocytes. Considering that DNA cytosine methylation and the hundreds of different nucleosomal histone side chain modifications can be differentially positioned in the genome it is theoretically possible that most neuronal cells are distinct in their epigenome. Thus, the problem of performing cell-type specific epigenetic analysis in the brain will be daunting. Although many of the epigenetic studies described herein were performed on sub-regions of the hippocampus, few examined purified neurons. A number of technical approaches have been applied to increase the cell type specificity of brain research, including immunofluorescence microscopy used to examine changes in the levels of particular PTMs within layers of neurons, neuron-specific electrophysiology in brain slice preparations (Bortolotto et al., 2011), laser capture micro-dissection of neurons (Blalock et al., 2011), culture of immortalized neuronal cells (Dalvi et al., 2011; Gingerich et al.,

2009), and fluorescence activated nuclear sorting of neuronal nuclei (Dammer et al., 2013; Yu et al., 2015), but of these approaches only brain slice electrophysiology (Jia et al., 2008) has been applied to epigenetic analysis of obesity related problems. Cell-type specific studies would greatly increase the statistical significance to these data as it does for leukocytes, allowing us to pinpoint how diet and obesity are reprogramming cells in the hippocampus and providing a wealth of new targets for the prevention and treatment of obesity.

Acknowledgements

We would like to thank Dr. Gaylen Edwards of the Department of Physiology and Pharmacology at the University of Georgia for his help revising this manuscript and Dr. Clifton A. Baile for his encouragement to initiate this synthesis.

References

Adebakin, A., Bradley, J., Gumusgoz, S., Waters, E.J., Lawrence, C.B., 2012. Impaired satiation and increased feeding behaviour in the triple-transgenic Alzheimer's disease mouse model. PLoS One 7, e45179.

Allfrey, V., Faulkner, R., Misrky, A., 1964. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. PNAS 51, 786-794.

Anan, F., Masaki, T., Shimomura, T., Fujiki, M., Umeno, Y., Eshima, N., Saikawa, T., Yoshimatsu, H., 2010. Abdominal visceral fat accumulation is associated with hippocampus volume in non-dementia patients with type 2 diabetes mellitus. Neuroimage 49, 57-62.

Awad, S., Adib Al-Haffar, K., Marashly, Q., Quijada, P., Kunhi, M., Al-Yacoub, N., Wade, F., Al-Dayel, F., Sutherland, G., Assiri, A., Sussman, M., Bers, D., Al-Habeeb, W., Poizat, C., 2015. Control of histone H3 phosphorylation by CaMKIIs in response to haemodynamic cardiac stress. J Pathol 235, 606-618.

Banks, W., Owen, J., Erickson, M., 2012. Insulin in the brain: There and back again. Pharmacol Therapeut 136, 82-93.

Bartholdy, S., Dalton, B., O'Daly, O.G., Campbell, I.C., Schmidt, U., 2016. A systematic review of the relationship between eating, weight and inhibitory control using the stop signal task. Neurosci Biobehav Rev 64, 35-62.

Beery, A., Kaufer, D., 2015. Stress, social behavior, and resilience: Insights from rodents. Neurobiol Stress 1, 116-127.

Berthoud, H., 2002. Multiple neural systems controlling food intake and body weight Neurosci Biobehav R 26, 393-428.

Bishop-Bailey, D., 2013. Mechanisms governing the health and performance benefits of exercise. Brit J Pharmacol 170, 1153-1166.

Blalock, E.M., Buechel, H.M., Popovic, J., Geddes, J.W., Landfield, P.W., 2011. Microarray analyses of laser-captured hippocampus reveal distinct gray and white matter signatures associated with incipient Alzheimer's disease. J Chem Neuroanat 42, 118-126.

Boitard, C., Cavaroc, A., Sauvant, J., Aubert, A., Castanon, N., Laye, S., Ferreira, G., 2014. Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. Brain Behav Immun 40, 9-17.

Bortolotto, Z.A., Amici, M., Anderson, W.W., Isaac, J.T., Collingridge, G.L., 2011. Synaptic plasticity in the hippocampal slice preparation. Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.] Chapter 6, Unit 6 13.

Bousiges, O., Vasconcelos, A., Neidl, R., Cosquer, B., Herbeaux, K., Panteleeva, I., Loeffler, J., Cassel, J., Boutillier, A., 2010. Spatial memory consolidation is associated with induction of several lysine-acetyltransferase (histone acetyltransferase) expression levels and H2B/H4 acetylation-dependent transcriptional events in the rat hippocampus. Neuropsychopharmacol 35, 2521-2537.

Bray, G., Lovejoy, J., Smith, S., DeLany, J., Lefevre, M., Hwang, D., Ryan, D., York, D., 2002. The influence of different fat and fatty acids on obesity, insulin resistance and inflammation. J Nutr 132, 2488-2491.

Broide, R., Redwine, J., Aftahi, N., Young, W., Bloom, F., Winrow, C., 2007. Distribution of histone deacetylases 1-11 in the rat brain. J Mol Neurosci 31, 47-58.

Brown, D.A., Johnson, M.S., Armstrong, C.J., Lynch, J.M., Caruso, N.M., Ehlers, L.B., Fleshner, M., Spencer, R.L., Moore, R.L., 2007. Short-term treadmill running in the rat: what kind of stressor is it? J Appl Physiol (1985) 103, 1979-1985.

Burns, J., Donnelly, J., Anderson, H., Mayo, M., Spencer-Gardner, L., Thomas, G., Cronk, B., Haddad, Z., Kilma, D., Hansen, D., Brooks, W., 2007. Peripheral insulin and brain structure in early Alzheimer disease. Neurology 69, 1094-1104.

Cadet, J., Wagner, J., 2014. TET enzymatic oxidation of 5-methylcytosine, 5-hydroxymethylcytosine, and 5-formylcytosine. Mutat Res 764-765, 18-35.

Cani, P., Amar, J., Iglesias, M., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A., Fava, F., Tuohy, K., Chabo, C., Waget, A., Delmee, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrieres, J., Tanti, J., Gibson, G., Casteilla, L., Delzenne, N., 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56, 00121797.

Cenquizca, L.A., Swanson, L.W., 2007. Spatial organization of direct hippocampal field CA1 axonal projections to the rest of the cerebral cortex. Brain Res Rev 56, 1-26.

Chaves, M., Camozzato, A., Ferreira, E., Piazenski, I., Kochhann, R., Dall'lgna, O., Mazzini, G., Souza, D., Portela, L., 2010. Serum levels of S100B and NSE proteins in Alzheimer's disease patients. J Neuroinflammation 7, 6.

Chouliaras, L., van den Hove, D., Kenis, G., Keitel, S., Hof, P., van Os, J., Steinbusch, H., Schmitz, C., Rutten, B., 2012. Age-related increase in levels of 5hmC in mouse hippocampus is prevented by caloric restriction. Curr Alzheimer Res 9, 536-544.

Clifton, P., Vickers, S., Somerville, E., 1998. Little and Often: Ingestive Behavior Patterns Following Hippocampal Lesions in Rats. Behav Neurosci 112, 502-511.

Crick, F., 1984. Memory and molecular turnover. Nature 312.

Dalvi, P.S., Nazarians-Armavil, A., Tung, S., Belsham, D.D., 2011. Immortalized neurons for the study of hypothalamic function. Am J Physiol Regul Integr Comp Physiol 300, R1030-1052.

Dammer, E.B., Duong, D.M., Diner, I., Gearing, M., Feng, Y., Lah, J.J., Levey, A.I., Seyfried, N.T., 2013. Neuron enriched nuclear proteome isolated from human brain. Journal of proteome research 12, 3193-3206.

Darling, J., Ross, A., Bartness, T., Parent, M., 2013. Predicting the effects of a high-energy diet on fatty liver and hippocampal-dependent memory in rats. Obesity 21, 910-917.

Davidson, T., Monnot, A., Neal, A., Martin, A., Horton, J., Zheng, W., 2012. The effects of a high-energy diet on hippocampal-dependent discrimination performance and blood-brain barrier integrity differ for diet-induced obese and diet-resistant rats. Physiol Behav 107, 26-33.

Day, J., Sweatt, J., 2011a. Epigenetic mechanisms in cognition. Neuron 70, 813-829.

Day, J.J., Sweatt, J.D., 2011b. Epigenetic mechanisms in cognition. Neuron 70, 813-829.

de Ruijter, A., van Gennip, A., Caron, H., Kemp, S., van Kiulenburg, A., 2003. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J 370, 737-749.

Deaton, A., Bird, A., 2011. CpG islands and the regulation of transcription. Genes Dev 25, 1010-1022.

Diano, S., Farr, S., Benoit, S., McNay, E., da Silva, I., Horvath, B., Gaskin, F., Nonaka, N., Jaeger, L., Banks, W., Morley, J., Pinto, S., Sherwin, R., Xu, L., Yamada, K., Sleeman, M., Tschop, M., Horvath, T., 2006. Ghrelin controls hippocampal spine density and memory performance. Nat Neurosci 9, 381-388.

Dinel, A., Andre, C., Aubert, A., Ferreira, G., Laye, S., Castanon, N., 2011. Cognitive and Emotional Alterations Are Related to Hippocampal Inflammation in a Mouse Model of Metabolic Syndrome. Plos One 6, e24325.

Dore, S., Kar, S., Rowe, W., Quirion, R., 1997. Distribution and levels of [125I]IGF-I, [125I]IGF-II and [125I]insulin receptor binding sites in the hippocampus of aged memory-unimpaired and -impaired rats. Neuroscience 80, 1033-1040.

Elsner, V.R., Lovatel, G.A., Bertoldi, K., Vanzella, C., Santos, F.M., Spindler, C., de Almeida, E.F., Nardin, P., Siqueira, I.R., 2011. Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus. Neuroscience 192, 580-587.

Elsner, V.R., Lovatel, G.A., Moyses, F., Bertoldi, K., Spindler, C., Cechinel, L.R., Muotri, A.R., Siqueira, I.R., 2013. Exercise induces age-dependent changes on epigenetic parameters in rat hippocampus: A preliminary study. Exp Gerontol 48, 136-139.

Erion, J., Woslski-Kuhn, M., Dey, A., Hao, S., Davis, C., Pollock, N., Stranahan, A., 2014. Obesity Elicits Interleukin 1-Mediated Deficits in Hippocampal Synaptic Plasticity. J Neurosci 34, 2618-2631.

Fall, T., Ingelsson, E., 2012. Genome-wide association studies of obesity and metabolic syndrome. Molecular and cellular endocrinology.

Frank, M.J., 2006. Hold your horses: a dynamic computational role for the subthalamic nucleus in decision making. Neural Netw 19, 1120-1136.

Fuller, N.R., Colagiuri, S., Schofield, D., Olson, A.D., Shrestha, R., Holzapfel, C., Wolfenstetter, S.B., Holle, R., Ahern, A.L., Hauner, H., Jebb, S.A., Caterson,

I.D., 2012. A within-trial cost-effectiveness analysis of primary care referral to a commercial provider for weight loss treatment, relative to standard care-an international randomised controlled trial. Int J Obes (Lond).

Gan, L., England, E., Yang, J., Toulme, N., Ambati, S., Hartzell, D., Meagher, R., Baile, C., 2015. A 72-hour high fat diet increases transcript levels of the neuropeptide galanin in the dorsal hippocampus of the rat. BMC Neurosci 16.

Garza, J., Guo, M., Zhang, W., Lu, X., 2008. Leptin increases adult hippocampal neurogenesis in vivo and in vitro. J Biol Chem 283, 18238-18247.

Gingerich, S., Wang, X., Lee, P.K., Dhillon, S.S., Chalmers, J.A., Koletar, M.M., Belsham, D.D., 2009. The generation of an array of clonal, immortalized cell models from the rat hypothalamus: analysis of melatonin effects on kisspeptin and gonadotropin-inhibitory hormone neurons. Neuroscience 162, 1134-1140.

Gomez-Pinilla, F., Zhuang, Y., Feng, J., Ying, Z., Fan, G., 2011a. Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci 33, 383-390.

Gomez-Pinilla, F., Zhuang, Y., Feng, J., Ying, Z., Fan, G., 2011b. Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. Eur J Neurosci 33, 383-390.

Good, M.A., Hale, G., 2007. The "Swedish" mutation of the amyloid precursor protein (APPswe) dissociates components of object-location memory in aged Tg2576 mice. Behav Neurosci 121, 1180-1191.

Good, M.A., Hale, G., Staal, V., 2007. Impaired "episodic-like" object memory in adult APPswe transgenic mice. Behav Neurosci 121, 443-448.

Gottlicher, M., Minucci, S., Zhu, P., 2001. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J 20, 6969-6978.

Graff, J., Kahn, M., Samiei, A., Gao, J., Ota, K., Rei, D., Tsai, L., 2013. A dietary regimen of caloric restriction or pharmacological activation of SIRT1 to delay the onset of neurodegenerations. J Neurosci 33, 8951-8960.

Gray, J., Rubin, T., Hunter, R., McEwen, B., 2014. Hippocampal gene expression changes underlying stress sensitization and recovery. Mol Psychiatr 19, 1171-1178.

Greenwood, C., Winocur, G., 1990. Learning and Memory Impairment in Rats Fed a High Saturated Fat Diet. Behav Neural Biol 53, 74-87.

Guo, J., Su, Y., Zhong, C., Ming, G., Song, H., 2011. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell 145, 423-434.

Guyenet, S., Nguyen, H., Hwang, B., Schwartz, M., Baskin, D., Thaler, J., 2013. High-fat diet feeding causes rapid, non-apoptotic cleavage of caspase-3 in astrocytes. Brain Research 1512, 97-105.

Guzel, A., Er, U., Tatli, M., Aluclu, U., Ozkan, U., Duzenli, Y., Satici, O., Guzel, E., Kemaloglu, S., Ceviz, A., Kaplan, A., 2008. Serum neuron-specific enolase as a predictor of short-term outcome and its correlation with Glasgow Coma Scale in traumatic brain injury. Neurosurg Rev 31, 439-444.

Haig, D., 2004. The (dual) origin of epigenetics. Cold Spring Harbor symposia on quantitative biology 69, 67-70.

Harvey, J., 2007. Leptin: a diverse regulator of neuronal function. J Neurochem 100, 307-313.

Hebben, N., Corkin, S., Eichenbaum, H., Shedlack, K., 1985. Diminished ability to interpret and report internal states after bilateral medial temporal resection: Case H. M. Behav Neurosci 98, 1031-1039.

Hebbes, T., Thorne, A., Crane-Robinson, C., 1988. A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 7, 1395-1402.

Helin, K., Dhanak, D., 2013. Chromatin proteins and modifications as drug targets. Nature 502, 480-488.

Henderson, Y., Smith, G., Parent, M., 2013. Hippocampal neurons inhibit meal onset. Hippocampus 23, 100-107.

Higgs, S., 2008. Cognitive influences on food intake: The effects of manipulating memory for recent eating. Physiol Behav 94, 734-739.

Holcomb, L., Gordon, M.N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C.M., Eckman, C., Younkin, S., Hsiao, K., Duff, K., 1998. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med 4, 97-100.

Holcomb, L.A., Gordon, M.N., Jantzen, P., Hsiao, K., Duff, K., Morgan, D., 1999. Behavioral changes in transgenic mice expressing both amyloid precursor protein and presenilin-1 mutations: lack of association with amyloid deposits. Behav Genet 29, 177-185.

Hollis, F., Wang, H., Dietz, D., Gunjan, A., Kabbaj, M., 2010. The effects of repeated social defeat on long-term depressive-like behavior and short-term histone modifications in the hippocampus in male Sprague-Dawley rats. Psychopharmacol 211, 69-77.

Hotamisligil, G., 2006. Inflammation and metabolic disorders. Nature 444, 860-867.

Irier, H., Street, R., Dave, R., Lin, L., Cai, C., Davis, T., Yao, B., Chen, Y., Jin, P., 2014. Environmental enrichment modulates 5-hydroxymethylcytosine dynamics in the hippocampus. Genomics 104, 376-382.

Jagust, W., Harvey, D., Mungas, D., Haan, M., 2005. Central obesity and the aging brain. JAMA Neurobiology 62, 1545-1548.

Jebb, S.A., Ahern, A.L., Olson, A.D., Aston, L.M., Holzapfel, C., Stoll, J., Amann-Gassner, U., Simpson, A.E., Fuller, N.R., Pearson, S., Lau, N.S., Mander, A.P., Hauner, H., Caterson, I.D., 2011. Primary care referral to a commercial provider for weight loss treatment versus standard care: a randomised controlled trial. Lancet 378, 1485-1492.

Jia, Y., Nguyen, T., Desai, M., Ross, M.G., 2008. Programmed alterations in hypothalamic neuronal orexigenic responses to ghrelin following gestational nutrient restriction. Reprod Sci 15, 702-709.

Jolly, K., Daley, A., Adab, P., Lewis, A., Denley, J., Beach, J., Aveyard, P., 2010. A randomised controlled trial to compare a range of commercial or primary care led weight reduction programmes with a minimal intervention control for weight loss in obesity: the Lighten Up trial. BMC Public Health 10, 439.

Jolly, K., Lewis, A., Beach, J., Denley, J., Adab, P., Deeks, J.J., Daley, A., Aveyard, P., 2011. Comparison of range of commercial or primary care led weight reduction programmes with minimal intervention control for weight loss in obesity: lighten Up randomised controlled trial. BMJ 343, d6500.

Kadriu, B., Guidotti, A., Chen, Y., Grayson, D., 2012. DNA methyltransferases 1 (DNMT1) and 3a (DNMT3a) colocalize with GAD67-positive neurons in the GAD67-GFP mouse brain. J Comp Neurol 520, 1951-1964.

Kaelin, W., Jr, McKnight, S., 2013. Influence of metabolism on epigenetics and disease. Cell 153, 56-69.

Kang, D.H., Heo, R.W., Yi, C.O., Kim, H., Choi, C.H., Roh, G.S., 2015. High-fat diet-induced obesity exacerbates kainic acid-induced hippocampal cell death. BMC Neurosci 16, 72.

Kanoski, S., Davidson, T., 2010. Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet. J Exp Psychol Anim B 36, 313-319.

Kanoski, S., Davidson, T., 2011 Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. Physiol Behav 103, 59-68.

Kanoski, S., Meisel, R., Mullins, A., Davidson, T., 2007. The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippcoampus and prefrontal cortex of the rat. Behav Brain Res 182, 57-66.

Kanoski, S., Zhang, Y., Zheng, W., Davidson, T., 2010. The Effects of a High-Energy Diet on Hippocampal Function and Blood-Brain Barrier Integrity in the Rat. J Alzheimers Dis 21, 207-219.

Knight, E.M., Brown, T.M., Gumusgoz, S., Smith, J.C., Waters, E.J., Allan, S.M., Lawrence, C.B., 2013. Age-related changes in core body temperature and activity in triple-transgenic Alzheimer's disease (3xTgAD) mice. Dis Model Mech 6, 160-170.

Knight, E.M., Verkhratsky, A., Luckman, S.M., Allan, S.M., Lawrence, C.B., 2012. Hypermetabolism in a triple-transgenic mouse model of Alzheimer's disease. Neurobiol Aging 33, 187-193.

Knutson, S., Kawano, S., Minoshima, Y., Warholic, N., Huang, K., Xiao, Y., Kadowaki, T., Uesugi, M., Kuznetsov, G., Kumar, N., Wigle, T., Klaus, C., Allain, C., Raimondi, A., Waters, N., Smith, J., Porter-Scott, M., Cheswort, R., Moyer, M., Copeland, R., Richon, V., Uenaka, T., Pollock, R., Kuntz, K., Yokoi, A., Keilhack, H., 2014. Selective inhibition EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. Mol Cancer Ther 13, 842-854.

Kohjima, M., Sun, Y., Chan, L., 2010. Increased food intake leads to obesity and insulin resistance in the tg2576 Alzheimer's disease mouse model. Endocrinology 151, 1532-1540.

Konycheva, G., Dziadek, M., Ferguson, L., Krageloh, C., Coolen, M., Davison, M., Breier, B., 2011. Dietary methyl donor deficiency during pregnancy in rats shapes learning and anxiety in offspring. Nutr Res 31, 790-804.

Kuzumaki, N., Ikegami, D., Tamura, R., Hareyama, N., Imai, S., Narita, M., Torigoe, K., Niikura, K., Takeshima, H., Ando, T., Igarashi, K., Kanno, J., Ushijima, T., Suzuki, T., Narita, M., 2011. Hippocampal epigenetic modification at the brain-

derived neurotrophic factor gene induced by an enriched environment. Hippocampus 21, 127-132.

Lambert, C., Zrinzo, L., Nagy, Z., Lutti, A., Hariz, M., Foltynie, T., Draganski, B., Ashburner, J., Frackowiak, R., 2012. Confirmation of functional zones within the human subthalamic nucleus: patterns of connectivity and sub-parcellation using diffusion weighted imaging. Neuroimage 60, 83-94.

Langie, S., Achterfeldt, S., Gorniak, J., Halley-Hogg, K., Oxley, D., van Schooten, F., Godschald, R., McKay, J., Mathers, J., 2013. Maternal folate depletion and high-fat feeding from weaning affects DNA methylation and DNA repair in brain of adult offspring. FASEB J 27, 3323-3334.

Laugerette, F., Vors, C., Geloen, A., Chauvin, M., Soulage, C., Lambert-Porcheron, S., Peretti, N., Alligier, M., Burcelin, R., Laville, M., Vidal, H., Michalski, M., 2011. Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. J Nutr Biochem 22, 53-59.

Li, S., Papale, L., Kintner, D., Sabat, G., Barrett-Wilt, G., Cengiz, P., Alisch, R., 2015. Hippocampal increases of 5-hmC in the glucocorticoid receptor gene following acute stress. Behav Brain Res 286, 236-240.

Lipsky, R., 2013. Epigenetic mechanisms regulating learning and long-term memory. Int J Devl Neuroscience 31, 353-358.

Lister, R., Mukamel, E., Nery, J., Urich, M., Puddifoot, C., Johnson, N., Lucero, J., Huang, Y., Dwork, A., Schultz, M., Yu, M., Tonti-Filippini, J., Heyn, H., Hu, S., Wu, J., Rao, A., Esteller, M., He, C., Haghighi, F., Sejnowski, T., Behrens, M.,

Ecker, J., 2013. Global epigenomic reconfiguration during mammalian brain development. Science 341, 1237905.

Lu, Y., Loos, R.J., 2013. Obesity genomics: assessing the transferability of susceptibility loci across diverse populations. Genome medicine 5, 55.

MacDonald, J., Roskams, A., 2009. Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. Prog Neurobiol 88, 170-183.

Maniam, J., Morris, M., 2012 The link between stress and feeding behaviour. Neuropharmacol 63, 97-110.

Martin, C., Han, H., Anton, S., Greenway, F., Smith, S., 2009. Effect of valproic acid on body weight, food intake, physical activity and hormones: results of a randomized control trial. J Psychopharmacol 23.

McNay, E., Ong, C., McCrimmon, R., Cresswell, J., Bogan, J., Sherwin, R., 2010. Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. Neurobiol Learn Mem 93, 546-553.

Meagher, R., 2014. 'Memory and molecular turnover,' 30 years after inception. Epigenetic Chrom 7, 37-46.

Meagher, R.B., 2010. The evolution of epitype. Plant Cell 22, 1658-1666.

Meagher, R.B., Mussar, K.J., 2012. The Influence of DNA Sequence on Epigenome-Induced Pathologies. Epigenetics Chromatin 5, 11-35.

Mehedint, M., Craciunescu, C., Zeisel, S., 2010. Maternal dietary choline deficiency alters angiogenesis in fetal mouse hippocampus. PNAS 107, 12834-12839.

Mielcarek, M., Zielonka, D., Carnemolla, A., Marcinkowski, J., Guidez, F., 2015. HDAC4 as a potential therapeutic target in neurodegenerative diseases: a summary of recent achievements. Front Cell Neurosci 9.

Mitchell, N.S., Ellison, M.C., Hill, J.O., Tsai, A.G., 2013. Evaluation of the effectiveness of making Weight Watchers available to Tennessee Medicaid (TennCare) recipients. J Gen Intern Med 28, 12-17.

Mody, N., Agouni, A., McIlroy, G.D., Platt, B., Delibegovic, M., 2011. Susceptibility to diet-induced obesity and glucose intolerance in the APP (SWE)/PSEN1 (A246E) mouse model of Alzheimer's disease is associated with increased brain levels of protein tyrosine phosphatase 1B (PTP1B) and retinol-binding protein 4 (RBP4), and basal phosphorylation of S6 ribosomal protein. Diabetologia 54, 2143-2151.

Molteni, R., Barnard, R., Ying, Z., Roberts, C., Gomez-Pinilla, F., 2002. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112, 803-814.

Moreno-Navarette, J., Ortega, F., Serino, M., Luche, E., Waget, A., Pardo, G., Salvador, J., Ricart, W., Fruhbeck, G., Burcelin, R., Fernandez-Real, J., 2012. Circulating lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. Int J Obesity 36, 1442-1449.

Mueller, K., Sacher, J., Arelin, K., Holiga, S., Kratzsch, J., Villringer, A., Schroeter, M.L., 2012. Overweight and obesity are associated with neuronal injury in the human cerebellum and hippocampus in young adults: a combined MRI, serum marker and gene expression study. Transl Psychiatry 2, e200.

Nanney, D.L., 1958. Epigenetic Control Systems. Proc Natl Acad Sci U S A 44, 712-717.

Naumann, N., Alpar, A., Ueberham, U., Arendt, T., Gartner, U., 2010. Transgenic expression of human wild-type amyloid precursor protein decreases neurogenesis in the adult hippocampus. Hippocampus 20, 971-979.

Oddo, S., Caccamo, A., Kitazawa, M., Tseng, B.P., LaFerla, F.M., 2003a. Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease. Neurobiol Aging 24, 1063-1070.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y., LaFerla, F.M., 2003b. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39, 409-421.

Odegaard, J., Chawla, A., 2013. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Science 339, 172-177.

Ogden, C., Carroll, M., Kit, B., Flegal, K., 2014. Prevalence of Childhood and Adult Obesity in the United States, 2011-2012. JAMA 311, 806-814.

Ogden, L.G., Stroebele, N., Wyatt, H.R., Catenacci, V.A., Peters, J.C., Stuht, J., Wing, R.R., Hill, J.O., 2012. Cluster analysis of the national weight control registry to identify distinct subgroups maintaining successful weight loss. Obesity (Silver Spring) 20, 2039-2047.

Paonessa, F., Latifi, S., Scarongella, H., Cesca, F., Benfenati, F., 2013. Specificity protein 1 (Sp1)-dependent activation of the synapsin I gene (SYN1) is modulated by RE1-silencing transcription factor (REST) and 5'-cytosine-phosphoguanine (CpG) methylation. J Biol Chem 288, 3227-3239.

Parent, M., Darling, J., Henderson, Y., 2014. Rembering to eat: hippcampal regulation of meal onset. Am J Physiol Regul Integr Comp Physiol 306, R701-R713.

Pasinetti, G., Eberstein, J., 2008. Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease. J Neurochem 106, 1503-1514.

Pelchat, M.L., Johnson, A., Chan, R., Valdez, J., Ragland, J.D., 2004. Images of desire: food-craving activation during fMRI. Neuroimage 23, 1486-1493.

Phiel, C., Zhang, F., Huang, E., Guenther, M., Lazar, M., Klein, P., 2001. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J Biol Chem 276, 36734-36741.

Pistell, P., Morrison, C., Gupta, S., Knight, A., Keller, J., Ingram, D., Bruce-Keller, A., 2010. Cognitive impairment following high fat diet consumption is associated with brain inflammation. J Neuroimmunology 219, 25-32.

Qi, C., Liu, S., Qin, R., Zhang, Y., Wang, G., Shang, Y., Liang, J., 2014. Coordinated regulation of dendrite arborization by epigenetic factors CDYL and EZH2. J Neurosci 34, 4494-4508.

Racette, S.B., Deusinger, S.S., Inman, C.L., Burlis, T.L., Highstein, G.R., Buskirk, T.D., Steger-May, K., Peterson, L.R., 2009. Worksite Opportunities for Wellness (WOW): effects on cardiovascular disease risk factors after 1 year. Prev Med 49, 108-114.

Raji, C.A., Ho, A.J., Parikshak, N.N., Becker, J.T., Lopez, O.L., Kuller, L.H., Hua, X., Leow, A.D., Toga, A.W., Thompson, P.M., 2010. Brain structure and obesity. Hum Brain Mapp 31, 353-364.

Rankinen, T., Bouchard, C., 2008. Gene-physical activity interactions: overview of human studies. Obesity (Silver Spring) 16 Suppl 3, S47-50.

Reichelt, A., Maniam, J., Westbrook, R., Morris, M., 2015. Dietary-induced obesity disrupts trace fear conditioning and decreases hippocampal reelin expression. Brain Behav Immun 43, 68-75.

Reinius, L.E., Acevedo, N., Joerink, M., Pershagen, G., Dahlen, S.E., Greco, D., Soderhall, C., Scheynius, A., Kere, J., 2012. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. PLoS One 7, e41361.

Ricobaraza, A., Cuadrado-Tejedor, M., Marco, S., Perez-Otano, I., Garcia-Osta, A., 2012. Phenylbutyrate rescues dendritic spine loss associated with memory deficits in a mouse model of Alzheimer disease. Hippocampus 22, 1040-1050.

Ricobaraza, A., Cuadrado-Tejedor, M., Perez-Mediavilla, A., Frechilla, D., Del Rio, J., Garcia-Osta, A., 2009. Phenylbutyrate ameliorates cognitive deficit and reduces tau pathology in an Alzheimer's disease mouse model. Neuropsychopharmacology 34, 1721-1732.

Robbins, T.W., 2007. Shifting and stopping: fronto-striatal substrates, neurochemical modulation and clinical implications. Philos Trans R Soc Lond B Biol Sci 362, 917-932.

Ross, A., Bruggeman, E., Kasamu, A., Mielke, J., Parent, M., 2012. Non-alcoholic fatty liver disease impairs hippocampal-dependent memory in male rats. Physiol Behav 106, 133-141.

Roth, T., Zoladz, P., Sweatt, J., Diamond, D., 2011. Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. J Psychiat Res 45, 919-926.

Rudenko, A., Dawlaty, M., Seo, J., Cheng, A., Meng, J., Le, T., Faull, K., Jaenisch, R., Tsai, L., 2013. Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. Neuron 79, 1109-1122.

Sable, P., Randhir, K., Kale, A., Chavan-Gautam, P., Joshi, S., 2015. Maternal micronutrients and brain global methylation patterns in the offspring. Nutr Neurosci 18, 30-36.

Sales, A.J., Biojone, C., Terceti, M.S., Guimaraes, F.S., Gomes, M.V., Joca, S.R., 2011. Antidepressant-like effect induced by systemic and intra-hippocampal administration of DNA methylation inhibitors. Br J Pharmacol 164, 1711-1721.

Sanchez-Hernandez, D., Poon, A., Kubant, R., Kim, H., Huot, P., Cho, C., Pannia, E., Pausova, Z., Anderson, G., 2015. A gestational diet high in fat-soluble vitamins alters expression of genes in brain pathways and reduces sucrose preference, but not food intake, in Wistar male rat offspring. Appl Physiol Nutr Metab 40, 424-431.

Sanchez-Molina, S., Estaras, C., Oliva, J., Akizu, N., Asensio-Juan, E., Rojas, J., Martinez-Balbas, M., 2014. Regulation of CBP and Tip60 coordinates histone acetylation at local and global levels in during Ras-induced transformation. Carcinogenesis 35, 2194-2202.

Sherwani, S., Khan, H., 2015. Role of 5-hydroxymethylcytosine in neurodegeneration. Gene 570, 17-24.

Sobesky, J., Barrientos, R., De May, H., Thompson, B., Weber, M., Watkins, L., Maier, S., 2014. High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1b, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism. Brain Behav Immun 42, 22-32.

Song, C., Szulwach, K., Fu, Y., Dai, Q., Yi, C., Li, X., Li, Y., Chen, C., Zhang, W., Jian, X., Wang, J., Zhang, L., Looney, T., Zhang, B., Godley, L., Hicks, L., Lahn, B., Jin, P., He, C., 2011. Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat Biotechnol 29, 68-72.

Stranahan, A., Norman, E., Lee, K., Cutler, R., Telljohan, R., Egan, J., Mattson, M., 2008. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus 18, 1085-1088.

Subbanna, S., Basavarajappa, B., 2014. Pre-administration of G9a/GLP inhibitor during synaptogenesis prevents postnatal ethanol-induced LTP deficitis and neurbehavioral abnormalities in adult mice. Exp Neurol 261, 34-43.

Szulwach, K., Li, X., Li, Y., Song, C., Wu, H., Dai, Q., Irier, H., Upadhyay, A., Gearing, M., Levey, A., Vasanthakumar, A., Godley, L., Chang, Q., Cheng, X., He, C., Jin, P., 2011. 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nat Neurosci 14, 1607-1616.

Tucsek, Z., Toth, P., Sosnowska, D., Gautam, T., Mitschelen, M., Koller, A., Szalai, G., Sonntag, W., Ungvari, Z., Csiszar, A., 2013. Obesity in aging exacerbates blood-brain barrier disruption, neuroinflammation, and oxidative stress in the mouse hippocampus: Effects on expression of genes involved in beta-amyloid generation and Alzheimer's disease. J Gerontol A Biol Sci Med Sci 177, 1-15.

Umka, J., Mustafa, S., ElBeltagy, M., Thorpe, A., Latif, L., Bennett, G., Wigmore, P., 2010. Valproic acid reduces spatial working memory and cell proliferation in the hippocampus. Neuroscience 166, 15-22.

Valladolid-Acebes, I., Fole, A., Martin, M., Morales, L., Victoria Cano, M., Ruiz-Gayo, M., Del Olmo, N., 2013. Spatial memory impairment and changes in hippocampal morphology are triggered by high-fat diets in adolescent mice. Is there a role of leptin? Neurobiol Learn Mem 106, 18-25.

Valladolid-Acebes, I., Stucchi, P., Cano, V., Fernandez-Alfonso, M., Merino, B., Gil-Ortega, M., Fole, A., Morales, I., Ruiz-Gayo, M., Del Olmo, N., 2011. High-fat diets impair spatial learning in the radial-arm maze in mice. Neurobiol Learn Mem 95, 80-85.

Vanhees, K., Vonhogen, I., van Schooten, F., Godschalk, R., 2014. You are what you eat, and so are your children: The impact of micronutrients on the epigenetic programming of offspring. Cell Mol Life Sci 71, 271-285.

Vaynman, S., Ying, Z., Gomez-Pinilla, F., 2004. Exercise induces BDNF and synapsin I to specific hippocampal subfields. J Neurosci Res 76, 356-362.

Verrotti, A., D'Egidio, C., Mohn, A., Coppola, G., Chiarelli, F., 2011. Weight gain following treatment with valproic acid: pathogenetic mechanisms and clinical implications. Obes Rev 12, e32-e43.

Villeneuve, L., Reddy, M., Lanting, L., Wang, M., Meng, L., Natarajan, R., 2008. Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. PNAS 105, 9047-9052.

Waddington, C.H., 1957. Chapter 2. The Cybernetics of Development. In. The strategy of the genes: A Discussion of Some Aspects of Theoretical Biology. Ruskin House, George Allen & Unwin LTD, London.

Wallner-Liebmann, S., Koschutnig, K., Reishofer, G., Sorantin, E., Blaschitz, B., Kruschitz, R., Unterrainer, H.F., Gasser, R., Freytag, F., Bauer-Denk, C., Schienle, A., Schafer, A., Mangge, H., 2010. Insulin and hippocampus activation in response to images of high-calorie food in normal weight and obese adolescents. Obesity (Silver Spring) 18, 1552-1557.

Watson, G., Craft, S., 2004. Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. Eur J Pharmacol 490, 97-113.

Woldemichael, B., Bohacek, J., Gapp, K., Mansuy, I., 2014. Epigenetics of memory and plasticity. Prog Mol Biol Transl 122, 305-340.

Wu, H., Zhang, Y., 2014. Reversing DNA methylation: mechanisms, genomics, and biological functions. Cell 156, 45-68.

Yu, P., McKinney, E.C., Kandasamy, M.M., Albert, A.L., Meagher, R.B., 2015. Characterization of brain cell nuclei with decondensed chromatin. Dev Neurobiol 75, 738-756.

Yu, X., Geng, Y., Liang, J., Zhang, S., Lei, H., Zhong, S., Lin, Q., Shan, Z., Lin, S., Li, Y., 2012. High levels of glucose induce "metabolic memory" in cardiomyocyte via epigenetic histone H3 lysine 9 methylation. Mol Biol Rep 39, 8891-8898.

Zhang, J., Ji, F., Liu, Y., Lei, X., Li, H., Ji, G., Yuan, Z., Jiao, J., 2014. Ezh2 regulates adult hippocampal neurogenesis and memory. J Neurosci 34, 5184-5199.

Zhang, R., Cui, Q., Murai, K., Lim, Y., Smith, Z., Jin, S., Ye, P., Rosa, L., Lee, Y., Wu, H., Liu, W., Xu, Z., Yang, L., Ding, Y., Tang, F., Meissner, A., Ding, C., Shi, Y., Xu, G., 2013 Tet1 regulates adult hippocampal neurogenesis and cognition. Cell Stem Cell 13, 237-245.

Zovkic, I., Guzman-Karlsson, M., Sweatt, J., 2013a. Epigenetic regulation of memory formation and maintenance. Learn Memory 20, 61-74.

Zovkic, I.B., Guzman-Karlsson, M.C., Sweatt, J.D., 2013b. Epigenetic regulation of memory formation and maintenance. Learn Mem 20, 61-74.

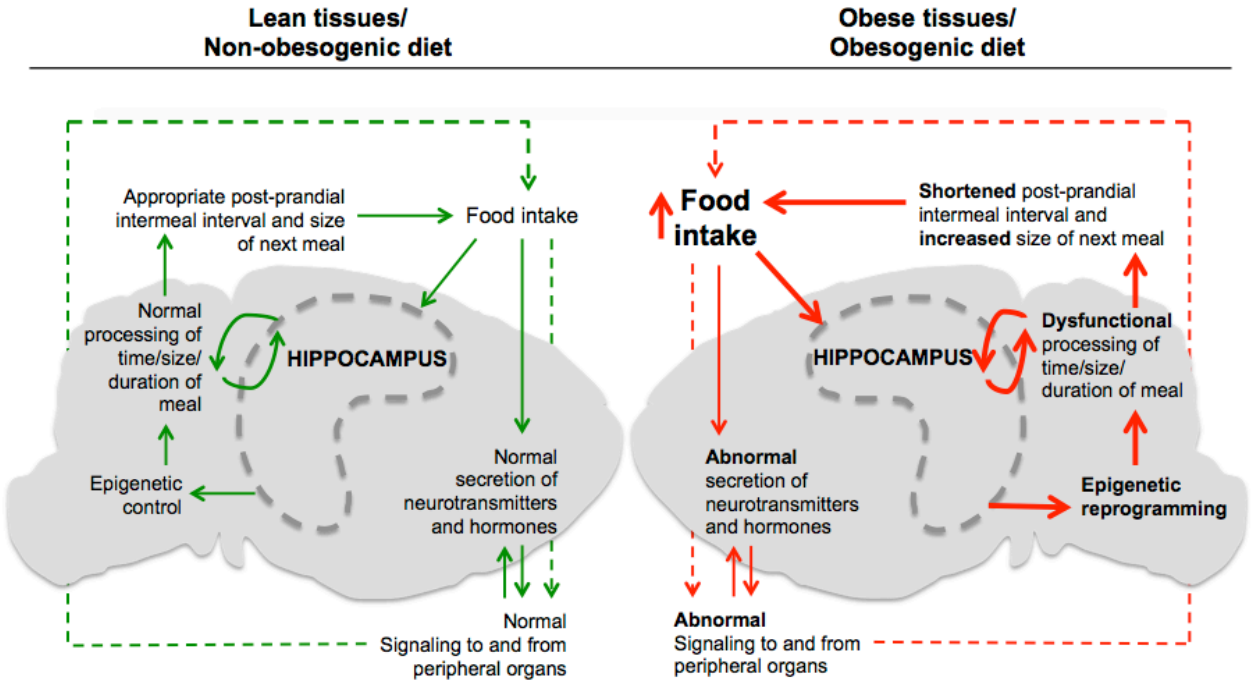


Figure 2.1. Involvement of the hippocampus in the cycle of food intake: the interplay of diet and obesity. In the normal/lean state (left side), the hippocampus records information about food intake including the time, size, and duration of a meal. Signals are sent from the hippocampus to other brain structures and to and from peripheral organs to delay consumption of the next meal. This normal/lean state is associated with specific chromatin modifications that play a role in the management and consistency of these signaling pathways. In the obese state (right side), obesogenic tissues and increased food intake give rise to altered signaling cascades between brain structures and to and from peripheral organs. These changes in synaptic and hormonal signaling, along with the information being processed by the hippocampus, result in epigenetic reprogramming that promotes food intake in the absence of physiological necessity and perpetuates the cycle of obesity. Pathways are superimposed on a cartoon image of the rodent brain with the hippocampus represented by a dotted outline.

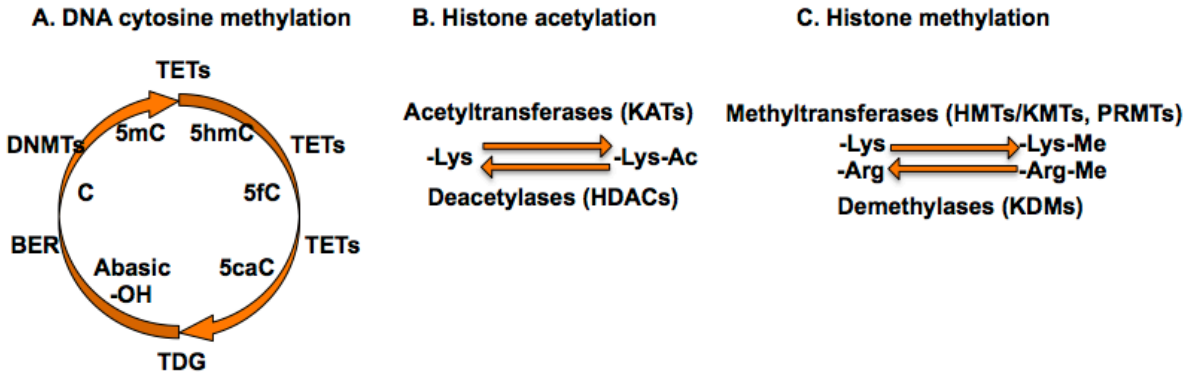


Figure 2.2. Cyclic modification of chromatin in the hippocampus.

In the cycle of DNA cytosine methylation, DNMTs catalyze the conversion of C to 5mC. TETs oxidize 5mC to the intermediates 5hmC, 5fC, and 5caC. TDG removes the carboxylated cystosine to create an abasic site, which is corrected to an unmodified C by base excision repair (A). Lysine residues with in the N-terminal tail of a histone can become acetylated or deacetylated (B) through the action of KATs or HDACs, respectively, or can become methylated/demethylated (C) via methyltransferases or demethylases, respectively.

CHAPTER 3

A 72-HOUR HIGH FAT DIET INCREASES TRANSCRIPT LEVELS OF THE NEUROPEPTIDE GALANIN IN THE DORSAL HIPPOCAMPUS OF THE RAT

England E.*^, Gan L.*, Yang J. Y., Toulme N., Ambati S., Hartzell D. L., Meagher R. B., and C. A. Baile. 2015. *BMC Neuroscience*. 16:51.

Reprinted here with permission of the publisher.

*Both authors contributed equally to this work.

^Please note that England is the maiden name of Emily Trunnell

Abstract

Recent evidence identifies the hippocampus, a brain structure commonly associated with learning and memory, as key to the regulation of food intake and the development and consequences of obesity. Intake of a high fat diet (HFD) results in altered consumptive behavior, hippocampal damage, and cognitive deficits. While many studies report the effects of HFD after chronic consumption and in the instance of obesity, few examine the events that occur following acute HFD consumption. In this study, male rats were fed either a control diet (10% fat by kcal) or HFD (45% fat by kcal) for 72 hours. At the end of the 72-hour period, serum and tissues were collected and weighed. Brains were rapidly frozen or formalin-fixed in preparation for qRT-PCR or immunohistochemistry, respectively. Acute intake of HFD resulted in higher serum levels of leptin and cholesterol, with no significant changes in final body weight or adipose tissue mass. In the dorsal hippocampus, transcription of the neuroprotective peptide *galanin* was significantly upregulated along with a trend for an increase in *brain-derived neurotrophic factor* and *histone deacetylase 2* in the rats fed HFD. In the ventral hippocampus, there was a significant increase in *histone deacetylase 4* and a decrease in *galanin receptor 1* in this group. Results from immunohistochemistry validate strong presence of the galanin peptide in the CA1/CA2 region of the dorsal hippocampus. These results provide evidence for a distinct response in specific functional regions of the hippocampus following acute HFD intake.

3.1 Introduction

Overweight and obesity are at epidemic levels in the United States and rates are rising in other countries that have adopted a more Western diet [1]. Consumption of diets rich in saturated fatty acids is commonplace in the United States and is pinpointed as a major factor in the development of obesity and resulting metabolic disease states [2]. Excess intake of saturated fat has also been implicated in the development of neurodegenerative diseases such as Alzheimer's [3, 4].

It is well established that the hippocampus is a major brain region involved in memory, particularly episodic and spatial memory [5]. However, the hippocampus is a heterogeneous structure with multiple projections to other areas of the brain involved in emotional motivation and feeding behaviors [6, 7]. A thorough review by Lathe highlighted the role of the hippocampus in monitoring the physiological environment and modulating an appropriate response, a major component of which is sensing of the endocrine and metabolic state of the blood and cerebral spinal fluid [8]. In fact, the blood-brain barrier in the vicinity of the hippocampus is particularly vulnerable to the exterior environment. Two studies have shown a reduction in blood brain barrier integrity in the vicinity of the hippocampus following high fat diet (HFD) consumption [9, 10]. Dietary fat and cholesterol are able to cross the blood-brain barrier and promote protein infiltration, a process that may contribute to the development of Alzheimer's [9]. Behavioral effects can be observed rapidly after this HFD-induced insult. Impairments in hippocampal-specific spatial memory are observed after only three to five days on a high-energy diet (higher in saturated fat and glucose), with no effects of the diet on memory

tasks that were not dependent on the hippocampus [11, 12]. This would suggest that the hippocampus responds more rapidly to dietary insult than other areas of the brain.

While many studies show the impact of chronic consumption of HFD on the molecular physiology of brain, few examine the acute response [13-16]. We investigated the effects of HFD consumption in a 72-hour time window, which for humans might be the amount of time a typically healthy individual spends eating high fat foods over a holiday or while on vacation. Three recent studies from the University of Washington showed that during the first three days of high fat feeding, hypothalamic inflammation, reactive gliosis and astrocytosis were present in both rats and mice at this time point, and that similar gliosis occurred in obese human subjects [12, 16, 17]. Other experiments involving 72-hour HFD consumption in rodent models have shown increased body weight, increased adipose tissue mass, and increases in markers of inflammation and oxidative stress in the brain, liver, and adipose tissue [12, 18, 19]. Little is known about changes in hippocampal gene expression following acute HFD intake.

It is now widely accepted that changes in the physical environment may be rapidly recorded in different regions of the brain by epigenetic mechanisms involving changes in chromatin structure [20]. For example, acetylation of nucleosomal histones is sensitive to dietary change and correlates with altered gene expression in a very rapid timeframe [21, 22]. Consequently, we chose to examine the expression levels of several enzymes that alter chromatin structure, as well as trophic factors, hormone receptors, genes associated with obesity and inflammation, and the neuropeptide galanin. Galanin is a small, highly conserved neuropeptide that is expressed throughout the mammalian central and peripheral nervous system [23, 24]. It is known to be involved in feeding, the

regulation of metabolism, neuronal excitability, neuroprotection, cognition, and stress, to name a few [25, 26]. In feeding studies, galanin is shown to be orexigenic, particularly stimulating the consumption of fat and intake of fat will alter levels of *galanin* mRNA in the hypothalamus [27]. Early changes in galanin expression in the hippocampus as well might help explain why animals do not self-regulate when offered a high fat diet, and will continue to consume until reaching a state of obesity.

We hypothesized that the gene expression profile of rats fed a high fat diet for 72 hours would be indicative of hippocampal damage and a propensity towards obesity, meaning that we expected to see a downregulation of neurotrophic and neuroprotective factors, an upregulation of certain epigenetic enzymes, and a downregulation of insulin and leptin receptors in rats fed a high fat diet for 72 hours. However, our results show a distinct response in specific functional poles of the hippocampus following acute HFD intake, with potential mediators of neuroprotection at play in the dorsal hippocampus.

3.2 Results

Energy Intake, Body and Tissue Weights

Male rats given a HFD *ad libitum* (45% kcal from fat) for 72 hours did not consume more food in grams than their control-fed counterparts (**Fig. 3.1a**), but they also did not reduce their food intake to account for increased caloric value of the HFD and consumed more energy on each day of the study ($p<0.001$; **Fig. 3.1b**). Results from a two-way ANOVA show that both dependent variables, food intake and energy intake, were normally distributed and that there was homogeneity of variance between groups as assessed by Levene's test for quality of variances. There was not a significant interaction

between the effects of day and treatment on food intake (g) ($F(2,48)=0.213$, $P=0.809$), or energy intake (kcal) ($F(2,48)=0.197$, $P=0.822$). HFD-fed rats did not weigh significantly more than rats fed a control diet (10% kcal from fat) at the conclusion of the study (Table 3), although they did gain more weight during the last 24-hour period (day 3) of the study ($p<0.05$, **Fig. 3.1c**). There was a significant interaction between the effect of day and treatment on the weight gain (g) of the rats ($F(2,48)=0.197$, $P=0.020$). There was no significant difference in the raw weights of white or brown adipose depots either alone or when combined (**Table 3.3**), or when they were calculated as a proportion of body weight (not shown). Liver weight was reduced in HFD-fed rats (**Table 3.3**) and this change was significant as a proportion of body weight ($p<0.05$, not shown).

Blood and Serum Measures

After 72-hours of a HFD, serum leptin ($p<0.01$) and total serum cholesterol ($p<0.05$) levels were significantly increased relative to controls (**Table 3.4**). There was no significant difference in either blood glucose or serum insulin levels between control- and HFD-fed rats (**Table 3.4**).

Gene Expression

In the dorsal hippocampus, there was a significant 20% increase in transcript levels of the neuropeptide *galanin* ($p<0.0488$; **Fig. 3.2a**). There was no change in transcript levels of either *galanin receptor 1* or *2* ($p=0.590$ and $p=0.818$ respectively; **Fig. 3.2b,c**). Also in the dorsal hippocampus there was a trend for an increase in *brain-derived neurotrophic factor* and *histone deacetylase 2* ($p=0.0593$ and $p=0.0604$ respectively; **Fig. 3.2d,e**) in HFD-fed rats compared to controls.

In the ventral hippocampus of HFD-fed rats, there was a significant 66% decrease in transcript levels of *galanin receptor 1* (p=0.0153; **Fig. 3.2b**) and a significant 14% increase in transcript levels of *histone deacetylase 4* (p=0.0497; **Fig. 3.2f**). There was no significant difference in levels of *galanin* or *galanin receptor 2* transcripts compared to controls (p=0.978 and p=0.220 respectively, **Fig. 3.2a,c**), or in transcript levels of other genes tested (**Table 3.2**) in either the dorsal or ventral hippocampus.

Galanin Immunohistochemistry

Immunostaining for the galanin protein in brain sections including the dorsal hippocampus showed a strong perinuclear pattern of staining in the pyramidal cells of the CA1 and CA2 regions (**Fig. 3.3a-f**), though differences in fluorescence intensity levels between treatment groups did not reach statistical significance (p=0.22, **Fig. 3.3g**).

3.3 Discussion

High intake of saturated fatty acids is identified as a factor leading to cognitive impairment later in life [29]. In this study, we found that rats fed a HFD for only 72 hours had significantly higher serum cholesterol than control-fed rats. Increased permeability of the blood-brain barrier has also been observed in rabbits with increased circulating cholesterol [30, 31]. Other studies have linked high fat and cholesterol diets to damage of the blood-brain barrier with corresponding impairments in hippocampal-dependent memory tasks [10].

Here we provide evidence that acute consumption of a HFD impacts the hippocampus. From our qRT-PCR results, we found that levels of *histone deacetylase* (*HDAC*) transcript were trending upward in the dorsal hippocampus and significantly

increased in the ventral hippocampus of HFD-fed rats. The epigenetic machinery tasked with modulating acetylation and methylation levels of DNA and histones, thus affecting gene expression, can be rapidly influenced by environment. HDAC activity is increased in instances of neuronal cell death and administration of HDAC-inhibitors has been proposed in the treatment of Alzheimer's disease [32, 33]. A recent review by Mielcarek, et al highlights the role of HDAC4 in neuronal cell death [34]. Interestingly, the activity of HDAC4 is regulated by its translocation from the cytoplasm to the nucleus. Wang, et al showed that in *Drosophila*, an important model organism for the study of obesity and diabetes, feeding activated the AMPK-family kinase SIK3, which phosphorylated HDAC4 and promoted its sequestration in the cytoplasm [35]. While upregulation of HDAC4 mRNA in the ventral hippocampus could be evidence of a compensatory response related lack of active HDAC4 in the nucleus of fed rats, the feeding-induced signal for this pathway was reported to be insulin [35], which was not significantly increased in our HFD-fed animals. Additionally, rats in our study were fasted for two hours before tissues were collected. Knowing that the mediator of apoptosis capsase-3 [17] and the inflammatory stimulator lipopolysaccharide (LPS) [36] are both upregulated by HFD, and that both of these signals trigger the degradation of HDAC4 [34], it is also possible that HFD-induced degradation of HDAC4 protein triggered the upregulation of HDAC4 transcripts that we observed in the ventral hippocampus of our HFD-fed rats.

Importantly we show that transcript levels of *galanin* are upregulated in the dorsal hippocampus after just 72 hours on a HFD. One of the many roles galanin is known to play in the central nervous system is that of a neurotrophic and neuroprotective factor [27, 37-40]. Specifically in the hippocampus, galanin has been implicated as having both

beneficial [26, 41, 42] and detrimental [25, 42, 43] effects regarding learning and memory, due mainly to its ability to regulate neural activity in the hippocampus through modulation of cholinergic transmission [44]. *Galanin* is also rapidly upregulated in septohippocampal neurons following either lesion of the area or blockade of neuronal activity [45]. Additionally, as previously stated, galanin is particularly involved in promoting the intake of fat [27], however we would expect this function of galanin to be associated primarily with the ventral hippocampus and we did not see a significant increase in transcript levels in this region. Also, we saw an increase in *galanin* only in the dorsal hippocampus, not in the ventral hippocampus where there was the most significant increase in *HDAC* transcripts. While the ventral hippocampus is reportedly involved in emotion and motivation, the dorsal hippocampus and its connections to the frontal cortex are critical for learning and memory [6, 7], and it is important to note that the function of galanin depends largely on the region of the brain in which it is acting [46-49]. Though our immunohistochemical results do not show a statistically significant increase in galanin protein signal in the dorsal hippocampus, there are some potential explanations for this discrepancy that would not necessarily negate our conclusions about *galanin* mRNA: 1) We are examining only an acute response (3 days) and the transcription of mRNA is more rapid than the translation of protein, 2) Immunohistochemistry is not as reliable a method for quantifying protein as other methods such as Western blot, therefore these results are most useful to display the location of galanin in the dorsal hippocampus, and 3) The small sample size (n=5) of our immunohistochemistry experiment was likely not powerful enough to generate significant results. Regional- and cell type-specific studies, along with more extensive

protein analysis, will be needed to further elucidate galanin's actions in the brain in response to acute HFD.

In addition to the significant upregulation of *galanin* in the dorsal hippocampus, this study also demonstrated a trend for an increase in the transcript levels of *brain-derived neurotrophic factor* (*BDNF*) in the same region. BDNF is known to enhance hippocampal function by increasing neurogenesis and neurite growth, enhancing long-term potentiation and spatial memory, protecting the hippocampus against excitotoxic injury, and for its involvement in neurodevelopment [26, 50, 51]. This finding is surprising considering the majority of the literature regarding BDNF and HFD points to a decrease in *BDNF* transcription following HFD, however in these experiments the animals consumed the HFD for 5 weeks or longer [51-53]. The upregulation of *BDNF* and *galanin* in the dorsal hippocampus suggests that the acute response to HFD is entirely different than the long-term response and requires further study.

Despite the increase in *galanin* transcript in the dorsal hippocampus, our qRT-PCR results did not show changes in either *galanin receptor 1* (*GALR1*) or *galanin receptor 2* (*GALR2*) transcripts in this region. We did observe a decrease in the transcript levels of *GALR1* in the ventral hippocampus. All three *galanin receptor* genes (1, 2, and 3) are expressed in both dorsal and ventral hippocampus [54, 55], with *GALR1* having exceptionally high expression in the ventral hippocampus when compared to the dorsal hippocampus and the brain as a whole [47, 48]. Pharmacological studies point to GALR1 as playing a larger role in feeding than either GALR2 or GALR3 [55]. GALR3 has a lower affinity for galanin than GALR1 or GALR2 and is postulated to have a greater role in the periphery; therefore it was not chosen for analysis in this study [55, 56]. Evidence

that galanin serves different functions in the dorsal versus ventral hippocampal regions is supported by our findings and leads us to believe that HFD differentially regulates the galanin pathway in these two regions [47, 49]. Galanin administration has completely opposite effects depending on the site of infusion, for example, decreasing basal acetylcholine release in the dorsal hippocampus and increasing release in the ventral hippocampus [49]. With GALR1 being reduced in the ventral hippocampus, we might expect that release of galanin here would be increased, causing a compensatory downregulation of the receptor. However, since we did not see a significant increase in galanin mRNA in the ventral hippocampus, meaning that this increased galanin is not coming from the ventral hippocampus itself, we hypothesize that there could be an increase in galanin peptide release into the ventral hippocampus coming from other areas of the brain, such as the locus coeruleus. The locus coeruleus is one of the major galanin-producing nuclei in the brain and sends direct galanergic projections to both the dorsal and ventral the hippocampus. However, we cannot verify this hypothesis with the remaining brain tissue from our study and do not know of studies showing a direct connection between high fat diet and increased galanin release from these neurons. Additionally, dopamine, the neurotransmitter most associated with motivation and reward, is activated following consumption of HFD and has long provided evidence for the rewarding effects of high fat and highly palatable foods [57, 58]. A recent study by Valdivia et al. showed that dopaminergic neurons in the ventral tegmental area (VTA), a reward-related brain area, are activated following only 2 hours of HFD intake [59]. Dopamine receptor stimulation has the ability to modulate the effects of GALR1 activation and, interestingly, dopamine-galanin heteromers in the hippocampus are found

only in the ventral pole, not the dorsal [47]. It is possible that an increase in VTA dopamine release following HFD could project, via the mesolimbic dopamine pathway, to the ventral hippocampus and result in a feedback downregulation of GALR1-expressing dopamine-responsive neurons in this area.

A possible confound in our interpretation of these results lies in the issue of novelty. In this experiment, animals were maintained on the control diet prior to start of the experiment and only the high fat group was switched to a new diet. To our current knowledge, no connections have been made between galanin and exposure to a novel food. However, the hippocampus itself responds strongly to a variety of novel events [60]. Learning and memory are critical components of feeding: an encounter with a food item prompts an animal to determine if he's ever encountered it before, and to remember the experience with the food item in case he is to encounter it again in the future [61]. Due to its classical role in learning and memory, the hippocampus cannot be separated from these processes. Importantly though, the issue of novelty also cannot be separated from the human experience with exposure to a highly palatable food. In cases of short-term high fat feeding in humans, such as in instances of vacation or holidays, exposure to and consumption of novel foods is likely a major contributor to caloric intake, and likely causes activation of similar brain pathways as does our model.

In this study we showed that rats fed a HFD for 72 hours had significantly higher serum leptin than rats fed a control diet. Importantly, this change was independent of a significant increase in fat mass. Many studies highlight a neuroprotective role for leptin in the central nervous system. Leptin reduces neuronal apoptosis, increases cell survival and proliferation, and reduces damage caused by stroke; specifically in the hippocampus,

leptin facilitates plasticity [62, 63]. A few studies have shown evidence of leptin receptors on both galanin- and BDNF-expressing neurons in the brain, particularly the hypothalamus, and that leptin indirectly mediates both galanin and BDNF release [64-66]. It is possible that in this 72-hour HFD model, the upregulation of *galanin* and *BDNF* are being mediated through the observed increased circulating leptin, but further studies are needed to determine the presence of this molecular interaction in hippocampal neurons. Chronic HFD consumption results in central and peripheral leptin resistance, thereby preventing the neuroprotective role of leptin in obesity and potentially contributing to the downregulation of *BDNF* after chronic HFD consumption, as mentioned earlier.

Our observance of a reduction in liver weight after 72 hours of high fat diet is surprising, however previous studies by Miller, et al. and Ren, et al. shed light on a possible mechanism. In those experiments, 72 hours of high fat feeding in rodents down-regulated hepatic lipogenesis, possibly due to an inhibitory effect of the dietary fat on certain hepatic enzymes [19, 67]. Additionally, compared to the HFD group, the control animals consumed a greater proportion of their calories as carbohydrate, which is likely to have caused increased glycogen storage in control livers [68] compared to livers of HFD animals, another possible explanation for the differences in final liver weight between the groups. It is well known that chronic high fat diet promotes lipid accumulation in the liver, leading eventually to hepatic steatosis and non-alcoholic fatty liver disease, but our current findings along with the previous studies shed light on the important differences between acute and chronic exposure models.

3.4 Conclusion

In conclusion, this study demonstrates a unique acute response to HFD consumption in the hippocampus of the rat. Prior to significant increases in fat mass or body weight, gene expression in the hippocampus is altered in a way that reflects a distinct response in specific functional poles of the hippocampus following acute HFD intake, with potential mediators of neuroprotection at play in the dorsal hippocampus. Future studies should examine structural changes in the hippocampus at this time point to determine the level of HFD-induced insult after 72 hours as well as examine the response of females to acute HFD, as there is evidence for sex differences in this model [19].

3.5 Methods

Animals and Feeding

Twenty male, 8-10 week-old Long-Evans rats (200-250 g) were purchased from Harlan (Indianapolis, IN, USA). Upon arrival rats were housed individually and adapted to the rodent facility and to a low-fat control diet (Control, **Table 3.1**, D12450B; Research Diets; New Brunswick, NJ, USA) for 11-16 days. Rats were weight-matched and either maintained on the control diet (n=10) or switched to a high-fat diet (HFD; n=10; Table 1, D12451; Research Diets; New Brunswick, NJ, USA). Rats had access to the diets and water *ad libitum* throughout the experiment. Food intake and body weight were monitored daily. Rooms were temperature (22°C ± 2°C) and humidity controlled and kept on a 12:12-hour light/dark cycle. All institutional and national guidelines for the care and use of laboratory animals were followed. All protocols for this experiment

were approved by the University of Georgia Institutional Animal Care and Use Committee (AUP #A2013 09-005-Y1-A0) prior to the start of this experiment.

Tissue Collection

After 72 hours of dietary treatment, rats were fasted for 2 hours then anesthetized with inhaled isoflurane anesthesia (2.5%) and euthanized by decapitation. Trunk blood was collected immediately for measurement of glucose (FreeStyle® Lite Blood Glucose Monitoring System; Abbot Diabetes Care, Abbot Park, IL, USA) and then allowed to clot for 30 minutes before serum was collected for further analysis. The brain was removed from the skull and weighed. The left hemisphere was rapidly frozen on dry ice for RNA isolation and the right hemisphere was fixed in 4% formaldehyde (Avantor; Center Valley, PA, USA) for 28 hours and flash frozen for immunohistochemistry. Inguinal, epididymal, retroperitoneal, omental, pericardial, and subscapular brown fat depots, along with the liver, were removed, weighed, and frozen in liquid nitrogen for long-term storage.

ELISA

Serum insulin was measured using a rat/mouse-specific ELISA kit (EZRMI-13K; Millipore; Billerica, MA, USA). Serum leptin was measured using a rat-specific ELISA kit (EZRL-83K; Millipore; Billerica, MA, USA). Total serum cholesterol was determined using a cholesterol reagent set (C7510; Pointe Scientific; Ann Arbor, MI, USA). All kits were used according to the manufacturers instructions and spectrophotometric measurements were made on a Flex Station 3 (Molecular Devices; Sunnyvale, CA, USA).

Quantitative Reverse Transcription Polymerase Chain Reaction

Total RNA from the dorsal and ventral hippocampus was isolated using E.Z.N.A. Microelute Total RNA Kit (Omega Bio-Tek; Norcross, GA, USA) and quantified using a Nanodrop spectrophotometer (ND-1000; Thermo Scientific; Wilmington, DE, USA). 100 ng of RNA went into each reverse transcription reaction using the High Capacity cDNA Reverse Transcription Kit (436814; Life Technologies; Grand Island, NY, USA) and a Thermocycler (Professional Thermocycler, Biometra; Goettingen, Germany) to synthesize cDNA. Using cDNA produced from a 5 ng equivalent per sample, expression levels of transcripts for *brain-derived neurotrophic factor (BDNF), fat mass and obesity-associated protein (FTO), galanin (GAL), galanin receptor 1 (GALR1), galanin receptor 2 (GALR2), histone acetyltransferase 1 (HAT1), histone deacetylase 2 (HDAC2), histone deacetylase 4 (HDAC4), insulin receptor (INSR), the long form of the leptin receptor (OBRB), orexin receptor (ORXR), ras homolog enriched in brain 1 (RHEB1), suppressor of cytokine signaling 3 (SOCS3), and synapsin 1 (SYN1)* were determined by qRT-PCR. Primers were designed using the NCBI online database (http://www.ncbi.nlm.nih.gov) and sequence specificity of each primer pair (**Table 3.2**) was confirmed using Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/index). Efficiency of primers for a single target sequence was determined by examining dissociation curves for each primer set and choosing the set that best amplified only our region of interest. In each RNA sample the level of *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) transcripts was used as an endogenous control. Quantitative reverse transcription PCR was performed with SYBR green reaction mix (4309155; Invitrogen; Carlsbad, CA, USA) using a 7500 system from Applied Biosystems to determine cycle threshold (C_T) values. For analysis of C_T values, each sample was run in triplicate and those triplicates were averaged to

assign C_T values for each sample and each gene. Relative quantity was determined using the ddCt method [28].

Immunohistochemistry

Ten μm-thick coronal brain sections taken on a cryostat (CM3050; Leica; Buffalo Grove, IL, USA) were used for immunofluorescence analysis to examine galanin (1:200 dilution, T-4334; Peninsula Laboratories T-4334; San Carlos, CA, USA) immunoreactivity in the hippocampus. Tissues were washed with PBST (PBS + 0.1% Triton X100) prior to antigen retrieval with 10mM sodium citrate (pH 6.0). Tissues were blocked in 3% PBST (PBS + 3% BSA + 0.4% Triton X100) for one hour before being incubated with the primary antibody overnight in a humidified chamber at 4°C. The next day tissues were washed with PBST then incubated with the secondary antibody (1:500, Alexa 488; Abcam; Cambridge, MA, USA) and DAPI (1 mg/ml diluted 1:500; Thermo Scientific; Waltham, MA, USA). Slides were washed again and coverslips were mounted with glycerol (G7893, 70% in water; Sigma-Aldrich; St. Louis, MO, USA). Images were captured on an Olympus IX81 Motorized Inverted Fluorescent Microscope (Center Valley, PA, USA) and quantified by calculating corrected total cell fluorescence (CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings)) using Image J software (NIH; Bethesda, MD, USA).

Statistical Analysis

The data are presented as the means ± standard error of the mean (SEM) for all measurements. A t-test (independent, by groups) was used to compare values between the Control and the HFD groups using Statistica software 7.1 (StatSoft; Tulsa, OK, USA). For food intake, energy intake, and weight gain over three days, a two-way

ANOVA was used to examine the effects of day and treatment, with variance between groups assessed by Levene's test for quality of variances and post-hoc Tukey's HSD test using SPSS Statistics 20 (IBM; New York City, NY, USA). A value of $p<0.05$ was is denoted with * while a value of $p<0.01$ is denoted with **.

Table 3.1. Diet composition (Research Diets)

Description of diets provided to rats for the duration of the study (n=10/diet).

Diet	**Control**	**High Fat Diet**
Catalog Number	D12450B	D12451
Form	Pelleted	Pelleted
Macronutrients (kcal%)		
Total Fat	10	45
Soybean oil	5.5	5.5
Lard	4.4	39.4
Protein	20	20
Carbohydrate	70	35
Cholesterol	167.8 mg/kcal	54.4 mg/kcal
Total kcal/gm	3.85	4.73
Fat and Carbohydrate Content (kcal%)		
Soybean Oil	5.55	5.55
Lard	4.44	39.3
Corn Starch	31.1	7.17
Maltodextrin 10	3.45	9.86
Sucrose	34.5	17.0

Table 3.2. Gene Primers

Sequences for all primers used in qRT-PCR reactions.

Gene Symbol	**Gene Name**	**Forward and Reverse**
GAPDH	*Glyceraldehyde 3-phosphate dehydrogenase*	GGGAAACCCATCACCATCTT
		CCAGTAGACTCCACGACATACT
BDNF	*Brain-derived neurotrophic factor*	GAGACAAGAACACAGGAGGAAA
		CCCAAGAGGTAAAGTGTAGAAGG
FTO	*Fat mass and obesity-associated protein*	CTGTGGAAGAAGATGGAGAGTG
		CAGGACGGCAGACAGAATTT

GAL	*Galanin*	CCATTGACAACCACAGATCATTTA
		CAACACTTCCTAGTCTCCCTTC
GALR1	*Galanin receptor 1*	GTTCCCATAGGTGTACAGAGTTC
		GGTGTCTTAGTCCACAGGATTAC
GALR2	*Galanin receptor 2*	GGACCAAAGGGCATCTAACA
		CCTACAATCCTCGGTCTTTAGC
HAT1	*Histone acetyltransferase 1*	TGTTTCTCCCGGGAAAGATTAC
		CCCGTCTAGCATGTTGCTTAT
HDAC2	*Histone deacetylase 2*	CTGTCAAAGGTCACGCTAAATG
		GTCCAACATCGAGCAACATTC
HDAC4	*Histone deacetylase 4*	AGCTGCAGGAGTTTGTTCTC
		CTGTGCTGTGTCTTCCCATAC
INSR	*Insulin receptor*	CCCTGTGACCCATGAAATCTT
		CGCCGATAGCTCACTTCATATAG
OBRB	*Leptin receptor, long form*	GGTTGGATGGACTAGGGTATTG
		CAGAATTCAGGCCCTCTCATAG
ORXR	*Orexin receptor*	CTCCTCATCGTGACACTGAAAG
		GAGGAAGAGAAACTCCCACAAG
RHEB1	*Ras homolog enriched in brain 1*	GAGCCCACCACCTCAATAAT
		GGGAAAGTGCAGATACCGATTA
SOCS3	*Suppressor of cytokine signaling 3*	ACCTTTCTTATCCGCGACAG
		CACTGGATGCGTAGGTTCTT
SYN1	*Synapsin 1*	GGACGGAAGGGATCACATTATT
		ACCACAAGTTCCACGATGAG

Table 3.3. Mean body parameters. After 72 hours of control (n=10) or high fat diet (n=10), animals were weighed and fasted for two hours before sacrifice. Inguinal, epididymal, retroperitoneal, omental, and pericardial white adipose depots; subscapular brown adipose; and livers were dissected and weighed. Statistics were performed using t-test.

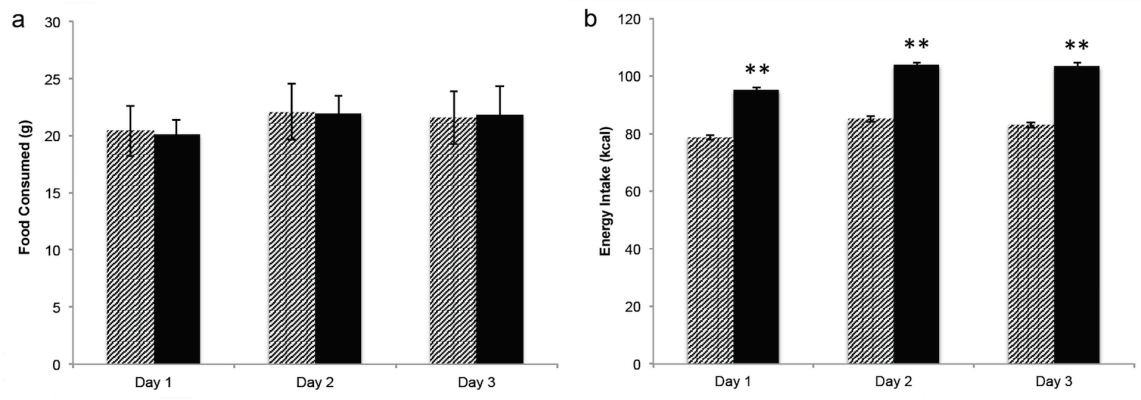
Measurement	**Control Diet**	**High Fat Diet**	**p value**
Body weights			
Body weight, initial (g)	299.4 ± 0.46	298.7 ± 0.66	0.802
Body weight, final (g)	318.4 ± 0.445	320.1 ± 0.772	0.528
72-Hour Weight gain (g)	19.05 ± 0.36	21.28 ± 0.30	0.093
Final Tissue Weights			
Inguinal adipose tissue (g)	6.32 ± 0.070	6.71 ± 0.101	0.326
Epididymal adipose tissue (g)	3.61 ± 0.049	3.89 ± 0.072	0.335
Retroperitoneal adipose tissue (g)	3.31 ± 0.029	3.53 ± 0.081	0.444

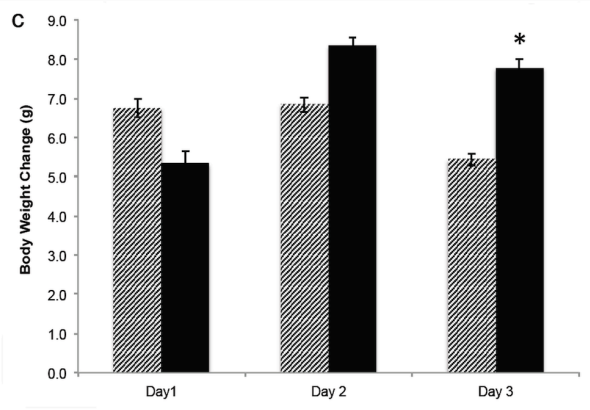
Omental adipose tissue (g)	0.183 ± 0.011	0.155 ± 0.004	0.460
Pericardial adipose tissue (g)	0.586 ± 0.009	0.534 ± 0.015	0.380
Total white adipose tissue (g)	14.2 ± 0.152	14.8 ± 0.178	0.376
Subscapular brown adipose tissue (g)	0.43 ± 0.006	0.43 ± 0.008	0.994
Liver weight (g)	14.4 ± 0.131	13.3 ± 0.080	0.050

Table 3.4. Blood and serum measures at endpoint. After 72 hours of control (n=10) or high fat diet (n=10), animals were fasted for two hours before sacrifice. At the time of sacrifice, trunk blood was used to measure blood glucose. Blood was allowed to clot for collection of serum. Serum insulin and leptin were measured via ELISA and total serum cholesterol was measured chemically. Statistics were performed using t-test. A p-value of $p < .05$ is denoted by * and a p-value of $p < 0.01$ is denoted by **.

Measurement	**Control Diet**	**High Fat Diet**	**p value**
Blood glucose (mg/dl)	123.0 ± 1.12	122.7 ± 1.15	0.952
Serum insulin (ng/ml)	2.40 ± 0.07	2.16 ± 0.05	0.365
Serum leptin (ng/ml)	1.55 ± 0.00	1.57 ± 0.00	0.005**
Serum cholesterol (mg/dl)	112.4 ± 0.96	128.0 ± 1.78	0.026*

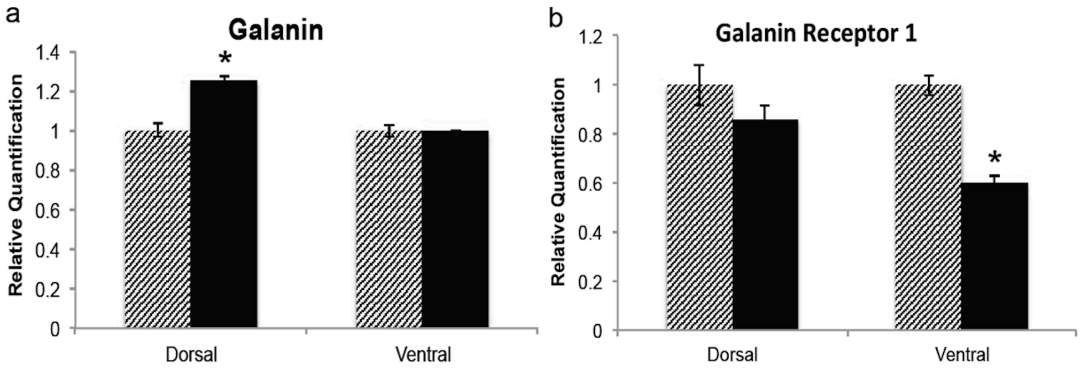
Figure 3.1. Energy intake, body and tissue weights

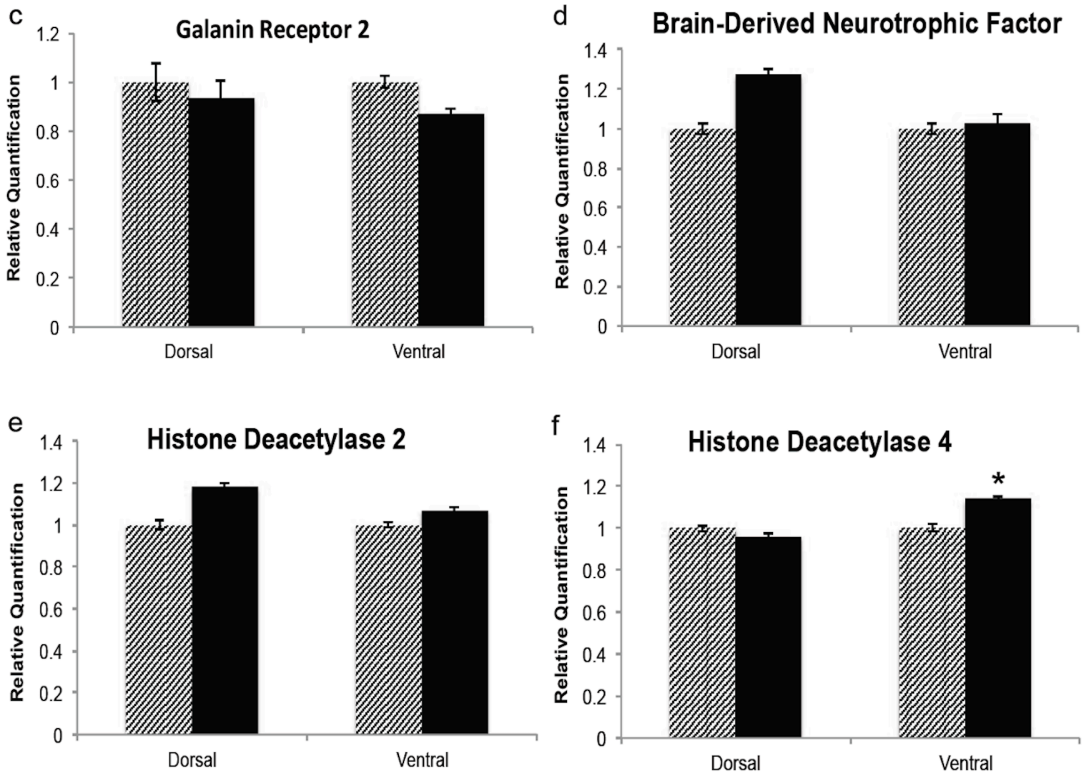




Eight to 10 week-old male Long-Evans rats were fed diets either low in fat (light bars, n=10) or high in fat (dark bars, n=10) for 72 hours. Food intake and body weight were measured daily following 24 hours (1 day), 48 hours (2 days), and 72 hours (3 days) on the diet. Statistics were performed using two-way ANOVA with post-hoc Tukey's HSD and Levene's test for equality of variances. A p-value of $p < .05$ is denoted by * and a p-value of $p < 0.01$ is denoted by **.

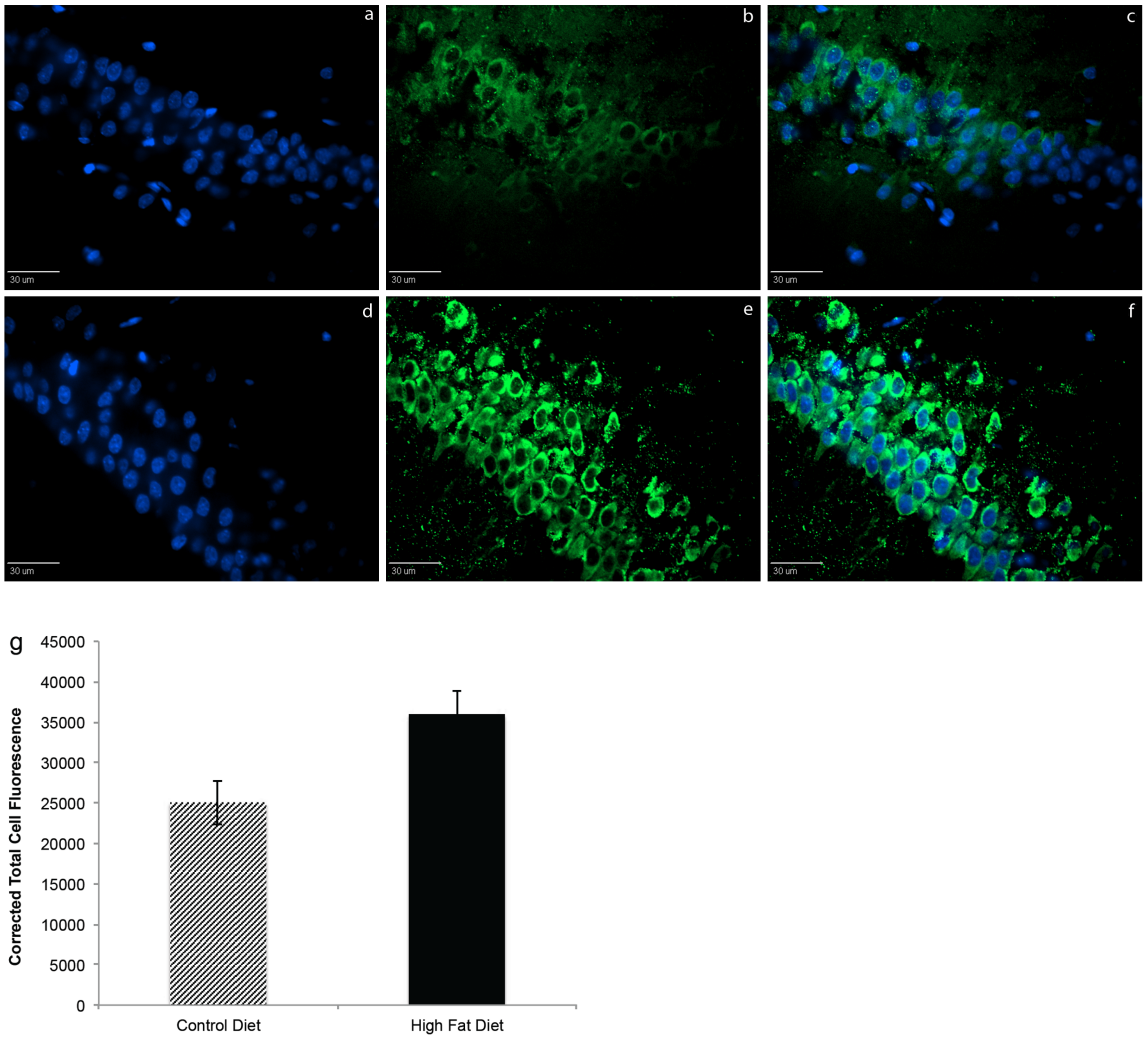
Figure 3.2. Gene Expression





Dorsal and ventral hippocampal qRT-PCR results for *galanin* (a), *galanin receptor 1* (b), *galanin receptor 2* (c), *brain-derived neurotrophic factor* (d), *histone deacetylase 2* (e), and *histone deacetylase 4* (f). GAPDH was used as an endogenous control. Light bars indicate control diet (n=10) and dark bars indicate high fat diet (n=10). Statistics were performed using t-test. A p-value of $p < .05$ is denoted by * and a p-value of $p < 0.01$ is denoted by **.

Figure 3.3. Galanin immunohistochemistry



Galanin immunostaining of dorsal hippocampal CA1/CA2 regions in one control (a-c) and one high fat-fed (d-f) rat after 72 hours. Scale bars set to 30 μm. 3a) and 3d) show DAPI staining in blue, 3b) and 3e) show galanin staining in green, and in 3c) and 3f) the images are merged. Quantification of GFP signal for high fat fed (n=3) and control fed (n=4) rats using Image J software (g). Light bars indicate control diet and dark bars indicate high fat diet. Statistics were performed using t-test.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM: **Prevalence of childhood and adult obesity in the United States, 2011-2012**. *JAMA : the journal of the American Medical Association* 2014, **311**(8):806-814.

2. Bray GA, Popkin BM: **Dietary fat intake does affect obesity!** *Am J Clin Nutr* 1998, **68**:1157-1173.

3. Pasinetti GM, Eberstein JA: **Metabolic syndrome and the role of dietary lifestyles in Alzheimer's disease**. *Journal of neurochemistry* 2008, **106**(4):1503-1514.

4. Grant WB, Campbell A, Itzhaki RF, Savory J: **The significance of environmental factors in the etiology of Alzheimer's disease**. *Journal of Alzheimer's disease : JAD* 2002, **4**:179-189.

5. Squire LR: **Memory systems of the brain: a brief history and current perspective**. *Neurobiology of learning and memory* 2004, **82**(3):171-177.

6. Berthoud H: **Multiple neural systems controlling food intake & body weight**. *Neurosci Biobehav R* 2002, **26**:36.

7. Fanselow MS, Dong HW: **Are the dorsal and ventral hippocampus functionally distinct structures?** *Neuron* 2010, **65**(1):7-19.

8. Lathe R: **Hormones and the Hippocampus**. *J Endocrinol* 2001, **169**:27.

9. Freeman LR, Granholm AC: **Vascular changes in rat hippocampus following a high saturated fat and cholesterol diet**. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism* 2012, **32**(4):643-653.

10. Kanoski SE, Zhang Y, Zheng W, Davidson TL: **The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat**. *Journal of Alzheimer's disease : JAD* 2010, **21**(1):207-219.

11. Kanoski SE, Davidson TL: **Different patterns of memory impairments accompany short- and longer-term maintenance on a high-energy diet**. *Journal of experimental psychology Animal behavior processes* 2010, **36**(2):313-319.

12. Beilharz JE, Maniam J, Morris MJ: **Short exposure to a diet rich in both fat and sugar or sugar alone impairs place, but not object recognition memory in rats**. *Brain, behavior, and immunity* 2014, **37**:134-141.

13. Winocur G, Greenwood CE: **Studies of the effects of high fat diets on cognitive function in a rat model**. *Neurobiology of aging* 2005, **26 Suppl 1**:46-49.

14. Boitard C, Cavaroc A, Sauvant J, Aubert A, Castanon N, Laye S, Ferreira G: **Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats**. *Brain, behavior, and immunity* 2014, **40**:9-17.

15. Sobesky JL, Barrientos RM, De May HS, Thompson BM, Weber MD, Watkins LR, Maier SF: **High-fat diet consumption disrupts memory and primes elevations in hippocampal IL-1beta, an effect that can be prevented with dietary reversal or IL-1 receptor antagonism**. *Brain, behavior, and immunity* 2014.

16. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR *et al*: **Obesity is associated with hypothalamic injury in rodents and humans**. *The Journal of clinical investigation* 2012, **122**(1):153-162.

17. Guyenet SJ, Nguyen HT, Hwang BH, Schwartz MW, Baskin DG, Thaler JP: **High-fat diet feeding causes rapid, non-apoptotic cleavage of caspase-3 in astrocytes**. *Brain research* 2013, **1512**:97-105.

18. Lee YS, Li P, Huh JY, Hwang IJ, Lu M, Kim JI, Ham M, Talukdar S, Chen A, Lu W *et al*: **Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance**. *Diabetes* 2011, **60**:2474-2483.

19. Miller CN, Morton HP, Cooney PT, Winters TG, Ramseur KR, Rayalam S, Della-Fera MA, Baile CA, Brown LM: **Acute exposure to high-fat diets increases hepatic expression of genes related to cell repair and remodeling in female rats**. *Nutrition research* 2014, **34**(1):85-93.

20. Zovkic IB, Guzman-Karlsson MC, Sweatt JD: **Epigenetic regulation of memory formation and maintenance**. *Learning & memory* 2013, **20**(2):61-74.

21. Funato H, Oda S, Yokofujita J, Igarashi H, Kuroda M: **Fasting and high-fat diet alter histone deacetylase expression in the medial hypothalamus**. *PloS one* 2011, **6**(4):e18950.

22. Elsner VR, Lovatel GA, Bertoldi K, Vanzella C, Santos FM, Spindler C, de Almeida EF, Nardin P, Siqueira IR: **Effect of different exercise protocols on histone acetyltransferases and histone deacetylases activities in rat hippocampus**. *Neuroscience* 2011, **192**:580-587.

23. Bedecs K, Berthoud M, Bartfai T: **Galanin- 10 years with a neuroendocrine peptide**. *Int J Biochem Cell Biol* 1995, **27**(337-349).

24. Mitsukawa K, Lu X, Bartfai T: **Galanin, galanin receptors and drug targets**. *Cellular and molecular life sciences : CMLS* 2008, **65**(12):1796-1805.

25. Kinney JW, Sanchez-Alavez M, Barr AM, Criado JR, Crawley JN, Behrens MM, Henriksen SJ, Bartfai T: **Impairment of memory consolidation by galanin correlates with in vivo inhibition of both LTP and CREB phosphorylation**. *Neurobiology of learning and memory* 2009, **92**(3):429-438.

26. Murray PS, Holmes PV: **An overview of brain-derived neurotrophic factor and implications for excitotoxic vulnerability in the hippocampus**. *International journal of peptides* 2011, **2011**:654085.

27. Beck B, Pourie G: **Ghrelin, neuropeptide Y, and other feeding-regulatory peptides active in the hippocampus: role in learning and memory**. *Nutrition reviews* 2013, **71**(8):541-561.

28. Livak KJ, Schmittgen TD: **Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method**. *Methods* 2001, **25**(4):402-408.

29. Eskelinen MH, Ngandu T, Helkala EL, Tuomilehto J, Nissinen A, Soininen H, Kivipelto M: **Fat intake at midlife and cognitive impairment later in life: a population-based CAIDE study**. *International journal of geriatric psychiatry* 2008, **23**(7):741-747.

30. Sparks DL, Kuo YM, Roher A, Martin T, Lukas RJ: **Alterations of Alzheimer's Disease in the Cholesterol-fed Rabbit, Including Vascular Inflammation**. *Ann N Y Acad Sci* 2000, **903**:335-344.

31. Ghribi O, Golovko MY, Larsen B, Schrag M, Murphy EJ: **Deposition of iron and beta-amyloid plaques is associated with cortical cellular damage in rabbits fed**

with long-term cholesterol-enriched diets. *Journal of neurochemistry* 2006, **99**(2):438-449.

32. Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJ, Zhou Y, Wang X, Mazitschek R *et al*: **HDAC2 negatively regulates memory formation and synaptic plasticity**. *Nature* 2009, **459**(7243):55-60.

33. Graff J, Rei D, Guan JS, Wang WY, Seo J, Hennig KM, Nieland TJ, Fass DM, Kao PF, Kahn M *et al*: **An epigenetic blockade of cognitive functions in the neurodegenerating brain**. *Nature* 2012, **483**(7388):222-226.

34. Mielcarek M, Zielonka D, Carnemolla A, Marcinkowski JT, Guidez F: **HDAC4 as a potential therapeutic target in neurodegenerative diseases- a summary of recent achievements**. *Front Cell Neurosci* 2015, **9**:00042.

35. Wang B, Moya N, Niessen S, Hoover H, Mihaylova MM, Shaw RJ, Yates JR, Fischer WH, Thomas JB, Montminy M: **A hormone-dependent module regulating energy balance**. *Cell* 2011, **145**:596-606.

36. Bray GA, Lovejoy JC, Smith SR, DeLany JP, Lefevre M, Hwang D, Ryan DH, York DA: **The influence of differents fats and fatty acids on obesity, insulin resistance and inflammation**. *J Nutr* 2002, **132**:2488-2491.

37. Hobson SA, Bacon A, Elliot-Hunt CR, Holmes FE, Kerr NC, Pope R, Vanderplank P, Wynick D: **Galanin acts as a trophic factor to the central and peripheral nervous systems**. *Cellular and molecular life sciences : CMLS* 2008, **65**(12):1806-1812.

38. Lang R, Kofler B: **The galanin peptide family in inflammation**. *Neuropeptides* 2011, **45**(1):1-8.

39. Sciolino NR, Dishman RK, Holmes PV: **Voluntary exercise offers anxiolytic potential and amplifies galanin gene expression in the locus coeruleus of the rat**. *Behavioural brain research* 2012, **233**(1):191-200.

40. Sciolino NR, Holmes PV: **Exercise offers anxiolytic potential: a role for stress and brain noradrenergic-galaninergic mechanisms**. *Neurosci Biobehav Rev* 2012, **36**(9):1965-1984.

41. Ogren SO, Kuteeva E, Elvander-Tottie E, Hokfelt T: **Neuropeptides in learning and memory processes with focus on galanin**. *European journal of pharmacology* 2010, **626**(1):9-17.

42. Schott PA, Hokfelt T, Ogren SO: **Galanin and spatial learning in the rat. Evidence for a differential role for galanin in subregions of the hippocampal formation**. *Neuropharmacology* 2000, **39**:1387-1403.

43. Crawley JN: **Galanin impairs cognitive abilities in rodents: relevance to Alzheimer's disease**. *Cellular and molecular life sciences : CMLS* 2008, **65**(12):1836-1841.

44. McDonald MP, Crawley JN: **Galanin-acetylcholine interactions in rodent memory tasks and Alzheimer's disease**. *J Psychiatry Neurosci* 1997, **22**(5):303-317.

45. Agoston DV, Komoly S, Palkovits M: **Selective up-regulation of neuropeptide synthesis by blocking the neuronal activity: galanin expression in septohippocampal neurons**. *Exp Neurol* 1994, **126**(2):247-255.

46. Gustafson EL, Smith KE, Durkin MM, Gerald C, Branchek TA: **Distribution of rat galanin receptor mRNA in rat brain**. *Neuroreport* 1996, **22**(7):953-957.

47. Moreno E, Vaz SH, Cai NS, Ferrada C, Quiroz C, Barodia SK, Kabbani N, Canela EI, McCormick PJ, Lluis C *et al*: **Dopamine-galanin receptor heteromers modulate cholinergic neurotransmission in the rat ventral hippocampus**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2011, **31**(20):7412-7423.

48. O'Donnell D, Ahmad S, Wahlestedt C, Walker P: **Expression of the novel galanin receptor subtype GALR2 in the adult rat CNS- distinct distribution from GALR1**. *The Journal of comparative neurology* 1999, **409**:469-481.

49. Yoshitake T, Yoshitake S, Savage S, Elvander-Tottie E, Ogren SO, Kehr J: **Galanin differentially regulates acetylcholine release in ventral and dorsal hippocampus: a microdialysis study in awake rat**. *Neuroscience* 2011, **197**:172-180.

50. Huang EJ, Reichardt LF: **Neurotrophins: roles in neuronal development and function**. *Annu Rev Neurosci* 2001, **24**:677-736.

51. Wu A, Molteni R, Ying Z, Gomez-Pinilla F: **A saturated-fat diet aggravates the outcome of traumatic brain injury on hippocampal plasticity and cognitive function by reducing brain-derived neurotrophic factor**. *Neuroscience* 2003, **119**(2):365-375.

52. Kanoski SE, Meisel RL, Mullins AJ, Davidson TL: **The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat**. *Behavioural brain research* 2007, **182**(1):57-66.

53. Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, Mattson MP: **Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats**. *Hippocampus* 2008, **18**(11):1085-1088.

54. Hawes JJ, Picciotto MR: **Characterization of GalR1, GalR2, and GalR3 immunoreactivity in catecholaminergic nuclei of the mouse brain**. *The Journal of comparative neurology* 2004, **479**(4):410-423.

55. Waters SM, Krause JE: **Distribution of galanin-1, -2, and -3 receptor messenger RNAs in central and peripheral rat tissues**. *Neuroscience* 2000, **95**(1):265-271.

56. Wang S, He C, Hashemi T, Bayne M: **Cloning and Expressional Characterization of a Novel Galanin Receptor: IDENTIFICATION OF DIFFERENT PHARMACOPHORES WITHIN GALANIN FOR THE THREE GALANIN RECEPTOR SUBTYPES**. *The Journal of biological chemistry* 1997, **272**(51):31949-31952.

57. Egecioglu E, Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Jerlhag E, Engel JA, Dickson SL: **Hedonic and incentive signals for body weight control**. *Reviews in endocrine & metabolic disorders* 2011, **12**(3):141-151.

58. Volkow ND, Wang GJ, Baler RD: **Reward, dopamine and the control of food intake: implications for obesity**. *Trends in cognitive sciences* 2011, **15**(1):37-46.

59. Valdivia S, Patrone A, Reynaldo M, Perello M: **Acute high fat diet consumption activates the mesolimbic circuit and requires orexin signaling in a mouse model**. *PloS one* 2014, **9**(1):e87478.

60. Nyberg L: **Any novelty in hippocampal formation and memory?** *Curr Opin Neurol* 2005, **18**:424-428.

61. Davidson TL, Altizer AM, Benoit SC, Walls EK, Powley TL: **Encoding and selective activation of "metabolic memories" in the rat**. *Behav Neurosci* 1997, **111**(1):1014-1030.

62. Folch J, Pedros I, Patraca I, Sureda F, Junyent F, Beas-Zarate C, Verdaguer E, Pallas M, Auladell C, Camins A: **Neuroprotective and anti-ageing role of leptin**. *Journal of molecular endocrinology* 2012, **49**(3):R149-156.

63. Garza JC, Guo M, Zhang W, Lu XY: **Leptin increases adult hippocampal neurogenesis in vivo and in vitro**. *The Journal of biological chemistry* 2008, **283**(26):18238-18247.

64. Cheung CC, Hohmann JG, Clifton DK, Steiner RA: **Distribution of galanin messenger RNA-expressing cells in murine brain and their regulation by leptin in regions of the hypothalamus**. *Neuroscience* 2001, **103**(2):423-432.

65. Laque A, Zhang Y, Gettys S, Nguyen TA, Bui K, Morrison CD, Munzberg H: **Leptin receptor neurons in the mouse hypothalamus are colocalized with the neuropeptide galanin and mediate anorexigenic leptin action**. *American journal of physiology Endocrinology and metabolism* 2013, **304**(9):E999-1011.

66. Liao GY, An JJ, Gharami K, Waterhouse EG, Vanevski F, Jones KR, Xu B: **Dendritically targeted Bdnf mRNA is essential for energy balance and response to leptin**. *Nature medicine* 2012, **18**(4):564-571.

67. Ren L, Chan SMH, Zeng X, Laybutt DR, Iseli TJ, Sun R, Kraegen EW, Cooney GJ, Turner N, Ye J: **Differing endoplasmic reticulul stress response to excess lipogenesis versus lipid oversupply in relation to hepatic steatosis and insulin resistance**. *PloS one* 2012, **7**(2):e30816.

68. Eaton RP, Kipnis DM: **Effects of high-carbohydrate diets on lipid and carbohydrate metabolism in the rat**. *Am J Physiol* 1969, **217**(4):1160-1168.

CHAPTER 4

CONDITIONED TASTE AVERSION IN RATS FED A HIGH FAT DIET: EFFECT OF SODIUM BUTYRATE

Trunnell E. R., Freeman, K., Meagher R. B., and G. L. Edwards. To be submitted to *Physiology and Behavior*.

Abstract

Conditioned taste aversion (CTA) is a robust learning paradigm relying on the innate necessity of animals to determine food safety. While obesogenic diets have been shown to reduce performance in other learning and memory tasks, their effect in CTA is largely unknown. In addition there is evidence that both CTA and diet-induced cognitive deficits may be modulated by chromatin modifications such as histone acetylation. We fed male Sprague-Dawley rats a chow or 45% high fat diet (HFD) and simultaneously trained them to consume a sweetened condensed milk (SCM) solution for 30 minutes each day. On the 5th day, all animals were offered flavored SCM, after which half were given an injection of lithium chloride to induce a taste aversion to the novel flavor. Additionally a subgroup of animals was administered sodium butyrate (NaB) prior to the novel taste exposure. Retrieval of CTA was tested 48 hours later and extinction was carried out over three additional days, during which NaB was also administered to select groups. There was no effect of peripheral NaB on CTA acquisition or extinction. With the caveat that all HFD-fed rats consumed less SCM than chow-fed rats, extinction of CTA was diminished in the HFD-fed groups. Additionally, HFD reduced transcript levels of *BDNF* in the ventral hippocampus. These findings provide evidence that acute HFD consumption reduces intake of other palatable food sources, lowers expression of an important neurotrophic factor, and may impact behavior.

4.1 Introduction

Taste aversion learning is a practical and evolutionarily advantageous trait (Rozin and Kalat, 1971). Recognition and avoidance of a food that has been associated with

previous illness can prevent future illness and possible death. Taste aversion learning is like other forms of learning in that it requires a series of molecular events modulating gene activity and leading to the formation of the memory and to its maintenance. A primary mechanism for regulating gene expression involves histone modifications such as histone acetylation, methylation, phosphorylation, ubiquitinylation, and ADP-ribosylation (Lipsky, 2013). In the case of histone acetylation, when acetyl groups are attached to lysine resides on the tails of DNA-wrapped histones, the DNA becomes more accessible to transcriptional machinery in the region of acetylated histones, typically promoting gene expression (Lipsky, 2013). Histone lysine acetylation levels are regulated by a balance between the activities of histone lysine acetyltransferases (KATs) and histone deacetylases (HDACs), enzymes that add or remove these acetyl groups promoting a more open or closed chromatin formation, respectively (Swank and Sweatt, 2001, Lipsky, 2013). Increased histone lysine acetylation has repeatedly shown to enhance memory formation and reconsolidation in a variety of learning and memory tasks (Levenson et al., 2004, Graff et al., 2012, Jarome and Lubin, 2014) through enhancement of long-term potentiation (LTP) and synaptic plasticity (Vecsey et al., 2007, Guan et al., 2009). A 2001 study by Swank and Sweatt showed that a single 10-minute exposure to a novel taste promoted histone lysine acetylation in the insular cortex of mice (Swank and Sweatt, 2001), indicating that histone acetylation plays a positive role in the formation of taste memories.

Conditioned Taste Aversion (CTA) is a behavioral paradigm where an animal is exposed to a novel taste (the conditioned stimulus – CS) which is paired with malaise (the unconditioned stimulus – US), typically induced by a pharmacological agent such as

lithium chloride (LiCl). The pairing of the novel taste with malaise results in a conditioned response (CR) involving subsequent avoidance of the novel taste (Gal-Ben-Ari and Rosenblum, 2011). Taste aversion learning can occur in a single trial and with substantially long CS-US delay periods (Rozin and Kalat, 1971). Under normal circumstances, the CR can then be extinguished by repeated presentation of the CS without the US. CTA is used as a robust and reliable method by which to study learning, memory, reconsolidation, and extinction, as well as to to help elucidate how taste is represented and processed in the brain. Taste information enters the CNS through cranial nerves VII, IX, and X, projecting to the nucleus of the solitary tract (NTS) in the brainstem (Gal-Ben-Ari and Rosenblum, 2011). In rodents, taste information is relayed through the parabrachial nucleus to the parvocellular portion of the ventroposteromedial nucleus of the thalamus and to the amygdala, hippocampus, lateral hypothalamus, nucleus accumbens, medial prefrontal cortex, and the agranular insular cortex (IC), also known as the gustatory cortex; many of these connections are reciprocal (Nunez-Jaramillo et al., 2010, Gal-Ben-Ari and Rosenblum, 2011, Maffei et al., 2012). A major signaling cascade by which CTA is established begins with glutamatergic stimulation of the N-methyl-D-aspartate receptor (NMDA) receptor N2RB and involves activation of the ERK/MAPK pathway and the cAMP response element-binding protein (CREB), leading to increases in gene transcription (Nunez-Jaramillo et al., 2010, Gal-Ben-Ari and Rosenblum, 2011). For a detailed review of signaling pathways implicated in CTA, see the 2012 review by Gal-Ben-Ari and Rosenblum. Histone acetylation in the hippocampus associated with memory formation is also dependent on NMDA activation and the ERK/MAPK pathway (Levenson et al., 2004, Zhang et al., 2014), suggesting that

ERK/MAPK signaling enhances gene transcription not only by the recruitment of transcription factors, but by the facilitation of a permissive chromatin structure. The relationship between ERK/MAPK and histone acetylation has also been shown for other brain regions (Niles et al., 2013, Zhao et al., 2013), tissues (Gao et al., 2015), and species (Danilova and Grinkevich, 2012).

ERK/MAPK signaling results in subsequent transcription of immediate early genes (IEGs) such as *c-fos*, *activity-regulated cytoskeleton-associated protein* (*Arc*), and *brain-derived neurotrophic factor* (*BDNF*) in the above-mentioned brain regions during CTA (Nunez-Jaramillo et al., 2010, Gal-Ben-Ari and Rosenblum, 2011, Uematsu et al., 2015). BDNF is a neurotrophic factor well known for its role in regulating synaptic plasticity in a variety of learning and memory paradigms, including CTA (Ma et al., 2011). BDNF expression is rapidly induced in the IC and central amygdala (CeA) during CTA learning (Ma et al., 2011) and in the IC during reconsolidation (Wang et al., 2012). Infusion of BDNF into the IC or CeA enhances both CTA learning (Moguel-Gonzalez et al., 2008, Martinez-Moreno et al., 2011, 2016) and extinction (Rodriguez-Serrano et al., 2014). BDNF is known to be regulated by a variety of chromatin modifications, including histone acetylation (Karpova, 2014). Induction of LTP via NMDA activation or high frequency stimulation promotes hyperacetylation of the *BDNF* promoter and increases in *BDNF* transcription (Tian et al., 2010, Sui et al., 2012). To date, the existence of an epigenetic mechanism of BDNF regulation during CTA is unknown. There are several pharmacological tools useful for enhancing histone lysine acetylation via the inhibition of HDACs, such as the widely used HDAC inhibitors sodium butyrate, trichostatin A, valproic acid, phenylbutryic acid (PBA), and

suberolyanilide hydroxamic acid (SAHA). Intlekofer and colleagues (Intlekofer et al., 2013) showed that the cognitive enhancing effects of the HDAC inhibitor NaB are dependent on BDNF upregulation. In the following experiment, we examined the ability of NaB to enhance CTA acquisition and extinction in two groups of rats, as well as how NaB treatment during CTA affects BDNF expression in the insular cortex, central amygdala, and dorsal and ventral hippocampus.

Also of interest to our research is the interplay of diet with the epigenetics of learning and memory. High fat diet (HFD) consumption in rodents has been shown to reduce performance on a variety learning and memory tasks (Kanoski and Davidson, 2011), but has not been studied extensively in CTA. One 1999 study by Singer and colleagues examined the ability of two inhibitors of fatty acid oxidation, mercaptoacetate and methyl palmoxirate to produce a CTA to saccharin in low- or high-fat fed rats. Both inhibitors produced a robust CTA, but no effect of diet was observed in this paradigm (Singer et al., 1999). Conversely, obese ob/ob mice show reduced extinction of CTA compared to lean mice (Thompson et al., 1993), and when obesity is induced by lesioning the ventromedial hypothalamus (VMH), rats display a slower rate of CTA extinction only when they reach a moderately obese state, and not before (Kramer et al., 1983). Since HFD and obesity have been shown to reduce BDNF (Molteni et al., 2004, Kanoski et al., 2007), a critical player in CTA acquisition and extinction, and because dietary fatty acids themselves may modulate the activity and expression of histone deacetylase (Das and Vaddadi, 2004, Gan et al., 2015), we asked whether or not HFD consumption during the CTA paradigm would affect the animals' performance on this task, what effect HFD

would have on *BDNF* in this context, and how NaB inhibition of acetylation would interact with these parameters.

4.2 Materials and Methods

Animals, housing, and diet

Sixty-four male Sprague-Dawley rats (Envigo; Indianapolis, IN, USA), approximately 280 g initial weight, were used in this study. Rats were housed individually upon arrival in a temperature- and humidity-controlled environment with a 12-h light/12-h dark cycle. Rats were allowed unlimited access to water and standard rat chow pellets (Purina PicoLab® Rodent Diet 20 5053, LabDiet; St. Louis, MO, USA) during the one-week acclimatization period. Following acclimatization rats were assigned to one of eight study groups (Chow (**C**), HFD (**H**), Chow + NaB (**CN**), HFD + NaB (**HN**), Chow + LiCl (**CL**), HFD + LiCl (**HL**), Chow + LiCl + NaB (**CLN**), HFD + LiCl + NaB (**HLN**)) in a manner to average body weight across the groups (n=8 per group). Experiments were performed in four cohorts of 16 rats each (n/group/cohort=2). Rats from the first cohort allowed us to improve the experimental design and were not included in behavioral and molecular analyses. Rats were weighed daily between 0800 and 1000 hours. On Day 1 of the study, H, HN, HL, and HLN rats were provided with pelleted 45% HFD diet (DIO Formula D12451, Research Diets; New Brunswick, NJ, USA) *ad libitum* in round ceramic bowls. C, CN, CL, and CLN rats were given standard rat chow pellets *ad libitum,* also in round ceramic bowls. Food intake was measured daily between 0800 and 1000 hours. All institutional and national guidelines for the care and use of laboratory animals were followed. All protocols for this experiment were

approved by the University of Georgia Institutional Animal Care and Use Committee (AUP #A2015 10-040-Y1-A3) prior to the start of this experiment.

Drug preparation and delivery

For Cohort 1, NaB (Sigma-Aldrich; St. Louis, MO, USA), was dissolved in deionized water at a concentration of 1g/ml and filter sterilized using a Steriflip® Disposable Vacuum Filtration System (Millipore; Darmstadt, DEU). For Cohorts 2-4, NaB was dissolved in deionized water at a concentration of 0.03 g/ml and filter sterilized in the same manner. NaB was administered to CN, HN, CLN, HLN rats via intraperitoneal injections at a dose of 1.2 g/kg (Cohort 1) or 300 mg NaB/kg body weight (Cohorts 2-4) and a volume of 1.2 ml/kg (Cohort 1) or 10 ml/kg (Cohorts 2-4) on Days 5, 8, 9, 10, and 11 (during Acquisition, Retrieval, and Extinction), 15 minutes before milk exposure. 0.9% sterile saline was administered to C, H, CL, and HL rats in the same manner. Saline was administered during Training (all training days for Cohort 1 or only Training Days 3 and 4 for Cohorts 2-4) to all animals at an equivalent volume and time frame in order to acclimate the animals to the injection regimen. The dose and delivery volume of NaB for Cohorts 2-4 was carefully chosen so as not to induce a hypertonic response in the animals upon injection, as was observed for the dose and volume given to Cohort 1.

LiCl (Sigma-Aldrich; St. Louis, MO, USA) was dissolved in deionized water at a concentration of 127 mg/ml and filter sterilized using the Steriflip® system. LiCl was administered to CL, HL, CLN, and HLN rats via intraperitoneal injections at a dose of 127 mg LiCl/kg body weight and a volume of 1 ml/kg on Day 5, 15 minutes following

milk exposure. 0.9% sterile saline was administered to C, H, CN, and HN rats in the same manner.

Conditioned taste aversion

A 40% sweetened condensed milk (Eagle Brand, Borden; Gahanna, OH, USA) solution was prepared in filtered water. On Days 1-4 (Training/T), rats were given access to the sweetened condensed milk solution (SCM) in a 25-mL plastic drinking tube for 30 minutes daily. On Days 3 and 4, 15 minutes before and after the 30-minute SCM exposure, rats were given 10 ml/kg body weight and 1 ml/kg body weight intraperitoneal injections of 0.9% sterile saline, respectively (or on all training days for Cohort 1). On Day 5 (Acquisition/ACQ), the diluted SCM was flavored with imitation banana extract (bSCM) (McCormick & Company; Sparks, MD, USA), 1.5 mL imitation banana per 1 L diluted SCM. 15 minutes before a 30-minute bSCM exposure, CN, HN, CLN, and HLN rats were given an intraperitoneal injection of NaB. C, H, CL, and HL rats were given an equal volume of saline. 15 minutes following the bSCM exposure, CL, HL, CLN, and HLN rats were given an intraperitoneal injection of LiCl while C, H, CN, and HN rats were given an equal volume of saline. No milk was provided on Days 6 or 7 (Rest Days/O). On Days 8-11 (Retrieval/RT + Extinction/E), rats were given daily 30-minute bSCM exposure and injected with either saline (C, H, CL, HL) or NaB (CN, HN, CLN, HLN) 15 minutes prior. See **Figure 4.1** for a graphical representation of the experimental design. All milk exposure occurred between 1000 and 1200 hours of the light cycle so as to avoid milk consumption due to normal feeding cycles. Water was

removed for approximately 2 hours prior to milk exposure to promote drinking. Milk consumption was measured at the end of the 30-minute drinking period.

Quantitative Reverse Transcription Polymerase Chain Reaction

On Day 11, following milk exposure, rats were euthanized via inhaled CO_2 and decapitation. The brain was removed from the skull and flash frozen in liquid nitrogen. Brains were thickly sliced (400-500 μm) on a CM1950 cryostat (Leica; Wetzlar, DEU) and punches were collected bilaterally from the rostral agranular insular cortex, central amygdala, dorsal hippocampus, and ventral hippocampus. For Cohorts 2-4, total RNA from brain samples was isolated using the E.Z.N.A. Microelute Total RNA Kit (Omega Bio-Tek; Norcross, GA, USA) and quantified using a Nanodrop spectrophotometer (Thermo Scientific; Wilmington, DE, USA). 25 or 50 ng of RNA went into each reverse transcription reaction using QScript cDNA Supermix (Quantabio; Beverly, MA, USA) according to the manufacturers instructions to synthesize cDNA. Using cDNA produced from a 5 ng equivalent per sample, expression level of transcripts for *brain-derived neurotrophic factor* (*BDNF,* forward 5'-GAGACAAGAACACAGGAGGAAA-3' and reverse 5'-CCCAAGAGGTAAAGTGTAGAAGG-3') were determined by qRT-PCR. In each RNA sample the level of *β–actin* (forward 5'-CTGTGCTATGTTGCCCTAGAC-3' and reverse 5'-GCTCATTGCCGATAGTGATGA-3') transcript was used as an endogenous control. Primers were designed using the NCBI online database (http://www.ncbi.nlm.nih.gov) and sequence specificity of each primer pair was confirmed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index). Efficiency of primers for a single target sequence was determined by examining

dissociation curves for each primer set and choosing the set that best amplified only our region of interest. qRT-PCR was performed with SYBR green reaction mix (Invitrogen; Carlsbad, CA, USA) using a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, Invitrogen; Foster City, CA, USA) to determine cycle threshold (CT) values. For analysis of CT values, each sample was run in triplicate and those triplicates were averaged to assign CT values for each sample and gene. Relative quantity (RQ) was determined using the ddCT method (Livak and Schmittgen, 2001).

Statistical Analysis

All data are presented as the mean ± standard error of the mean (SEM) for all experiments. Analyses comparing the main effects of Diet, NaB treatment, and LiCl treatment, as well as the interactions of these main effects, were determined by Factorial ANOVA followed by Tukey's Honest Significant Difference (HSD) test. A MANOVA was performed to report any significant effects of body weight or milk consumption over the entire course of the experiment, or over a multi-day section of the experiment. A one-way ANOVA was used in instances where the main effect of diet prior to taste aversion induction was determined, also with Tukey's test. Statistica software (Dell; Round Rock, TX, USA) was used for analyses while Prism 7 (GraphPad Software, Inc; La Jolla, CA, USA) was used to create figures. Statistical significance is denoted in the following manner: * ($p<005$), ** ($p<0.01$), *** ($P<0.001$).

4.3 Results

NaB Dosing

For the first cohort of this study (n=2/group), we performed an intraperitoneal injection of NaB at 1.2 g/kg, the dose most commonly used in current literature (Levenson et al., 2004, Dash et al., 2009, Govindarajan et al., 2011, Itzhak et al., 2012, Itzhak et al., 2013, Ji et al., 2014, Zhong et al., 2014). Only two published studies provided the volume at which NaB was delivered or the concentration at which it was prepared, 1 ml/kg (Ji et al., 2014) and 120 mg/ml (Dash et al., 2009), respectively. Considering 1 ml/kg is a commonly used injection volume, we made our first attempt under this parameter. However, upon injection the animals exhibited behavior consistent with abdominal pain and cramping, suggesting the solution was hypertonic. This observation was not reported in previous studies and presented an obvious confound to our experimental design, as NaB itself induced an aversive response in the animals due to the pain associated with this injection. We then increased our injection volume to 10 ml/kg, the volume used in the study by Dash, et al (Dash et al., 2009), but still observed a hypertonic response. These results should not be surprising considering the high molarity of both preparations (9 M and 1 M). Moving forward we lowered our dose of NaB to 300 mg/kg in a 10-ml/kg injection volume to achieve a concentration that was more isotonic to blood (0.3 M) and that did not appear to induce aversion or pain in the animals. In support of using lower NaB dose, Sailaja, et al found that 100 mg/kg NaB induced behavioral alterations as well as acetylation changes in the hippocampi of mice (Sailaja et al., 2012). Future studies should take care to report concentrations and injection volumes of NaB in order to more accurately interpret results and compare

results across studies. For consistency, only Cohorts 2-4 are included in the remaining results.

Body weights and food intake

After only 24 hours on their respective diets, there was a significant effect of diet on body weight ($F(1,46)=5.80$, $p=0.020132$), with high fat-fed animals weighing more than chow-fed animals, and this effect remained significant throughout the course of the experiment ($F(11,30)=14.513$, $p<0.000000$, Wilk's $\Lambda=0.158194$; **Figure 4.2a**). By the end of the 11-day study, HFD-fed animals had gained significantly more weight than their chow-fed counterparts ($F(1,40)=25.4138$, $p=0.000010$; **Figure 4.2b**). There was no difference among groups in the amount of food consumed by weight ($F(1,40=2.229$, $p=0.143326$, **Figure 4.2c**) but HFD-fed animals ate significantly more kilocalories, approximately 20 kcal more per day on average, than there chow-fed counterparts ($F(1,40)=81.170$, $p<0.000000$, **Figure 4.2c**), due to the higher caloric content of the HFD. There were no significant main effects of NaB or LiCl on body weight or food consumption, nor were there significant interactions of either of these factors with diet or with each other.

Conditioned taste aversion

During the first four days of this experiment, rats were trained to drink a diluted sweetened condensed milk solution (SCM) during a 30-minuted period in their light cycle. During the training period, we observed a main effect of diet, where HFD-fed animals consumed significantly less SCM than chow-fed animals ($F(4,35=23.9116$,

p<0.000000, Wilk's Λ=0.0267899, **Figure 4.3a**). This observation held true on the CTA acquisition day upon first exposure to the banana-flavored (bSCM) and to NaB injections (F(1,38)=41.8305, p<0.000000), prior to LiCl-induced taste aversion (**Figure 4.3b**). Two days later, upon taste aversion retrieval, there was a significant effect of LiCl treatment (F(1,38)=114.0956, p<0.000000) on bSCM consumption, indicating that a taste aversion to bSCM was induced in these animals (**Figure 4.3c-d**). Throughout extinction (where bSCM was presented with no subsequent LiCl injection), there remained a significant effect of diet (F(3,36)=14.21413, p=0.000003, Wilk's Λ=0.0.457768), LiCl treatment (F(3,36)=25.20494, p<0.000000, Wilk's Λ=0.322538), and a significant interaction between diet and LiCl treatment (F(3,36)=6.51601, p=0.001234, Wilk's Λ=0.648088) on levels of bSCM consumption. LiCl treatment reduced bSCM consumption, with the greatest effect of LiCl observed in the HFD-fed animals, whose extinction was impaired compared to chow-fed controls (**Figure 4.3d**).

Gene expression

Following three days of extinction, animals were euthanized and the rostral agranular insular cortex, central amygdala, and dorsal and ventral hippocampi were collected to measure levels of *BDNF* transcript via qRT-PCR (**Figure 4.4a**). There was a significant main effect of diet on *BDNF* in the ventral hippocampus (F(1,34)=5.5334, p=0.024585), with HFD resulting in an overall reduction in *BDNF* transcript in this region (**Figure 4.4c**). Despite an apparent numerical increase in relative quantity of *BDNF* in the dorsal hippocampi of HFD-fed, LiCl-exposed animals (**Figure 4.4b**), there was no significant interaction between diet and LiCl treatment in this region

($F(1,39)=3.3912$, $p=0.073162$) nor did post-hoc testing reveal a significant difference in this group compared to any others ($p>0.05$). There were no significant main effects of diet, NaB, or LiCl on *BDNF* levels in the agranular insular cortex (**Figure 4.4d**) or central amygdala (**Figure 4.4e**).

4.4 Discussion

By employing the fundamental ability of animals to learn and recall the safety of particular tastes, the conditioned taste aversion behavioral paradigm has paved the way for much of the current knowledge regarding learning and memory processes (Rozin and Kalat, 1971, Garcia et al., 1985). In this experiment, we asked whether or not acquisition or extinction of CTA could be altered by diet or enhanced by peripheral administration of the HDAC inhibitor sodium butyrate, as it has shown to enhance performance in other learning and memory tasks (Dash et al., 2009, Intlekofer et al., 2013, Itzhak et al., 2013, Zhong et al., 2014). In a version of CTA using banana flavor as the conditioned stimulus and lithium chloride as the unconditioned stimulus, we did not observe any effect of NaB on CTA acquisition. A caveat in the assessment of this finding lies in the observation that bSCM consumption upon retrieval in all LiCl-injected animals was remarkably low, with only one subject consuming greater than one milliliter of SCM. With levels of consumption so consistently low among all LiCl-receiving groups, detecting an effect of NaB in this narrow range becomes subject to our method of measurement, which was limited to units of 0.25 ml using modified graduated cylinders. However, a 2014 study by Núñez-Jaramillo and colleagues supports this finding: 10, 50, or 100 μg NaB injected directly into the insular cortex of rats did not affect acquisition in a saccharin-LiCl CTA

paradigm (Nunez-Jaramillo et al., 2014). In this study, researchers went on to determine if any of the three doses of NaB would impact CTA extinction. Interestingly, they found that extinction was delayed with *decreasing* doses of intra-IC NaB (Nunez-Jaramillo et al., 2014). In contrast, CTA extinction is accelerated in *HDAC2* knockout mice (Morris et al., 2013). In the present experiment, we did not observe a significant effect of peripheral NaB on extinction of CTA in either chow- or high fat-fed animals. However, continuation of the extinction phase beyond three days in future studies will supply additional data. Unlike the Núñez-Jaramillo, et al study, CTA in our experiment was not completely extinguished by the third day, possibly due to differing doses of LiCl used during acquisition (their 10 mg/kg LiCl vs. our 127 mg/kg) (Nunez-Jaramillo et al., 2014). To follow up, we plan to continue extinction until the intake of LiCl-treated animals increases to levels similar to their controls (saline-injected animals).

Perhaps one of our most interesting findings is that animals on HFD consumed significantly less SCM during the entirety of the study, going against the notion that high fat-fed or obese animals may be more inclined to over consume palatable foods (Volkow et al., 2011). At first thought, one might attribute the reduced SCM consumption in HFD animals to increased satiety brought on by consumption of a higher calorie food source. However, on the first day of the experiment, HFD was provided between 0800 and 1000 hours, during the animal's light cycle. SCM was first provided between 1000 and 1200 hours on the same day, providing little time for consumption of the HFD, particularly in a species known to exhibit neophobia in the presence of novel foods and flavors (Modlinska et al., 2015). Food intake between introduction of the HFD and 30-minute SCM session was not measured at the time to validate this hypothesis. When given

restricted access to a palatable food source, rats will typically reduce consumption of standard chow in favor of "bingeing" on the palatable food (Cottone et al., 2008, Johnson and Kenny, 2010). Daily food consumption decreased slightly for chow-fed rats during SCM training while SCM consumption rose (**Figure 4.3a**). A similar trend was observed for high fat-fed rats, with a sharper initial decline in food intake and an attenuated increase in SCM consumption over the four days of training, compared to chow-fed rats (**Figure 4.3a**).

In Johnson and Kenny's 2010 study, rats were subject to three dietary paradigms, chow only, restricted access to a cafeteria-like diet (one hour per day), or extended access to a cafeteria-like diet (18-23 hours per day), for approximately 40 days. Following this period, all rats were restricted to only 30-minute daily exposure to the cafeteria diet. Chow-only and restricted access rats binged on the palatable food during the 30-minute access while extended access rats did not (Johnson and Kenny, 2010), suggesting that a previous history of unlimited access to palatable foods inhibits the development of binge-like feeding behavior. One possible explanation for reduced consumption of palatable food in high-fat fed rats is what some researchers have deemed as "reward hypofunction," where increasing obesity is associated with reduced sensitivity to rewarding stimuli (Johnson and Kenny, 2010); however our data do not support the theory that reward hypofunction contributes to increased consumption of palatable foods as has been suggested (Volkow et al., 2011). Similar to our study, diet-induced overweight and obese mice exhibited increased latency to consume a sweetened condensed milk reward as compared to control mice (Harb and Almeida, 2014). In addition, rats fed a HFD for 16 weeks showed decreased motivation to consume solutions

with low concentrations of corn oil or sucrose compared to chow-fed rats, but this motivation increased with increasing concentrations of the palatable ingredients (Shin et al., 2011), suggesting that high-fat fed rats have a higher threshold for assigning preference to food items.

A major disparity between the previously mentioned studies and ours is that decreased motivation to consume the SCM occurred on the same day as presentation of the HFD, prior to the feeding period and prior to weight gain. It is possible that during the window of time between first HFD presentation and SCM training, the rats consumed the novel HFD to satiety, decreasing the motivation to drink SCM. However, after four days of training, when rats could reasonably begin to anticipate daily SCM exposure, high fat-fed rats still did not reach SCM consumption levels near that of chow-fed rats during the 30-minute sessions. A 2015 study by Tracy, et al attempted to clarify discrepancies regarding the effects of obesogenic diets on food-motivated behavior. It was found that shorter HFD exposure (3 weeks) was associated with increased motivation to obtain a sucrose reinforcer while longer exposure (6 weeks) reduced this motivation (Tracy et al., 2015). Additionally, rats maintained on HFD did not develop a conditioned place preference (CPP) for a context previously paired with a sucrose reward, while chow-maintained rats did (Tracy et al., 2015), indicating that HFD reduced the saliency of the sucrose reward. This last point could provide insight into our findings. Prior to dilution, sweetened condensed milk contains 0.08% fat and 56% sugar by weight; after dilution to 40% with deionized water, the SCM solution used in our experiments is approximately 0.03% fat and 22% sugar. Considering these figures it is reasonable to assume that the most palatable feature of the SCM is its sweetness, as the solution is

relatively low in fat. The HFD used in our experiments was 17% sugar (sucrose), compared to 5% sugar (sucrose, lactose, fructose, and glucose) found in standard chow. Providing that the rats did sample the HFD upon first exposure prior to the first SCM training session, and since HFD and SCM contained reasonably similar sugar concentrations, the rats may not have deemed the SCM any more palatable than the HFD and therefore were not motivated to binge during the SCM sessions. If this hypothesis is correct, scheduling the daily SCM sessions before the presentation of HFD should result in SCM consumption levels closer to that of chow-fed animals. Confirmation of this hypothesis may also explain the reluctance to extinguish the taste aversion in HFD animals, whereby the SCM was not deemed rewarding enough promote extinction the conditioned response.

HFD-induced reduction of ventral hippocampal *BDNF* has been previously reported (Kanoski et al., 2007), though not in this rapid of a time frame (11 days). In our study, we did not observe any HFD-induced cognitive impairments in CTA acquisition; however these observations with other learning paradigms have typically been associated with a longer duration HFD intake prior to behavioral training or testing (Beilharz et al., 2015). The impairment in extinction observed in HFD rats could be attributed to the reductions in ventral hippocampal *BDNF* observed at the study's conclusion. If extinction represents not an erasure of one truth (i.e. that the banana-flavored milk is unsafe), but the learning of a new truth (i.e. that the banana-flavored milk is safe), then reduced ventral hippocampal BDNF could be involved in the impaired extinction learning seen in HFD-fed animals. While as a whole the hippocampus is associated with memory formation, the dorsal and ventral hippocampal poles are thought to serve slightly

different functions. The dorsal hippocampus is associated with contextual and situational memory and the ventral hippocampus is thought to aid in the contribution of emotional salience to certain memories and tasks and to be more associated with memories that are associated with visceral responses (Fanselow and Dong, 2010). In addition the ventral hippocampus is highly interconnected with diencephalic brain structures involved in autonomic and endocrine regulation (Moser and Moser, 1998, Fanselow and Dong, 2010), and may play a role in meal timing (Henderson et al., 2013). Importantly, reduced ventral hippocampal *BDNF* transcript levels may not immediately reflect a reduction in BDNF protein levels but could predict reduced BDNF protein with longer dietary exposure. Further experiments will determine if BDNF protein levels were altered under this paradigm as well.

In conclusion, our findings support the notion that unlimited access to a palatable food source may reduce binge-like consumption of other palatable foods and impair taste aversion extinction. Future studies wishing to examine the relationship between HFD and CTA might employ a paradigm where the CS and diet are more distinct in their perceived reward levels or involve a period of food restriction prior to CS exposure in an attempt to increase consumption of the CS in the HFD-fed groups. Peripheral NaB inhibition of HDACs did not impact food intake, body weight, CTA performance, or *BDNF* in the brain regions examined. Importantly, we found that ventral hippocampal *BDNF* levels were reduced by only 11 days of HFD consumption. While it is known that chronic HFD consumption and obesity are associated with reduced hippocampal function (Kanoski and Davidson, 2011), how rapidly these changes occur is still unclear.

Acknowledgements

We would like to thank Dr. Jesse Schank of the Department of Physiology and Pharmacology at the University of Georgia for the use of his equipment in our gene expression experiments.

References

Beilharz JE, Maniam J, Morris MJ (2015) Diet-Induced Cognitive Deficits: The Role of Fat and Sugar, Potential Mechanisms and Nutritional Interventions. Nutrients 7:6719-6738.

Cottone P, Sabino V, Steardo L, Zorrilla EP (2008) Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food. Neuropsychopharmacology 33:524-535.

Danilova AB, Grinkevich LN (2012) Failure of long-term memory formation in juvenile snails is determined by acetylation status of histone H3 and can be improved by NaB treatment. PLoS One 7:e41828.

Das UN, Vaddadi KS (2004) Essential fatty acids in Huntington's disease. Nutrition 20:942-947.

Dash PK, Orsi SA, Moore AN (2009) Histone deactylase inhibition combined with behavioral therapy enhances learning and memory following traumatic brain injury. Neuroscience 163:1-8.

Fanselow MS, Dong HW (2010) Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65:7-19.

Gal-Ben-Ari S, Rosenblum K (2011) Molecular mechanisms underlying memory consolidation of taste information in the cortex. Front Behav Neurosci 5:87.

Gan L, England E, Yang JY, Toulme N, Ambati S, Hartzell DL, Meagher RB, Baile CA (2015) A 72-hour high fat diet increases transcript levels of the neuropeptide galanin in the dorsal hippocampus of the rat. BMC Neurosci 16:51.

Gao W, Pan B, Liu L, Huang X, Liu Z, Tian J (2015) Alcohol exposure increases the expression of cardiac transcription factors through ERK1/2-mediated histone3 hyperacetylation in H9c2 cells. Biochem Biophys Res Commun 466:670-675.

Garcia J, Lasiter PS, Bermudez-Rattoni F, Deems DA (1985) A general theory of aversion learning. Ann N Y Acad Sci 443:8-21.

Govindarajan N, Agis-Balboa RC, Walter J, Sananbenesi F, Fischer A (2011) Sodium butyrate improves memory function in an Alzheimer's disease mouse model when administered at an advanced stage of disease progression. J Alzheimers Dis 26:187-197.

Graff J, Rei D, Guan JS, Wang WY, Seo J, Hennig KM, Nieland TJ, Fass DM, Kao PF, Kahn M, Su SC, Samiei A, Joseph N, Haggarty SJ, Delalle I, Tsai LH (2012) An epigenetic blockade of cognitive functions in the neurodegenerating brain. Nature 483:222-226.

Guan JS, Haggarty SJ, Giacometti E, Dannenberg JH, Joseph N, Gao J, Nieland TJ, Zhou Y, Wang X, Mazitschek R, Bradner JE, DePinho RA, Jaenisch R, Tsai LH (2009) HDAC2 negatively regulates memory formation and synaptic plasticity. Nature 459:55-60.

Harb MR, Almeida OF (2014) Altered motivation masks appetitive learning potential of obese mice. Front Behav Neurosci 8:377.

Henderson YO, Smith GP, Parent MB (2013) Hippocampal neurons inhibit meal onset. Hippocampus 23:100-107.

Intlekofer KA, Berchtold NC, Malvaez M, Carlos AJ, McQuown SC, Cunningham MJ, Wood MA, Cotman CW (2013) Exercise and sodium butyrate transform a subthreshold learning event into long-term memory via a brain-derived neurotrophic factor-dependent mechanism. Neuropsychopharmacology 38:2027-2034.

Itzhak Y, Anderson KL, Kelley JB, Petkov M (2012) Histone acetylation rescues contextual fear conditioning in nNOS KO mice and accelerates extinction of cued fear conditioning in wild type mice. Neurobiol Learn Mem 97:409-417.

Itzhak Y, Liddie S, Anderson KL (2013) Sodium butyrate-induced histone acetylation strengthens the expression of cocaine-associated contextual memory. Neurobiol Learn Mem 102:34-42.

Jarome TJ, Lubin FD (2014) Epigenetic mechanisms of memory formation and reconsolidation. Neurobiol Learn Mem 115:116-127.

Ji M, Dong L, Jia M, Liu W, Zhang M, Ju L, Yang J, Xie Z, Yang J (2014) Epigenetic enhancement of brain-derived neurotrophic factor signaling pathway improves cognitive impairments induced by isoflurane exposure in aged rats. Mol Neurobiol 50:937-944.

Johnson PM, Kenny PJ (2010) Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat Neurosci 13:635-641.

Kanoski SE, Davidson TL (2011) Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol Behav 103:59-68.

Kanoski SE, Meisel RL, Mullins AJ, Davidson TL (2007) The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat. Behav Brain Res 182:57-66.

Karpova NN (2014) Role of BDNF epigenetics in activity-dependent neuronal plasticity. Neuropharmacology 76 Pt C:709-718.

Kramer TH, Sclafani A, Kindya K, Pezner M (1983) Conditioned taste aversion in lean and obese rats with ventromedial hypothalamic knife cuts. Behav Neurosci 97:110-119.

Levenson JM, O'Riordan KJ, Brown KD, Trinh MA, Molfese DL, Sweatt JD (2004) Regulation of histone acetylation during memory formation in the hippocampus. J Biol Chem 279:40545-40559.

Lipsky RH (2013) Epigenetic mechanisms regulating learning and long-term memory. Int J Dev Neurosci 31:353-358.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408.

Ma L, Wang DD, Zhang TY, Yu H, Wang Y, Huang SH, Lee FS, Chen ZY (2011) Region-specific involvement of BDNF secretion and synthesis in conditioned taste aversion memory formation. J Neurosci 31:2079-2090.

Maffei A, Haley M, Fontanini A (2012) Neural processing of gustatory information in insular circuits. Curr Opin Neurobiol 22:709-716.

Martinez-Moreno A, Rodriguez-Duran LF, Escobar ML (2011) Late Protein Synthesis-Dependent Phases in CTA Long-Term Memory: BDNF Requirement. Front Behav Neurosci 5:61.

Martinez-Moreno A, Rodriguez-Duran LF, Escobar ML (2016) Brain-derived neurotrophic factor into adult neocortex strengthens a taste aversion memory. Behav Brain Res 297:1-4.

Modlinska K, Stryjek R, Pisula W (2015) Food neophobia in wild and laboratory rats (multi-strain comparison). Behav Processes 113:41-50.

Moguel-Gonzalez M, Gomez-Palacio-Schjetnan A, Escobar ML (2008) BDNF reverses the CTA memory deficits produced by inhibition of protein synthesis. Neurobiol Learn Mem 90:584-587.

Molteni R, Wu A, Vaynman S, Ying Z, Barnard RJ, Gomez-Pinilla F (2004) Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral plasticity associated to the action of brain-derived neurotrophic factor. Neuroscience 123:429-440.

Morris MJ, Mahgoub M, Na ES, Pranav H, Monteggia LM (2013) Loss of histone deacetylase 2 improves working memory and accelerates extinction learning. J Neurosci 33:6401-6411.

Moser MB, Moser EI (1998) Functional differentiation in the hippocampus. Hippocampus 8:608-619.

Niles LP, Pan Y, Kang S, Lacoul A (2013) Melatonin induces histone hyperacetylation in the rat brain. Neurosci Lett 541:49-53.

Nunez-Jaramillo L, Ramirez-Lugo L, Herrera-Morales W, Miranda MI (2010) Taste memory formation: latest advances and challenges. Behav Brain Res 207:232-248.

Nunez-Jaramillo L, Reyes-Lopez J, Miranda MI (2014) Sodium butyrate into the insular cortex during conditioned taste-aversion acquisition delays aversive taste memory extinction. Neuroreport 25:386-390.

Rodriguez-Serrano LM, Ramirez-Leon B, Rodriguez-Duran LF, Escobar ML (2014) Acute infusion of brain-derived neurotrophic factor in the insular cortex promotes conditioned taste aversion extinction. Neurobiol Learn Mem 116:139-144.

Rozin P, Kalat JW (1971) Specific hungers and poison avoidance as adaptive specializations of learning. Psychol Rev 78:459-486.

Sailaja BS, Cohen-Carmon D, Zimmerman G, Soreq H, Meshorer E (2012) Stress-induced epigenetic transcriptional memory of acetylcholinesterase by HDAC4. Proc Natl Acad Sci U S A 109:E3687-3695.

Shin AC, Townsend RL, Patterson LM, Berthoud HR (2011) "Liking" and "wanting" of sweet and oily food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic predisposition. Am J Physiol Regul Integr Comp Physiol 301:R1267-1280.

Singer LK, York DA, Berthoud HR, Bray GA (1999) Conditioned taste aversion produced by inhibitors of fatty acid oxidation in rats. Physiol Behav 68:175-179.

Sui L, Wang Y, Ju LH, Chen M (2012) Epigenetic regulation of reelin and brain-derived neurotrophic factor genes in long-term potentiation in rat medial prefrontal cortex. Neurobiol Learn Mem 97:425-440.

Swank MW, Sweatt JD (2001) Increased histone acetyltransferase and lysine acetyltransferase activity and biphasic activation of the ERK/RSK cascade in insular cortex during novel taste learning. J Neurosci 21:3383-3391.

Thompson CI, Margules DL, Kreider JW, Boha SP, Rejer RE, Jr., Quirey RA, Reitz JA (1993) Propensity to form conditioned taste aversions augments anorexia in obese (ob/ob) mice with B16 melanoma. Behav Neurosci 107:786-798.

Tian F, Marini AM, Lipsky RH (2010) Effects of histone deacetylase inhibitor Trichostatin A on epigenetic changes and transcriptional activation of Bdnf promoter 1 by rat hippocampal neurons. Ann N Y Acad Sci 1199:186-193.

Tracy AL, Wee CJ, Hazeltine GE, Carter RA (2015) Characterization of attenuated food motivation in high-fat diet-induced obesity: Critical roles for time on diet and reinforcer familiarity. Physiol Behav 141:69-77.

Uematsu A, Kitamura A, Iwatsuki K, Uneyama H, Tsurugizawa T (2015) Correlation Between Activation of the Prelimbic Cortex, Basolateral Amygdala, and Agranular Insular Cortex During Taste Memory Formation. Cereb Cortex 25:2719-2728.

Vecsey CG, Hawk JD, Lattal KM, Stein JM, Fabian SA, Attner MA, Cabrera SM, McDonough CB, Brindle PK, Abel T, Wood MA (2007) Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB:CBP-dependent transcriptional activation. J Neurosci 27:6128-6140.

Volkow ND, Wang GJ, Baler RD (2011) Reward, dopamine and the control of food intake: implications for obesity. Trends Cogn Sci 15:37-46.

Wang Y, Zhang TY, Xin J, Li T, Yu H, Li N, Chen ZY (2012) Differential involvement of brain-derived neurotrophic factor in reconsolidation and consolidation of conditioned taste aversion memory. PLoS One 7:e49942.

Zhang Q, Xu X, Li T, Lu Y, Ruan Q, Lu Y, Wang Q, Dong F, Yang Y, Zhang G (2014) Exposure to bisphenol-A affects fear memory and histone acetylation of the hippocampus in adult mice. Horm Behav 65:106-113.

Zhao Y, Xing B, Dang YH, Qu CL, Zhu F, Yan CX (2013) Microinjection of valproic acid into the ventrolateral orbital cortex enhances stress-related memory formation. PLoS One 8:e52698.

Zhong T, Qing QJ, Yang Y, Zou WY, Ye Z, Yan JQ, Guo QL (2014) Repression of contexual fear memory induced by isoflurane is accompanied by reduction in histone acetylation and rescued by sodium butyrate. Br J Anaesth 113:634-643.

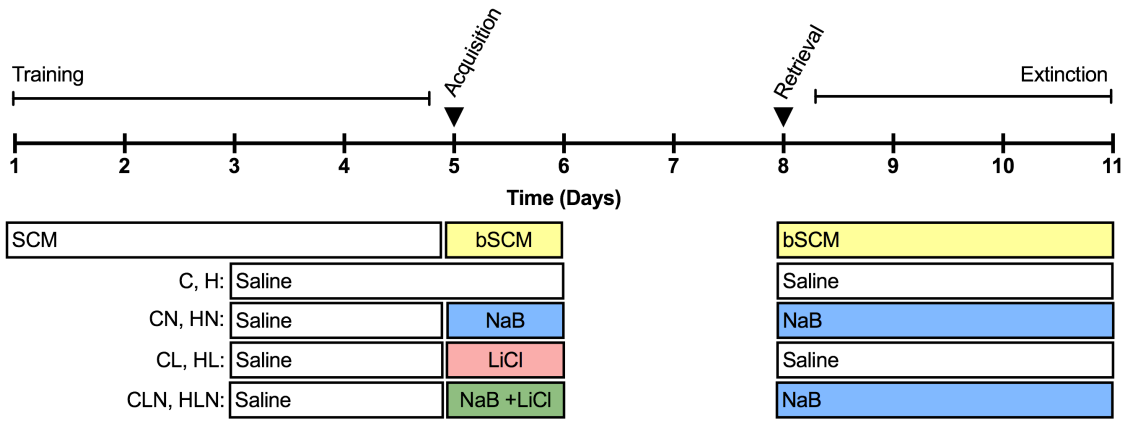


Figure 4.1. Conditioned taste aversion experimental design, Cohorts 2-4. On Days 1-4, rats were given access to a sweetened condensed milk solution (SCM) for 30 minutes daily. On Days 3 and 4, 15 minutes before and after the 30-minute SCM exposure, rats were given intraperitoneal injections of saline. On Day 5, the diluted SCM was flavored with imitation banana extract (bSCM). Fifteen minutes before a 30-minute bSCM exposure, CN, HN, CLN, and HLN rats were given an intraperitoneal injection of NaB (300 mg/kg). C, H, CL, and HL rats were given an equal volume of saline. 15 minutes following the bSCM exposure, CL, HL, CLN, and HLN rats were given an intraperitoneal injection of LiCl (127 mg/kg) while C, H, CN, and HN rats were given an equal volume of saline. No milk was provided on Days 6 or 7. On Days 8-11, rats were given daily 30-minute bSCM exposure and injected with either saline (C, H, CL, HL) or NaB (CN, HN, CLN, HLN) 15 minutes prior.

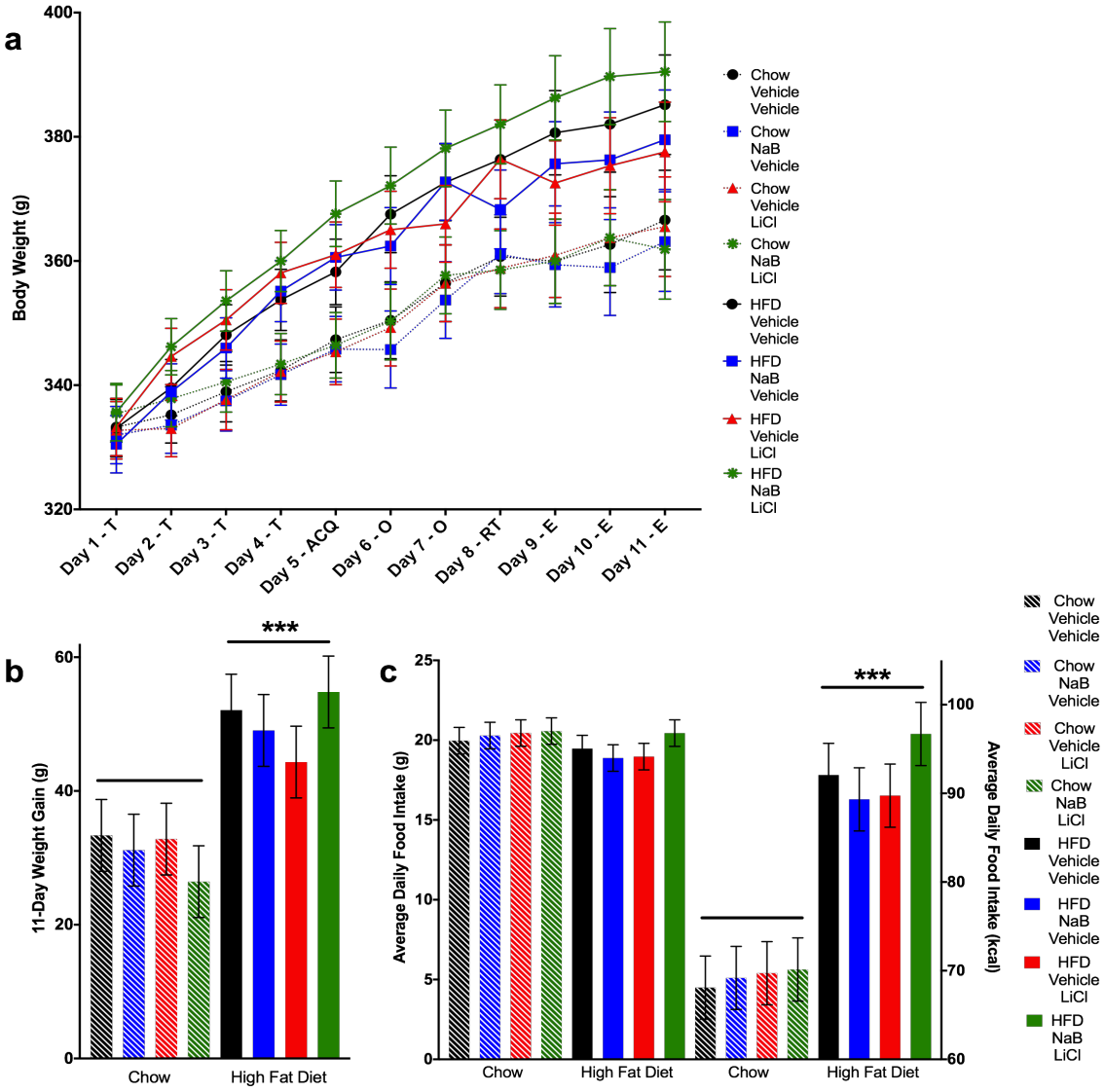
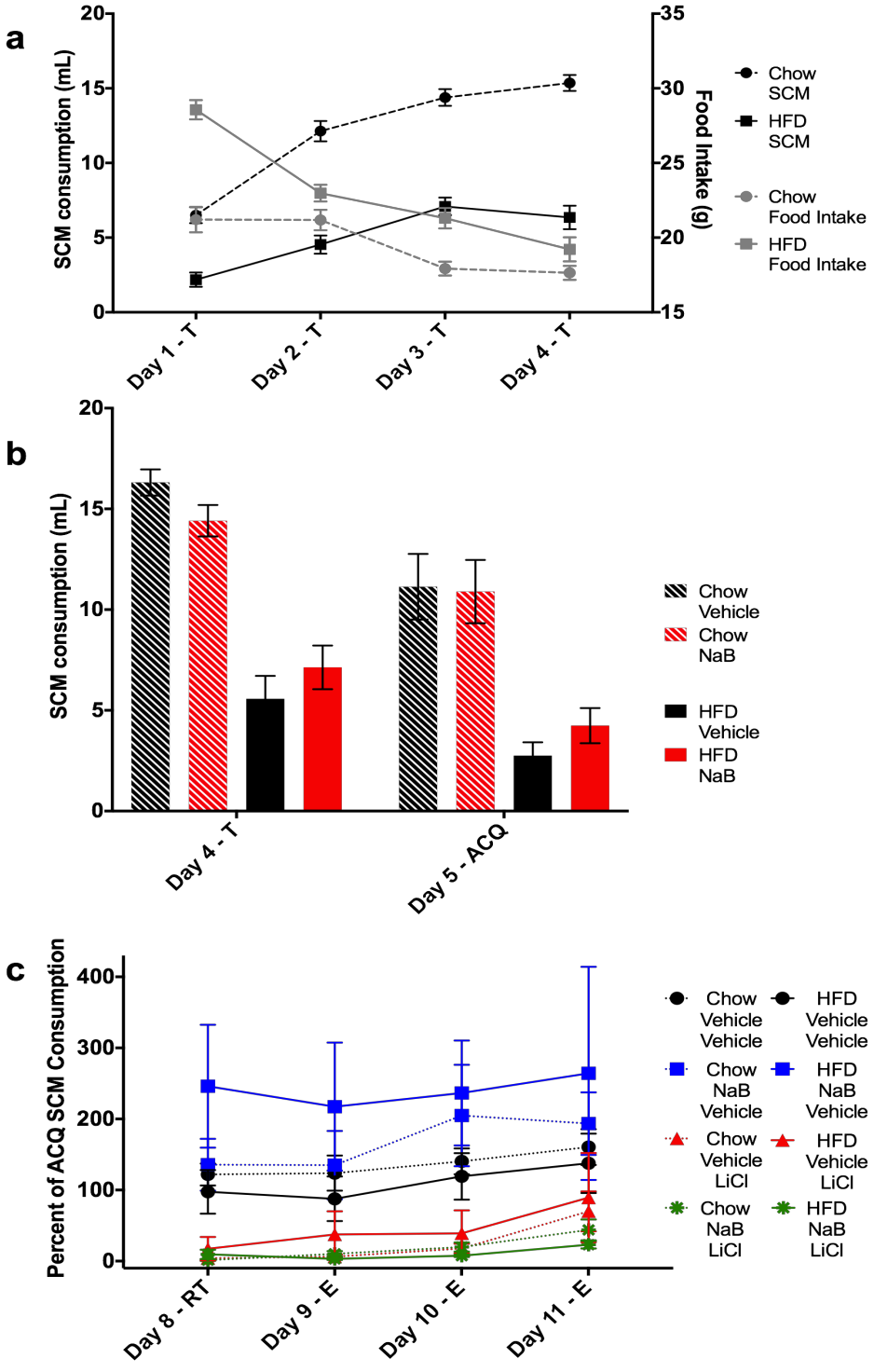


Figure 4.2. Body weight and food intake. Body weights were measured daily for all chow-fed (dashed lines) and HFD-fed (solid lines) rats (a). HFD-fed rats (solid bars) gained more weight over the course of the 11-day experiment than did chow-fed rats (striped bars) (b). On average, HFD-fed rats (solid bars) and chow-fed (striped bars) rats consumed a similar amount of food by weight daily (left y axis) but HFD-fed rats consumed more energy per day (right y axis) than chow-fed rats (c). Statistics were

performed by MANOVA with Wilk's test (a) or three-way factorial ANOVA (b,c) with post-hoc Tukey's HSD. A p-value of less than 0.001 is denoted by a triple asterisk.



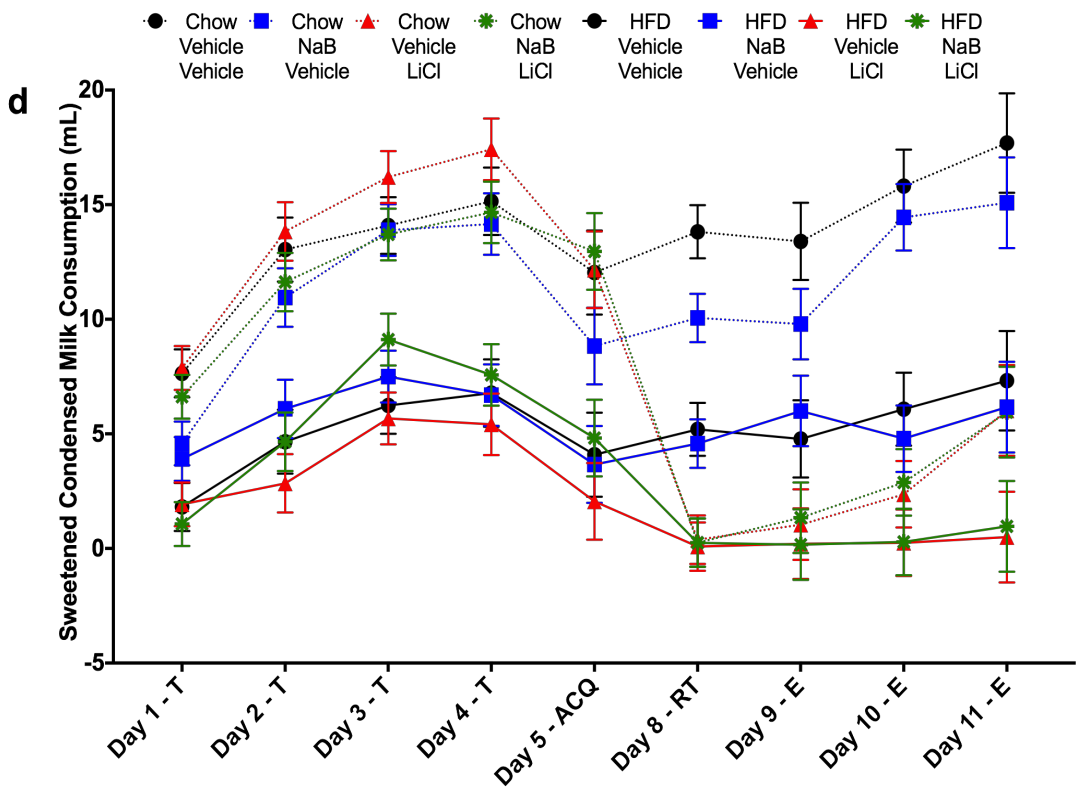


Figure 4.3. Conditioned taste aversion. Volume of SCM consumed (left y-axis, black lines) and food consumed (right y-axis, grey lines) in chow-fed (dashed lines) and HFD-fed (solid lines) rats during CTA training (T) (a). SCM consumption on the last day of training compared with consumption of bSCM on CTA Acquisition day (ACQ) in Chow (striped bars) and HFD rats (solid bars) treated with vehicle (black bars) or NaB (red bars) on the ACQ day. Only saline vehicle injections were given on Training day 4 (b). bSCM consumption upon CTA retrieval (RT) and during extinction (E) as a percentage of consumption levels on the CTA acquisition day (c). Volume of SCM consumed over the entire 11-day conditioned taste aversion experiment (d). Statistics were performed by MANOVA with Wilk's test to determine main effects of diet, NaB, or LiCl over the course of the experiment, or by three-way factorial ANOVA with post-hoc Tukey's HSD to determine main effects of the same independent variables on individual days.

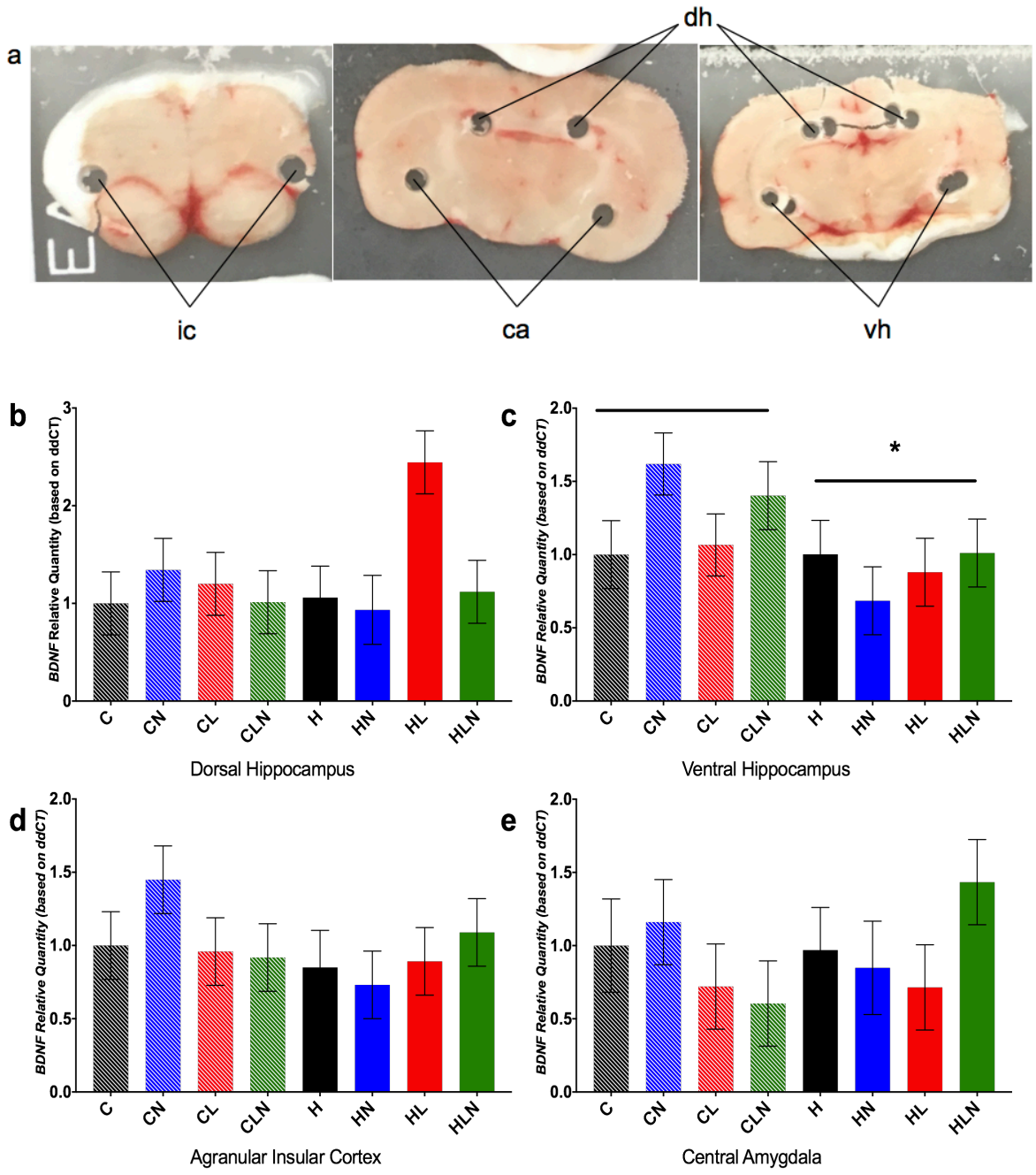


Figure 4.4. Quantitative real-time polymerase chain reaction. Verification of brain regions punched for qRT-PCR analysis: dorsal hippocampus (dh), ventral hippocampus (vh), agranular insular cortex (ic), and central amygdala (ca) (a). qRT-PCR for *brain-derived neurotrophic factor* (*BDNF*) was measured in the dorsal hippocampus (b), ventral hippocampus (c), agranular insular cortex (d), and central amygdala (e) in chow-fed

(striped bars) and HFD-fed (solid bars) rats. C=Chow, CN = Chow/NaB/Vehicle, CL=Chow/Vehicle/LiCl, CLN=Chow/NaB/LiCl, H=HFD, HN=HFD/NaB/Vehicle, HL=HFD/Vehicle/LiCl, HLN=HFD/NaB/LiCl. Statistics were performed by three-way factorial ANOVA with post-hoc Tukey's HSD. p-values are denoted in the following manner: less than 0.05 with a single asterisk, less than 0.01 with a double asterisk, and less than 0.001 is denoted by a triple asterisk.

CHAPTER 5

CHANGES IN HIPPOCAMPAL GENE EXPRESSION IN OFFSPRING EXPOSED TO MATERNAL HIGH FAT DIET AND AN IMMUNOMODULATORY GLYCAN

Trunnell E. R., Krishna S., Lin Z., de La Serre C. B., Harn D. H., Baile C. A., and N. M. Filipov. To be submitted to *Brain, Behavior, and Immunity.*

Abstract

With currently one third of women of childbearing age in the United States classified as obese, it is imperative to examine the effects of maternal obesity on the developing offspring. It is known now that chronic obesity and high-fat diet (HFD) intake have a negative impact on the hippocampus, a brain region recognized for its pivotal role in learning and memory. The current literature only provides us with isolated, and sometimes confounding, ideas about effects of maternal HFD (MHFD) on the hippocampi of offspring. We fed female mice a 10% or 60% fat diet before, during, and after gestation and performed qRT-PCR analysis on dorsal and ventral hippocampal samples from male and female offspring at the time of weaning (PND21) and two weeks later after switching the offspring to a low-fat diet (PND35). We also treated a subset of HFD-fed dams with the immunomodulatory glycan LNFPIII to determine if this natural, anti-inflammatory compound could reverse aberrant, and potentially detrimental, gene expression induced by the MHFD exposure. We found significant effects of treatment on transcript levels of *brain-derived neurotrophic factor* (*BDNF*), *glucocorticoid receptor*, *insulin receptor*, *leptin receptor*, the serotonin receptor *5HT1a*, and the marker of endoplasmic reticulum stress *XBP1s*; effects of offspring sex on transcript levels of *BDNF* and *interleukin-10*; and interaction effects of treatment and sex on transcript levels of *BDNF* and *glucocorticoid receptor*. Our data suggest that gene transcription in the developing hippocampus is influenced by MHFD in a sex-specific manner, that it can be modified by LNFPIII, and that changes in transcript levels can be abolished or reversed by removal of the stimuli.

5.1 Introduction

The Fetal Origins Hypothesis, first proposed by British physician and epidemiologist David J. Barker, states that the intrauterine environment plays a major role in adult development of degenerative disease (Barker, 1990). Early studies supporting Barker's hypothesis looked at the effects of maternal undernutrition on the development of cardiovascular disease and other aspects of metabolic syndrome in offspring. This work eventually led Barker and his colleague Hales to coin the *thrifty phenotype hypothesis*, stating that "the epidemiological associations between poor fetal and infant growth and the subsequent development of type 2 diabetes and the metabolic syndrome result from the effects of poor nutrition in early life" (Hales and Barker, 2001). The *thrifty phenotype hypothesis* works under the idea that nutritional deficits experienced in the womb program the developing fetus for optimal survival in a nutritionally deficient environment, and that metabolic problems arise when the later environment turns out to be nutritionally plentiful instead.

Taking into consideration current trends in modern developed and developing nations, many researchers have begun to ask about the consequences of maternal *overnutrition* in the context of the Fetal Origins Hypothesis. Currently one in three women of reproductive age in the United States are classified as obese (BMI $\geq$ 30) (Honein et al., 2013). Not only is obesity dangerous for the mother during pregnancy, contributing to gestational diabetes, preeclampsia, incidence of cesarean delivery, and other complications, it is also associated with adverse outcomes for the fetus during the pregnancy, in delivery, and throughout life (Leddy et al., 2008, Honein et al., 2013). In fact, children of obese parents are twice as likely to become obese themselves (Whitaker

et al., 1997) and so are at greater risk to develop obesity-related comorbidities. Animal models of maternal obesity typically involve using a high-fat (HFD) or Western-like (WD) diet to induce obesity in female rodents, allowing them to mate, and then maintaining them on the HFD/WD throughout gestation and often afterward. The fetus, including the central nervous system, develops in this obese physiological context.

Just as obesity and HFD/WD negatively impact hippocampal function in adult animals (see **Chapter 2** and (Kanoski and Davidson, 2011)), these stimuli also have consequences in the developing organism. Male rodent offspring born to HFD-fed dams (MHFD males) have decreased hippocampal neurogenesis at weaning (Tozuka et al., 2009) and into adulthood (Lepinay et al., 2015). Transcript and protein analysis of the hippocampus reveals decreased expression of plasticity-related factors such as nerve growth factor, brain-derived neurotrophic factor (BDNF) and activity-regulated cytoskeletal-associated protein (Page et al., 2014); of cell signaling molecules such as synaptotagmin and synaptophysin (Page et al., 2014, Lepinay et al., 2015) and the NMDA subunit N2RB (Page et al., 2014); as well as decreased expression of insulin receptor and glucose transporter 3 (Lepinay et al., 2015) in MHFD males. These data suggest that MHFD offspring may have reduced hippocampal function and therefore impaired learning and memory. Hippocampal-dependent spatial memory testing in these subjects using the Morris water maze has been inconclusive. In this task, rodents are trained over consecutive trials to use cues to find a hidden platform on which they can rest in an otherwise deep and inescapable pool of water. Performance on the task is typically measured by the animal's latency to find the platform, number of platform crossings, and time spent in or away from the quadrant of the pool in which the platform

is located. One study reports that MHFD males display worse performance on the task compared to their low-fat diet-exposed (MLFD) counterparts (Page et al., 2014), another shows that MHFD males perform better on the task than MLFD males (Bilbo and Tsang, 2010), another says there is no difference between the groups in the acquisition of the task but that MHFD males have reduced retention of this learning when tested later (White et al., 2009), and still another demonstrates that differences between the groups do not arise unless MHFD males are maintained on a HFD after weaning and into adulthood (Lepinay et al., 2015). Discrepancies in study design, such as the use of rats vs. mice, differences in maternal diet duration or maternal diet fat composition and quantity, and differences in the age at which offspring were tested, may account for some of these differences. Nonetheless the effect of MHFD on spatial memory in the offspring is not clear.

Only one of the above-mentioned experiments also included female offspring (MHFD females and MLFD females) for analysis. Bilbo and Tsang found that like their male siblings in this same experiment, MHFD females showed improved performance on the Morris water maze task compared to MLFD females (Bilbo and Tsang, 2010). Increased brain inflammatory markers and microglial activation were also present in the hippocampus of MHFD offspring and these results were similar in both sexes (Bilbo and Tsang, 2010). In this study, differences arose in the elevated plus maze where MHFD males spent significantly less time in the open arms than MLFD males or MHFD/MLFD females, an outcome interpreted to mean that MHFD males felt increased anxiety during this task (Bilbo and Tsang, 2010). Only a few other experiments have examined the sex differences in the hippocampi of offspring exposed to MHFD. A research group from

the University of Toronto performed several experiments examining anxiety behavior and markers of glucocorticoid and inflammatory signaling in the hippocampus and amygdala of MHFD males and females at two different developmental time points (Sasaki et al., 2013, Sasaki et al., 2014). Interestingly, both adolescent MHFD males and females (35-45 days old) demonstrated reduced anxiety in the open field test and elevated plus maze paradigms (Sasaki et al., 2014), while adult MHFD offspring (110 days old) were seemingly more anxious and performance outcomes on the behavioral tests were sexually dimorphic: MHFD males displayed a more anxious phenotype in the open field test, while MHFD females did so in the elevated plus maze (Sasaki et al., 2013). Sex differences were also apparent in gene expression analysis of offspring hippocampal tissue. Transcript levels of glucocorticoid receptor and markers of inflammatory response (both pro- and anti-inflammatory) NF-κB, interleukin-6, IκBα, interleukin receptor antagonist, and MAP kinase phosphatase-1 were significantly altered as a function of maternal diet, sex, and age (Sasaki et al., 2013, Sasaki et al., 2014). The behavioral results contrast with a similar experiment performed by Kang and colleagues where 32- to 35-day old MHFD females displayed increased anxiety in the open field test and MHFD males did not (Kang et al., 2014). These data and others suggest that male and female offspring respond differently to MHFD exposure and that these effects depend on the developmental window examined.

For the experiment presented herein, we asked the question: How do hippocampal gene expression profiles of MHFD males and females compare at weaning and how do these expression profiles change two weeks after weaning and without further exposure to HFD? We focused specifically on genes implicated in synaptic

plasticity, metabolic processes, oxidative stress, and inflammation. We asked if perturbations in gene profiles of offspring induced by MHFD could be ameliorated by maternal treatment with the immunomodulatory glycan Lacto-*N*-fucopentaose III (LNFPIII), containing the macrophage-initiator Lewis X (LeX) trisaccharide. LNFPIII is a natural component of breast milk and has been shown by members of our group to induce an anti-inflammatory response and improve glucose tolerance and insulin sensitivity in diet-induced obese mice (Atochina et al., 2008, Bhargava et al., 2012). Chronic inflammation is thought to be a major factor in obesity-related cognitive and behavioral disruption (Bilbo and Tsang, 2010, Cai, 2013, Bolton and Bilbo, 2014). In an effort to reverse the inflammatory state of the maternal environment, we sought to determine the impact of maternal treatment with LNFPIII on hippocampal gene expression profiles of MHFD males and females.

5.2 Methods

Animals and Diet

Young adult female C57BL/6 mice (6-7 weeks old, Harlan, Indianapolis, IN, USA) were housed (4-5 per cage) in an environmentally controlled room (22–24 °C) with food (standard chow) and water available *ad libitum* on a 12h light/dark cycle in an AAALAC accredited facility throughout the study. All procedures involving animal handling in this study were carried out according to the latest NIH guidelines (8th edition, NRC, 2010) and were approved in advance by the Institutional Animal Care and Use Committee (IACUC) of the University of Georgia. After 1 week of acclimatization, female mice (n = 62; mean initial weight: 16.0 ± 0.14 g; mean ± SEM) were randomly

divided into two groups and placed on either a low-fat diet (LFD; 10% fat by kCal, D12450J, Research Diets, Inc., New Brunswick, NJ, USA; n = 22) or a high-fat diet (HFD; 60% fat by kCal, D12492, Research Diets, Inc.; n = 42) for the entire duration of the study. Diet composition is presented in **Table 5.1**. After six weeks on their respective diets, the mean body weight of the LFD group was 19.6 ± 0.21 g (mean ± SEM); the mean body weight of the HFD group was 24.0 ± 0.40 g and weight of the two groups was statistically different ($P < 0.001$). After 6-weeks on the diets, mice from the HFD group were randomly sub-divided into two: HFD-dextran and HFD-glycan groups. Mice from all three groups were mated with control C57BL/6 males fed regular chow by placing one male with two females overnight. Plug-positive females were housed individually for the duration of pregnancy and lactation and fed their respective diets. Dextran vehicle (to LFD and HFD females) or the glycan (to HFD-LNFPIII females) treatments were administered subcutaneously twice a week (Tue. AM and Fri. PM), beginning the day before initial mating and continuing through weaning. Male and female offspring were weaned at post-natal day 21 and either euthanized at this time or placed on low-fat diet and euthanized two weeks later, at post-natal day 35.

Gene Expression

Total RNA from the dorsal and ventral hippocampus was isolated using E.Z.N.A. Microelute Total RNA Kit (Omega Bio-Tek; Norcross, GA, USA) and quantified using a Nanodrop spectrophotometer (Wilmington, DE, USA). 100 ng of RNA went into each reverse transcription reaction using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, ThermoFisher Scientific; Grand Island, NY, USA) and a Thermocycler (Biometra; Goettingen, DEU) to synthesize cDNA. Using cDNA produced

from a 5 ng equivalent per sample, expression levels of transcripts were determined by qRT-PCR. Primers were designed using the NCBI online database (U.S. National Library of Medicine, NIH; Bethesda, MD, USA), generated by IDT© (Coralville, IA, USA) and sequence specificity of each primer pair was confirmed using Primer-BLAST (U.S. National Library of Medicine, NIH; Bethesda, MD, USA). Primer sequences are listed in **Table 5.2**. In each RNA sample the level of 18S ribosomal RNA transcripts was used as an endogenous control. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed with SYBR green reaction mix (Applied Biosystems, ThermoFisher Scientific; Grand Island, NY, USA) using an Applied Biosystems 7500 system (ThermoFisher Scientific; Grand Island, NY, USA) to determine cycle threshold (CT) values. For analysis of CT values, each sample was run in triplicate and those triplicates were averaged to assign CT values for each sample and each gene. Normalized CT values were calculated using the following formula: 40 – average CT value for gene of interest – average CT value for 18S. Forty is the total number of cycles for which the PCR was run. Any negative values were inverted for graphical representation. Statistics were performed on the Statistica software (StatSoft, Dell; Aliso Viejo, CA, USA) using a factorial ANOVA with post-hoc Newman-Keuls test to determine differences between individual groups. Main effects of sex and treatment and the interaction sex*treatment are reported in the text. Significance level was set at $p<0.05$ with findings reported as "trends" fitting the criteria of $p<0.10$.

5.3 Results

Hippocampal gene expression profile of male and female offspring exposed to HFD in utero and prior to weaning (PND21)

In the dorsal hippocampus, there was a significant main effect of treatment on transcript levels of *BDNF* ($F(2,29)=4.213$, $p=0.02476$), *insulin receptor* ($F(2,29)=8.0593$, $p=0.00165$) and *glucocorticoid receptor* ($F(2,29)=11.9360$, $p=0.00017$) as well as a treatment*sex interaction for *BDNF* ($F(2,29)=6.979$, $p=0.00336$) and *glucocorticoid receptor* ($F(2,29)=4.3567$, $p=0.02216$). Post-hoc testing showed that MHFD + LNFPIII females had increased dorsal hippocampal *BDNF* relative to their MHFD counterparts ($p=0.00414$ and $p=0.00790$, respectively; **Figure 5.1a**). In MHFD + LNFPIII females, *insulin receptor* was increased compared to MLFD females ($p=0.00389$, **Figure 5.1a**). MHFD increased *glucocorticoid receptor* in males above their MLFD counterparts ($p=0.04641$) levels and this effect was sustained in MHFD + LNFPIII males ($p=0.02903$) (**Figure 5.1a**). The effect of treatment on *glucocorticoid receptor* in the dorsal hippocampi of females was different, with MHFD + LNFPIII females alone having significantly higher transcript levels of *glucocorticoid receptor* over their MLFD ($p=0.00141$) and MHFD ($p=0.00372$) counterparts (**Figure 5.1a**). There was a significant main effect of sex on dorsal hippocampal transcript levels of *BDNF* ($F(1,29)=7.065$, $p=0.01266$) and *IL-10* ($F(1,29)=5.2585$, $p=0.02928$) (**Figure 5.1**).

In the ventral hippocampus, there was a significant main effect of treatment on transcript levels of *glucocorticoid receptor* ($F(2,26)=5.3051$, $p=0.01169$). Post-hoc analysis showed that levels of ventral hippocampal *glucocorticoid receptor* transcript

were trending higher in MHFD + LNFPIII females when compared to their MLFD and MHFD counterparts (p=0.06330 and p=0.06667, respectively; **Figure 5.2a**).

There were no significant effects of maternal treatment or sex on dorsal or ventral hippocampal transcript levels of *leptin receptor*; *insulin receptor substrate 1*; *PPAR*α; the *serotonin receptors 1a*, *1b*, or *2b*; the apoptosis factor *Bcl2-11*; or the marker of endoplasmic reticulum stress *XBP1s* in the PND21 cohort.

Hippocampal gene expression profile of HFD-exposed male and female offspring two weeks post-weaning (PND35)

In the dorsal hippocampus, there was a significant main effect of treatment on transcript levels of *BDNF* (F(2,25)=7.6780, p=0.00251) and a treatment*sex interaction (F(2,25)=4.5904, p=0.02004). Post-hoc testing showed that both MHFD and MHFD + LNFPIII males had decreased dorsal hippocampal *BDNF* compared to MLFD males of this age cohort (p=0.02400 and p=0.00218, respectively; **Figure 5.3a**). There was also a significant main effect of treatment on transcript levels of *leptin receptor* (F(2,25)=5.794, p=0.00856), *insulin receptor* (F(2,26)=4.3833, p=0.02286, *glucocorticoid receptor* (F(2,25)=17.3239, p=0.00002), and *XBP1s* (F(2,30)=3.6534, p=0.03802) in this region. Compared to MLFD, MHFD + LNFPIII decreased *leptin receptor*, *insulin receptor*, and *XBP1s* transcript in the dorsal hippocampus of males (p=0.02462, p=0.04692, and p=0.03802, respectively; **Figures 5.3a and 5.3c**). *Glucocorticoid receptor* transcript levels were lower in both MHFD and MHFD + LNFPIII males and females compared to their MLFD counterparts (males: p=0.00694 and p=0.00025, respectively; females: p=0.05622 and p=0.05160, respectively; **Figure 5.3a**).

In the ventral hippocampi of PND35 offspring, there was a significant effect of treatment on transcript levels of *insulin receptor* ($F(2,30)=3.429$, $p=0.04557$), *glucocorticoid receptor* ($F(2,30)=3.7669$, $p=0.03471$), *5HT1a* ($F(2,30)=4.726$, $p=0.01644$), and *XBP1s* ($F(2,30)=14.9736$, $p=0.00003$). The combination MHFD + LNFPIII treatment reduced levels of *XBP1s* in both males and females compared to their MLFD and MHFD counterparts (males: $p=0.00594$ and $p=0.01048$, respectively; females: $p=0.01428$ and $p=0.01837$, respectively; **Figure 5.4c**).

There were no significant effects of maternal treatment or sex on dorsal or ventral hippocampal transcript levels of *insulin receptor substrate 1*, *PPAR*α, the *serotonin receptors 1b* or *2b*, *IL-10*, or the apoptosis factor *Bcl2-11* in the PND35 cohort.

5.4 Discussion

High fat diet intake in adult animals has been shown to negatively impact hippocampal function. The effect of maternal exposure to HFD (MHFD) in utero and prior to weaning on the hippocampi of offspring is less well studied, particularly in a way that compares the effects on both sexes and takes into account pre- and post-weaning time points. Here we analyzed the effect of MHFD on gene expression in the dorsal and ventral hippocampi of male and female offspring, both at the time of weaning and two weeks post-weaning, after the offspring were switched to a LFD. We also assayed the effect of maternal treatment with the immunomodulatory glycan LNFPIII, a natural and anti-inflammatory component of breast milk, on these parameters in the MHFD context.

Exposure to MHFD in utero and during weaning resulted in higher transcript levels of *glucocorticoid receptor* (*GR*) in the dorsal hippocampi of male offspring, but not female.

This effect was reversed in animals that were switched to a LFD for two weeks following weaning. Reduced *GR* transcript levels were observed at PND35 in MHFD females as well. In both males and females, MHFD + LNFPIII treatment resulted in significantly higher *GR* levels in the dorsal hippocampus at PND21 and this was true for the ventral hippocampus in females as well. The *GR* increase was reversed after two weeks on a LFD with reduced *GR* expression observed in both male and female offspring in the MHFD + LNFPIII treatment group at PND35.

Appropriate levels of glucocorticoids (not too high or too low) are required for neuronal maintenance, synaptic integrity, and dendritic spine stabilization in the developing hippocampus (Daskalakis et al., 2015) and are involved in the regulation of neurogenesis, migration, and cell death in the dentate gyrus, a hippocampal subregion (Gould et al., 1991). Environmentally-induced glucocorticoid alterations in offspring have mainly been studied in the context of early life stress and maternal care, where lack of maternal care, separation from the mother, and lack of experimenter handling result in lower expression of GR in the hippocampi of offspring (Meaney et al., 1985, Meaney et al., 1991, Maniam and Morris, 2010, Daskalakis et al., 2015). Normally, a stressor induces an acute rise in circulating corticosterone in rodents. This corticosterone binds GRs in the brain and has a dampening effect on stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, preventing stress hyper-responsiveness (Meaney et al., 1985, Herman et al., 2005, Zhu et al., 2014). The rise in GR transcript induced by MHFD (males) and MHFD + LNFPIII (males and females) prior to weaning could be interpreted as a sign of increased stress resilience in these animals. Interestingly, a HFD given to adolescent male rats immediately post-weaning ameliorated the stress effects of

early maternal separation (Maniam and Morris, 2010), also supporting the idea that HFD could contribute to stress resilience in this age group. In rats, Sasaki and colleagues found that GR expression was increased in the hippocampi of both male and female MHFD offspring after they had spent 2-3.5 weeks on a control chow diet (Sasaki et al., 2014). This discrepancy with our data showing that 2 weeks on a control diet reversed the MHFD and MHFD + LNFPIII impact on GR transcript could be attributed to species-specific effects and the fact that different control diets (their 13.5% fat vs. our 10% fat, sucrose-matched diet) were used in both studies. Additionally, and conversely from our study, maternal undernutrition (50% caloric restriction) results in reduced GR expression in the hippocampi of newborn pups (Lesage et al., 2001).

The observed increases in GR transcripts presented here were transient, meaning they either depended on presence of the maternal stimuli (HFD and HFD + LNFPIII) and/or were a factor of this developmental time point, since the opposite expression profile was found at PND35. Lower dorsal hippocampal *GR* in older MHFD and MHFD + LNFPIII offspring may signify that these early life exposures reduce resilience to stress later in life. This hypothesis is supported by other data showing that MHFD offspring demonstrate increased anxiety on elevated plus maze and open field tasks post-weaning (Bilbo and Tsang, 2010, Sasaki et al., 2013, Kang et al., 2014, Ramirez-Lopez et al., 2015). These data together provide evidence for the concept that MHFD exposure puts offspring at higher risk for mental health disorders (Sullivan et al., 2014).

Precise levels of GR are required for neuronal survival in the developing brain (Gould et al., 1991), and appropriate crosstalk between glucocorticoids and BDNF signaling is essential for early life HPA programming, neurotrophin signaling

(Daskalakis et al., 2015), and hippocampal excitability (Morsink et al., 2006). In this study, MHFD + LNFPIII treatment resulted in an increase in *BDNF* transcripts in the dorsal hippocampus of female offspring, but not males, and this effect was abolished when pups were switched to a LFD for two weeks after weaning. In post-weaning males, *BDNF* in the dorsal hippocampus was reduced by both MHFD and MHFD + LNFPIII treatments. Typically, glucocorticoid signaling is thought to downregulate BDNF (Smith et al., 1995, Morsink et al., 2006, Gourley et al., 2008, Gourley et al., 2009), however *GR* showed similar expression patterns as *BDNF* in our study. BDNF is a neurotrophin and a major regulator of activity-dependent synaptic plasticity, particularly in the hippocampus, where it is involved in long-term potentiation (Lebrun et al., 2006, Vanevski and Xu, 2013, Edelmann et al., 2014, Leal et al., 2015). In current literature, the effect of MHFD on offspring hippocampal BDNF is contested. Two studies, one in mice and one in rats, showed that MHFD reduced hippocampal BDNF levels at PND21 (Tozuka et al., 2010) and PND110-118 (Page et al., 2014), respectively, in male offspring. The control/LFD vs. HFDs used in these experiments had a smaller difference in fat content (28-35% difference) than what was used in our study (50% difference) and neither of the HFDs was matched in sucrose content to the control/LFD as ours was (Tozuka et al., 2010, Page et al., 2014). White and colleagues compared the effects of a 10% to 60% fat maternal diet (similar to ours but non-sucrose-matched) on the level of BDNF protein in the cortex of male rat offspring and saw no change in BDNF levels (White et al., 2009). Combining data from both male and female mouse offspring, Peleg-Raibstein, et al. showed an increase in *BDNF* transcript in the dorsal, but not ventral, hippocampus (Peleg-Raibstein et al., 2012) around PND90. As you can see, given the variety of experimental designs, it

not currently possible to draw any major conclusions about the effect of MHFD on hippocampal BDNF. However, MHFD-induced hippocampal *BDNF* reduction as observed in PND35 males agree most with data on HFD-exposed adult animals and reinforce findings demonstrating that HFD exposure impairs learning and memory.

In the present study, an increase *BDNF* transcript only arose in the dorsal hippocampus of PND21 females when HFD-fed dams were administered LNFPIII. One possible explanation lies in the mechanisms of LNFPIII's action, which involve an increase in IL-10 production by macrophages and dendritic cells (Bhargava et al., 2012). Our results show a significant effect of sex on *IL-10* expression. Though we did not observe a significant increase in *IL-10* transcript in the dorsal hippocampus in MHFD + LNFPIII females, we expect that the IP injections of LNFPIII increased IL-10 in the serum as has been previously shown (Bhargava et al., 2012). IL-10 can influence BDNF, particularly when an immune challenge is involved. The presence of IL-10 rescued lipopolysaccharide (LPS)-induced reductions in BDNF *in vitro* (Zhu et al., 2016) and *in vivo* (Richwine et al., 2009). de Almeida and colleagues showed that high intensity exercise during development increased hippocampal BDNF protein levels only in the developmental window when the stimulus also produced an increase in hippocampal IL-10 (de Almeida et al., 2013), providing evidence that through IL-10, maternal LNFPIII treatment could have a positive influence on BDNF expression. Why this effect was only seen in female offspring in our study remains to be determined. We have no reason to believe that the peripheral IL-10 stimulating function of LNFPIII is sex-specific.

Sex-specific effects were also observed in transcript levels of *insulin receptor* and *PPARα* in the dorsal hippocampus of PND21 offspring. The dual MHFD + LNFPIII

treatment significantly increased expression of *insulin receptor* in PND21 females and decreased *insulin receptor* expression in PND35 males, compared to their MLFD counterparts. While glucose utilization in the brain is not insulin-dependent, insulin modulates synaptic plasticity in the hippocampus and intrahippocampal insulin delivery or blockade can enhance or impair spatial memory, respectively (McNay, 2007). Hippocampal insulin resistance has been named as a mediator of cognitive dysfunction in both Type II Diabetes Mellitus and Alzheimer's Disease (Biessels and Reagan, 2015). LNFPIII has been shown to increase insulin sensitivity in a diet-induced obese mouse model (Bhargava et al., 2012), though this is the first evidence of this LNFPIII's possible insulin-sensitizing effect in the brain. Insulin pathways in females may be more responsive to certain stimuli, as estrogen treatment and LPS exposure can increase insulin sensitivity in females but not males (Nilsson et al., 2002, Pratchayasakul et al., 2011), and prenatal stress alters the expression of genes in the insulin pathway only in female offspring (Van den Hove et al., 2013). In humans, intranasal insulin improves hippocampus-dependent memory functions more so in females than in males (Benedict et al., 2008). It is possible that biochemically females are more receptive to the effects of LNFPIII, as evidenced by fact that the compounded effect of MHFD + LNFPIII treatment had a more significant impact on gene expression in female offspring compared to males at weaning. However, this possible insulin-sensitizing effect was transient, as it was not seen in PND35 females. The opposite effect was observed in PND35 male dorsal hippocampi. There was a trend ($p=0.07395$) for MHFD alone to reduce dorsal hippocampal *insulin receptor* in this group, which was previously shown at PND17 (Hami et al., 2013). These data provide support to other data demonstrating a negative

long-term impact of HFD on hippocampal insulin signaling (Pratchayasakul et al., 2011) and suggest a potential additive effect of LNFPIII on modulation of hippocampal *INSR* by HFD.

In the dorsal and ventral hippocampus of PND35 males, and in the ventral hippocampus of PND35 females, MHFD + LNFPIII treatment reduced expression of the marker for endoplasmic reticulum (ER) stress, the spliced form of X-box protein 1 (XPB1s). Upon initiation of the unfolded protein response during ER stress, *XBP1* mRNA becomes spliced and translocates to the nucleus of the cell to function as a transcription factor. XBP1s initiates the transcription of genes involved in many pathophysiological processes, including those observed in inflammatory diseases such as obesity (Wu et al., 2015). In the brain, presence of XBP1s has been correlated with cell death in injury models (Paschen et al., 2004, Ibuki et al., 2012) and known stressors such as restraint and exercise increase hippocampal XBP1s in rodents (Kim et al., 2010, Zhang et al., 2014). Reduction of *XBP1s* in the presence of LNFPIII is not surprising. LNFPIII's anti-inflammatory effects are mediated by activator protein 1 (AP1) (Bhargava et al., 2012), a member of the ERK-pathway that also regulates XBP1 expression and processing (Kim et al., 2006, Cunha et al., 2014). Lower *XBP1s* levels in MHFD + LNFPIII groups suggests that this combinatory treatment confers a reduction in hippocampal ER stress, though whether LNFPIII would provide this protection in a LFD scenario remains to be determined.

Finally, we observed a decrease in *leptin receptor* transcript levels in the dorsal hippocampi of MHFD + LNFPIII PND35 males when compared to their MLFD counterparts. While a decrease in leptin receptor could signify increased hippocampal

leptin resistance, without information regarding circulating leptin levels or leptin receptor functionality we cannot rightly draw this conclusion. One possible scenario in which LNFPIII might reduce leptin receptor expression in offspring is through its immunomodulatory effects in the dam. Considering that ER stress plays an important role in the development of leptin resistance (Ozcan et al., 2009), if LNFPIII enhanced leptin signaling profiles in HFD-fed dams through reduction of inflammation and ER stress, reduced *leptin receptor* in the offspring may be the result of compensatory down-regulation due to enhanced products of leptin receptor signaling in the maternal environment. This speculative hypothesis would be improved by collection of data regarding leptin signaling profiles of the dams and the offspring.

In summary, our data and others show that a maternal high fat diet influences the developing hippocampus and highlights the importance of 1) examining effects of stimuli on both sexes in biological research and 2) examining effects of stimuli on both sexes separately, as combining potentially opposing effects could lead to unintelligible results. We plan to replicate our study to enhance its statistical power and to add a MLFD + LNFPIII treatment group so that we can tease apart the effects of maternal diet against the effects of maternal treatment with the immunomodulatory glycan, and to extend our molecular and behavioral analysis of these offspring. With our current data, it is impossible to know if the effects of MHFD + LNFPIII on expression of *BDNF*, *insulin receptor*, and *glucocorticoid receptor* in PND21 females is due to actions of LNFPIII alone or a synergistic combination of MHFD and LNFPIII treatment. Another important conclusion is that the effects of treatment were either abolished or reversed by weaning the mice onto a LFD. Other findings have demonstrated that the most profound and long-

lasting effects of MHFD appear when the offspring is directly exposed to a HFD in adolescence and adulthood (Kruse et al., 2013, Kang et al., 2014, Page et al., 2014, Lepinay et al., 2015) calling back into frame the *thrifty phenotype hypothesis*. It may be the case that offspring exposed to an obese physiological state in utero are, like offspring exposed to maternal undernutrition, primed to differently respond to an obesogenic environment, increasing their own likelihood for obesity and perpetuating the cycle of overweight and obesity that we so drastically see in developed and developing nations.

Table 5.1. Maternal diet composition. Data of interest regarding maternal diets as provided by Research Diets and available online. Unlisted ingredients that were present to the same extent in both diets include Mineral Mix S10026, dicalcium phosphate, calcium carbonate, potassium citrate 1 H2O, Vitamin Mix V10001, choline bitartate, and FD&C food colorings. Dams were fed respective diets for six weeks prior to mating, throughout gestation, and lactation up to age P21 of the offspring.

	MLFD		**MHFD**	
	Gram %	*kCal %*	Gram %	*kCal %*
Macronutrients				
Protein	19.2	*20*	26	*20*
Carbohydrate	67.3	*70*	26	*20*
Fat	4.3	*10*	35	*60*
kCal/gram	4.73		5.24	
Ingredients of Interest				
Casein, 30 Mesh	200	*800*	200	*800*
L-Cysteine	3	*12*	3	*12*
Cornstarch	506.2	*2024.8*	0	*0*
Maltodextrin 10	125	*500*	125	*500*
Sucrose	68.8	*275.2*	68.8	*275*
Cellulose, BW200	50	*0*	50	*0*
Soybean Oil	25	*225*	25	*225*
Lard	20	*180*	245	*2205*

Table 5.2. Gene abbreviations and primer sequences. Primer sequences generated by IDT© and used for quantitative real-time polymerase chain reaction experiments.

Gene abbreviation	Gene name	Primer sequence
5HT1a	*5-hydroxytryptamine receptor (serotonin receptor) subtype 1a*	CTCCCTCTTGCTCTAGAAACATC GGGCACCATAACCCAAAGTA
5HT1b	*5-hydroxytryptamine receptor (serotonin receptor) subtype 1b*	CCTGGTGATGCCTATCTGTAAG CCTCATTGGACATGGTGTAGAT
5HT2b	*5-hydroxytryptamine receptor (serotonin receptor) subtype 2b*	CAGACTCAGTAGCAGAGGAAATG GGGTATTATCACCGCGAGTATC
BCL2-11	*B-cell lymphoma 2-like protein 11*	GAGCTGGGAGTCTTGTGTACTA GCCCATATGCTGGGTGTATTT
BDNF	*Brain-derived neurotrophic factor*	CTGAGCGTGTGTGACAGTATTA CTTTGGATACCGGGACTTTCTC
GR, NR3C1	*Glucocorticoid receptor*	CCAGACTCAGCATGGAGAATTA GTATCGCCTTTGCCCATTTC
IL-10	*Interleukin-10*	CCAAGACCAAGGTGTCTACAA GGAGTCCAGCAGACTCAATAC
INSR	*Insulin receptor*	CCACACTCACACTTCCAGATT GACTCCTTGTTCACCACTTTCT
IRS1	*Insulin receptor substrate 1*	GAGAGTGGTGGAGTTGAGTTG TGTAGTCACCACGGCTATTTG
OBRB	*Leptin receptor, long-form*	CTCTGCACTCACAGACAACA CAGCACTCTATGTCCCAGTTT
PPARα	*Peroxisome proliferator-activated receptor alpha*	GGTTCCTGGTGCCGATTTAT CACAGACTAGCATCCCACTTAAT
XBP1s	*X-box binding protein 1, spliced form*	CCTTCAGTGACATGTCTTCTCC CCCAGTGTTATGTGGCTCTTTA

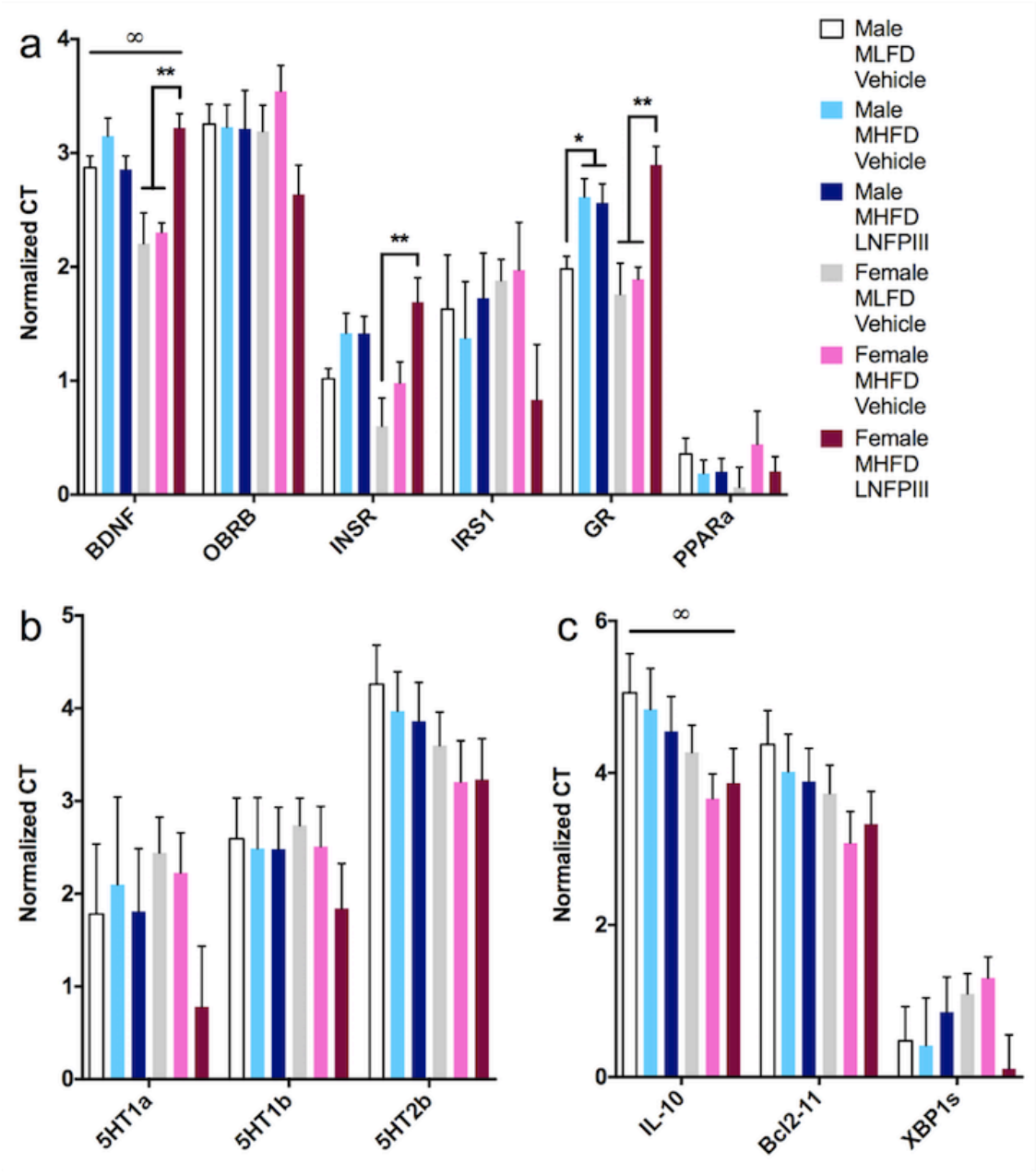


Figure 5.1. qRT-PCR analysis of dorsal hippocampus in PND21 offspring. We examined the transcript expression for genes related to synaptic plasticity and metabolic processes (**a**), serotonin receptors (**b**), and inflammation and metabolic stress (**c**). White bars represent MLFD male offspring, light blue bars represent MHFD males while light pink bars represent MHFD female offspring, and dark blue bars represent MHFD + LNFPIII male while burgundy bars represent MHFD + LNFPIII female offspring.

Statistics were performed using a two-way ANOVA. Statistical significance of $p<0.05$ is represented by one asterisk (*) while $p<0.01$ is represented by two asterisks (**). Main effect of sex is denoted with the infinity symbol (∞).

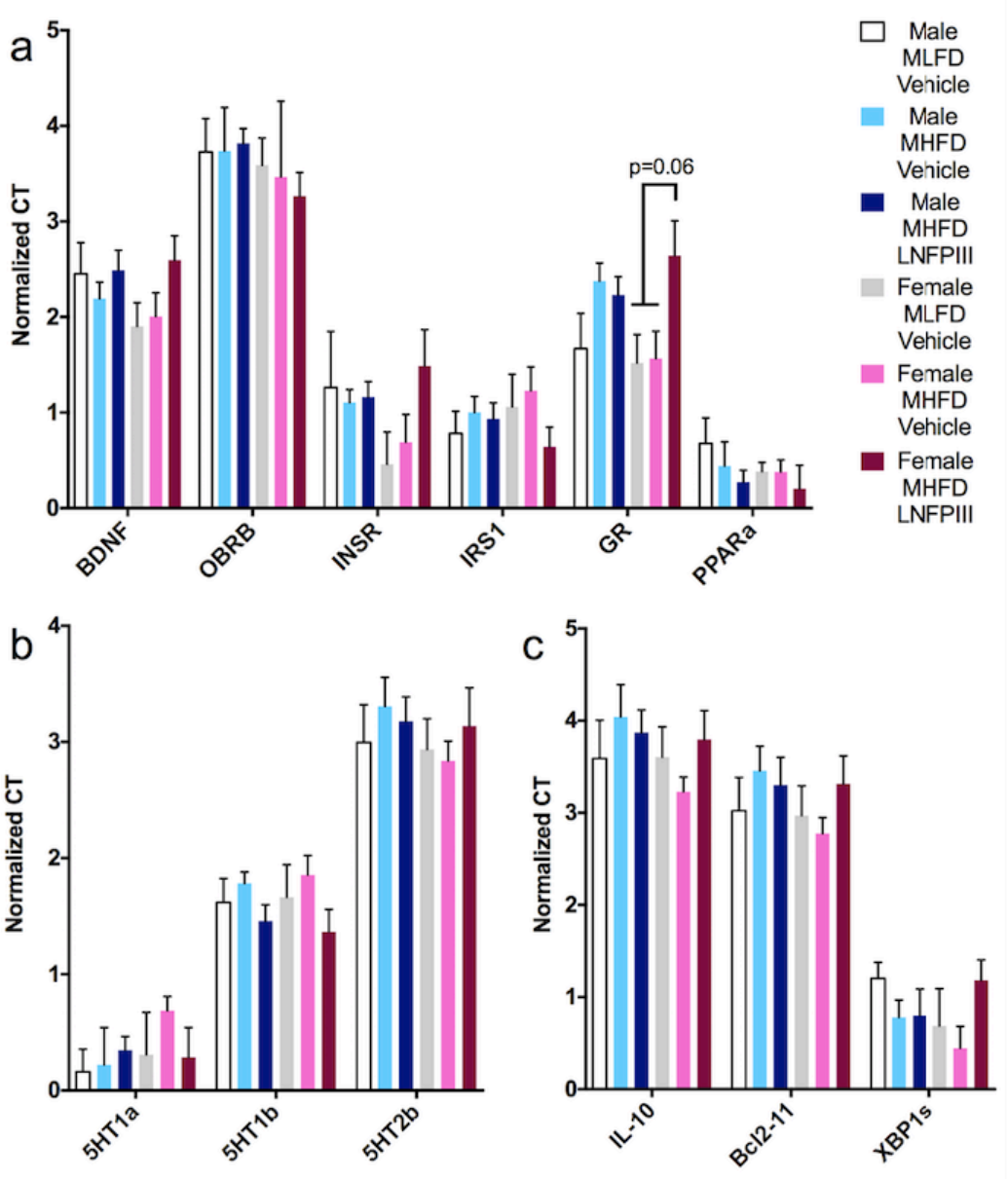


Figure 5.2. qRT-PCR analysis of ventral hippocampus in PND21 offspring. We examined the transcript expression for genes related to synaptic plasticity and metabolic

processes (**a**), serotonin receptors (**b**), and inflammation and metabolic stress (**c**). White bars represent MLFD male offspring, light blue bars represent MHFD males while light pink bars represent MHFD female offspring, and dark blue bars represent MHFD + LNFPIII male while burgundy bars represent MHFD + LNFPIII female offspring. Statistics were performed using a two-way ANOVA. Statistical significance of $p<0.05$ is represented by one asterisk (*) while $p<0.01$ is represented by two asterisks (**). Main effect of sex is denoted with the infinity symbol (∞).

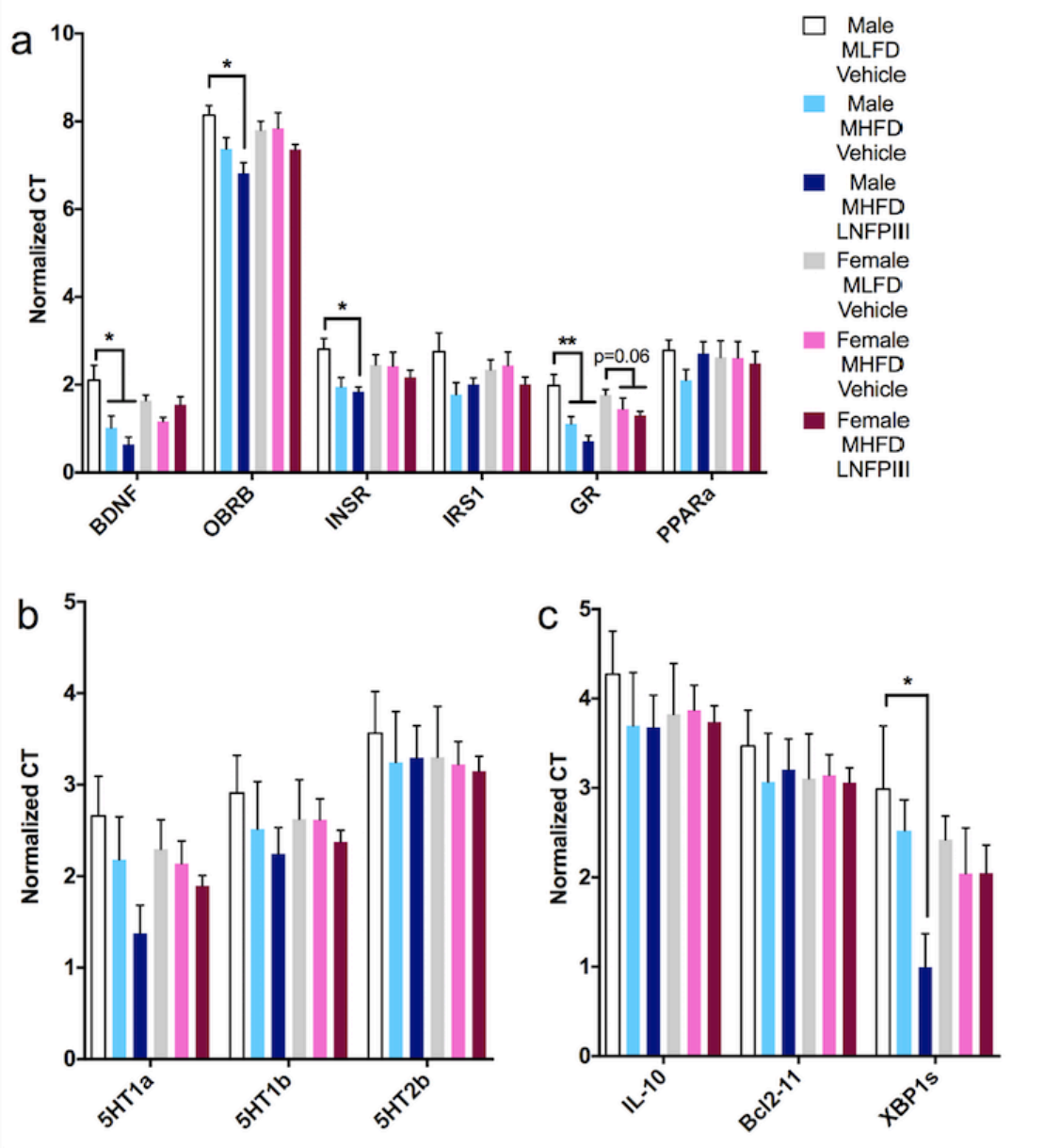


Figure 5.3. qRT-PCR analysis of dorsal hippocampus in PND35 offspring. We examined the transcript expression for genes related to synaptic plasticity and metabolic processes (**a**), serotonin receptors (**b**), and inflammation and metabolic stress (**c**). White bars represent MLFD male offspring, light blue bars represent MHFD males while light pink bars represent MHFD female offspring, and dark blue bars represent MHFD + LNFPIII male while burgundy bars represent MHFD + LNFPIII female offspring.

Statistics were performed using a two-way ANOVA. Statistical significance of $p<0.05$ is represented by one asterisk (*) while $p<0.01$ is represented by two asterisks (**). Main effect of sex is denoted with the infinity symbol (∞).

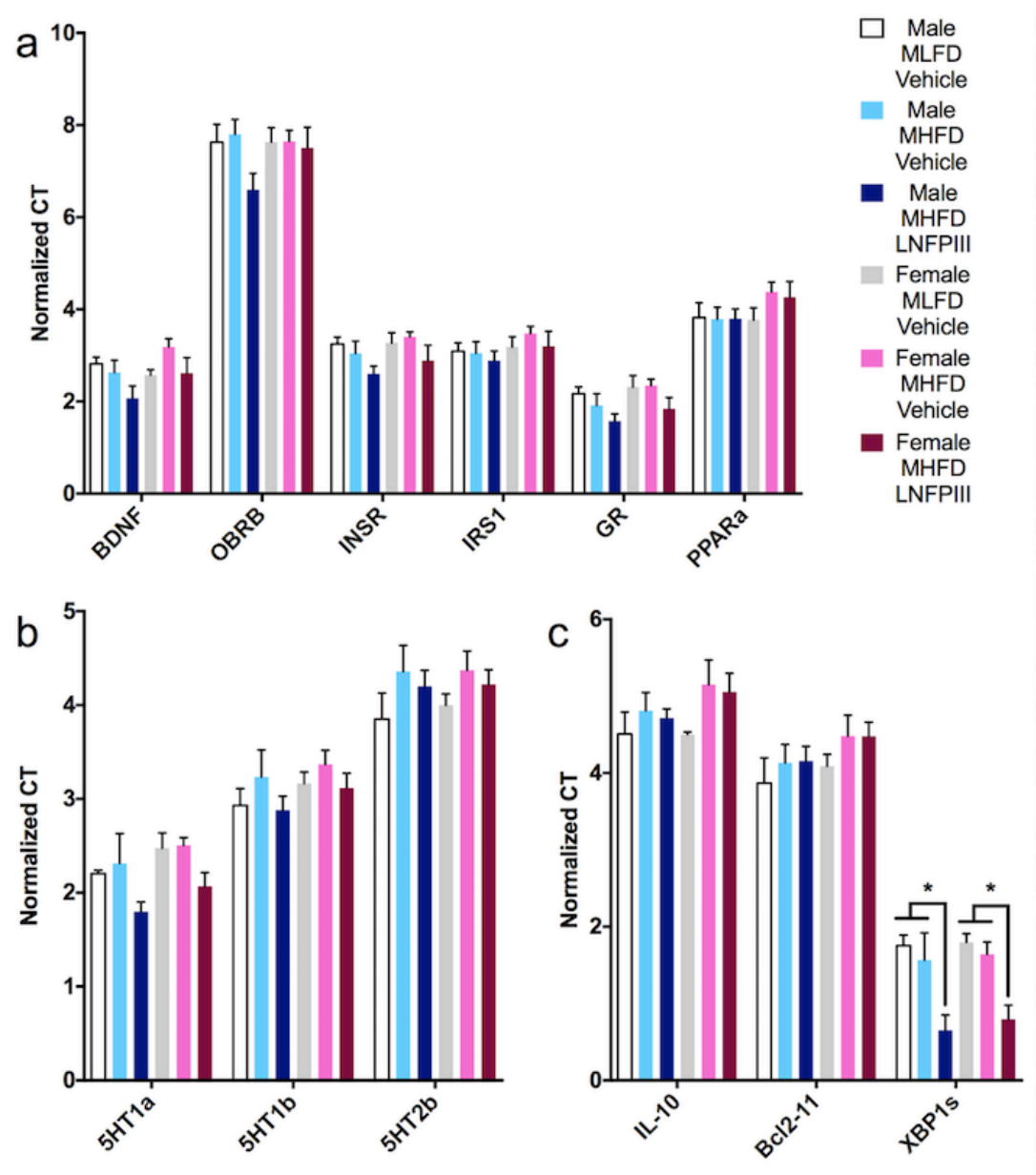


Figure 5.4. qRT-PCR analysis of ventral hippocampus in PND35 offspring. We examined the transcript expression for genes related to synaptic plasticity and metabolic

processes (**a**), serotonin receptors (**b**), and inflammation and metabolic stress (**c**). White bars represent MLFD male offspring, light blue bars represent MHFD males while light pink bars represent MHFD female offspring, and dark blue bars represent MHFD + LNFPIII male while burgundy bars represent MHFD + LNFPIII female offspring. Statistics were performed using a two-way ANOVA. Statistical significance of $p<0.05$ is represented by one asterisk (*) while $p<0.01$ is represented by two asterisks (**). Main effect of sex is denoted with the infinity symbol (∞).

References

Atochina O, Da'dara AA, Walker M, Harn DA (2008) The immunomodulatory glycan LNFPIII initiates alternative activation of murine macrophages in vivo. Immunology 125:111-121.

Barker DJ (1990) The fetal and infant origins of adult disease. BMJ 301:1111.

Benedict C, Kern W, Schultes B, Born J, Hallschmid M (2008) Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. J Clin Endocrinol Metab 93:1339-1344.

Bhargava P, Li C, Stanya KJ, Jacobi D, Dai L, Liu S, Gangl MR, Harn DA, Lee CH (2012) Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. Nat Med 18:1665-1672.

Biessels GJ, Reagan LP (2015) Hippocampal insulin resistance and cognitive dysfunction. Nat Rev Neurosci 16:660-671.

Bilbo SD, Tsang V (2010) Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. FASEB J 24:2104-2115.

Bolton JL, Bilbo SD (2014) Developmental programming of brain and behavior by perinatal diet: focus on inflammatory mechanisms. Dialogues Clin Neurosci 16:307-320.

Cai D (2013) Neuroinflammation and neurodegeneration in overnutrition-induced diseases. Trends Endocrinol Metab 24:40-47.

Cunha DA, Gurzov EN, Naamane N, Ortis F, Cardozo AK, Bugliani M, Marchetti P, Eizirik DL, Cnop M (2014) JunB protects beta-cells from lipotoxicity via the XBP1-AKT pathway. Cell Death Differ 21:1313-1324.

Daskalakis NP, De Kloet ER, Yehuda R, Malaspina D, Kranz TM (2015) Early Life Stress Effects on Glucocorticoid-BDNF Interplay in the Hippocampus. Front Mol Neurosci 8:68.

de Almeida AA, Gomes da Silva S, Fernandes J, Peixinho-Pena LF, Scorza FA, Cavalheiro EA, Arida RM (2013) Differential effects of exercise intensities in hippocampal BDNF, inflammatory cytokines and cell proliferation in rats during the postnatal brain development. Neurosci Lett 553:1-6.

Edelmann E, Lessmann V, Brigadski T (2014) Pre- and postsynaptic twists in BDNF secretion and action in synaptic plasticity. Neuropharmacology 76 Pt C:610-627.

Gould E, Woolley CS, McEwen BS (1991) The hippocampal formation: morphological changes induced by thyroid, gonadal and adrenal hormones. Psychoneuroendocrinology 16:67-84.

Gourley SL, Kedves AT, Olausson P, Taylor JR (2009) A history of corticosterone exposure regulates fear extinction and cortical NR2B, GluR2/3, and BDNF. Neuropsychopharmacology 34:707-716.

Gourley SL, Wu FJ, Kiraly DD, Ploski JE, Kedves AT, Duman RS, Taylor JR (2008) Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. Biol Psychiatry 63:353-359.

Hales CN, Barker DJ (2001) The thrifty phenotype hypothesis. Br Med Bull 60:5-20.

Hami J, Sadr-Nabavi A, Sankian M, Balali-Mood M, Haghir H (2013) The effects of maternal diabetes on expression of insulin-like growth factor-1 and insulin receptors in male developing rat hippocampus. Brain Struct Funct 218:73-84.

Herman JP, Ostrander MM, Mueller NK, Figueiredo H (2005) Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Prog Neuropsychopharmacol Biol Psychiatry 29:1201-1213.

Honein MA, Devine O, Sharma AJ, Rasmussen SA, Park S, Kucik JE, Boyle C (2013) Modeling the potential public health impact of prepregnancy obesity on adverse fetal and infant outcomes. Obesity (Silver Spring) 21:1276-1283.

Ibuki T, Yamasaki Y, Mizuguchi H, Sokabe M (2012) Protective effects of XBP1 against oxygen and glucose deprivation/reoxygenation injury in rat primary hippocampal neurons. Neurosci Lett 518:45-48.

Kang SS, Kurti A, Fair DA, Fryer JD (2014) Dietary intervention rescues maternal obesity induced behavior deficits and neuroinflammation in offspring. J Neuroinflammation 11:156.

Kanoski SE, Davidson TL (2011) Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. Physiol Behav 103:59-68.

Kim R, Emi M, Tanabe K, Murakami S (2006) Role of the unfolded protein response in cell death. Apoptosis 11:5-13.

Kim Y, Park M, Boghossian S, York DA (2010) Three weeks voluntary running wheel exercise increases endoplasmic reticulum stress in the brain of mice. Brain Res 1317:13-23.

Kruse M, Seki Y, Vuguin PM, Du XQ, Fiallo A, Glenn AS, Singer S, Breuhahn K, Katz EB, Charron MJ (2013) High-fat intake during pregnancy and lactation exacerbates high-fat diet-induced complications in male offspring in mice. Endocrinology 154:3565-3576.

Leal G, Afonso PM, Salazar IL, Duarte CB (2015) Regulation of hippocampal synaptic plasticity by BDNF. Brain Res 1621:82-101.

Lebrun B, Bariohay B, Moyse E, Jean A (2006) Brain-derived neurotrophic factor (BDNF) and food intake regulation: a minireview. Auton Neurosci 126-127:30-38.

Leddy MA, Power ML, Schulkin J (2008) The impact of maternal obesity on maternal and fetal health. Rev Obstet Gynecol 1:170-178.

Lepinay AL, Larrieu T, Joffre C, Acar N, Garate I, Castanon N, Ferreira G, Langelier B, Guesnet P, Bretillon L, Parnet P, Laye S, Darnaudery M (2015) Perinatal high-fat diet increases hippocampal vulnerability to the adverse effects of subsequent high-fat feeding. Psychoneuroendocrinology 53:82-93.

Lesage J, Blondeau B, Grino M, Breant B, Dupouy JP (2001) Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. Endocrinology 142:1692-1702.

Maniam J, Morris MJ (2010) Voluntary exercise and palatable high-fat diet both improve behavioural profile and stress responses in male rats exposed to early life stress: role of hippocampus. Psychoneuroendocrinology 35:1553-1564.

McNay EC (2007) Insulin and ghrelin: peripheral hormones modulating memory and hippocampal function. Curr Opin Pharmacol 7:628-632.

Meaney MJ, Aitken DH, Bodnoff SR, Iny LJ, Tatarewicz JE, Sapolsky RM (1985) Early postnatal handling alters glucocorticoid receptor concentrations in selected brain regions. Behav Neurosci 99:765-770.

Meaney MJ, Mitchell JB, Aitken DH, Bhatnagar S, Bodnoff SR, Iny LJ, Sarrieau A (1991) The effects of neonatal handling on the development of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. Psychoneuroendocrinology 16:85-103.

Morsink MC, Steenbergen PJ, Vos JB, Karst H, Joels M, De Kloet ER, Datson NA (2006) Acute activation of hippocampal glucocorticoid receptors results in different waves of gene expression throughout time. J Neuroendocrinol 18:239-252.

Nilsson C, Jennische E, Ho HP, Eriksson E, Bjorntorp P, Holmang A (2002) Postnatal endotoxin exposure results in increased insulin sensitivity and altered activity of neuroendocrine axes in adult female rats. Eur J Endocrinol 146:251-260.

Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, Myers MG, Jr., Ozcan U (2009) Endoplasmic reticulum stress plays a central role in development of leptin resistance. Cell Metab 9:35-51.

Page KC, Jones EK, Anday EK (2014) Maternal and postweaning high-fat diets disturb hippocampal gene expression, learning, and memory function. Am J Physiol Regul Integr Comp Physiol 306:R527-537.

Paschen W, Yatsiv I, Shoham S, Shohami E (2004) Brain trauma induces X-box protein 1 processing indicative of activation of the endoplasmic reticulum unfolded protein response. J Neurochem 88:983-992.

Peleg-Raibstein D, Luca E, Wolfrum C (2012) Maternal high-fat diet in mice programs emotional behavior in adulthood. Behav Brain Res 233:398-404.

Pratchayasakul W, Chattipakorn N, Chattipakorn SC (2011) Effects of estrogen in preventing neuronal insulin resistance in hippocampus of obese rats are different between genders. Life Sci 89:702-707.

Ramirez-Lopez MT, Vazquez M, Bindila L, Lomazzo E, Hofmann C, Blanco RN, Alen F, Anton M, Decara J, Ouro D, Orio L, Suarez J, Lutz B, Rodriguez de Fonseca F, Gomez de Heras R (2015) Exposure to a Highly Caloric Palatable Diet During Pregestational and Gestational Periods Affects Hypothalamic and Hippocampal Endocannabinoid Levels at Birth and Induces Adiposity and Anxiety-Like Behaviors in Male Rat Offspring. Front Behav Neurosci 9:339.

Richwine AF, Sparkman NL, Dilger RN, Buchanan JB, Johnson RW (2009) Cognitive deficits in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide. Brain Behav Immun 23:794-802.

Sasaki A, de Vega W, Sivanathan S, St-Cyr S, McGowan PO (2014) Maternal high-fat diet alters anxiety behavior and glucocorticoid signaling in adolescent offspring. Neuroscience 272:92-101.

Sasaki A, de Vega WC, St-Cyr S, Pan P, McGowan PO (2013) Perinatal high fat diet alters glucocorticoid signaling and anxiety behavior in adulthood. Neuroscience 240:1-12.

Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768-1777.

Sullivan EL, Nousen EK, Chamlou KA (2014) Maternal high fat diet consumption during the perinatal period programs offspring behavior. Physiol Behav 123:236-242.

Tozuka Y, Kumon M, Wada E, Onodera M, Mochizuki H, Wada K (2010) Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring. Neurochem Int 57:235-247.

Tozuka Y, Wada E, Wada K (2009) Diet-induced obesity in female mice leads to peroxidized lipid accumulations and impairment of hippocampal neurogenesis during the early life of their offspring. FASEB J 23:1920-1934.

Van den Hove DL, Kenis G, Brass A, Opstelten R, Rutten BP, Bruschettini M, Blanco CE, Lesch KP, Steinbusch HW, Prickaerts J (2013) Vulnerability versus resilience to prenatal stress in male and female rats; implications from gene expression profiles in the hippocampus and frontal cortex. Eur Neuropsychopharmacol 23:1226-1246.

Vanevski F, Xu B (2013) Molecular and neural bases underlying roles of BDNF in the control of body weight. Front Neurosci 7:37.

Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH (1997) Predicting obesity in young adulthood from childhood and parental obesity. N Engl J Med 337:869-873.

White CL, Pistell PJ, Purpera MN, Gupta S, Fernandez-Kim SO, Hise TL, Keller JN, Ingram DK, Morrison CD, Bruce-Keller AJ (2009) Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: contributions of maternal diet. Neurobiol Dis 35:3-13.

Wu R, Zhang QH, Lu YJ, Ren K, Yi GH (2015) Involvement of the IRE1alpha-XBP1 pathway and XBP1s-dependent transcriptional reprogramming in metabolic diseases. DNA Cell Biol 34:6-18.

Zhang Y, Liu W, Zhou Y, Ma C, Li S, Cong B (2014) Endoplasmic reticulum stress is involved in restraint stress-induced hippocampal apoptosis and cognitive impairments in rats. Physiol Behav 131:41-48.

Zhu LJ, Liu MY, Li H, Liu X, Chen C, Han Z, Wu HY, Jing X, Zhou HH, Suh H, Zhu DY, Zhou QG (2014) The different roles of glucocorticoids in the hippocampus and hypothalamus in chronic stress-induced HPA axis hyperactivity. PLoS One 9:e97689.

Zhu Y, Chen X, Liu Z, Peng YP, Qiu YH (2016) Interleukin-10 Protection against Lipopolysaccharide-Induced Neuro-Inflammation and Neurotoxicity in Ventral Mesencephalic Cultures. Int J Mol Sci 17.

CHAPTER 6

CONCLUSIONS AND FUTURE DIRECTIONS

In the previous chapters we learned that a diet high in fat influences hippocampal gene expression in rodents during development and in adulthood. Following only 72 hours on a high fat diet, adult male rats show distinct gene transcription differences in functional poles of the hippocampus, with increased *galanin* and *brain-derived neurotrophic factor* in the dorsal hippocampus and increased *histone deacetylase 4* in the ventral hippocampus of high fat-fed rats. In addition, *galanin receptor 1* was decreased in the ventral hippocampus of high-fat fed rats. These animals also had increased circulating leptin and cholesterol prior to significant increases in body weight or adipose tissue mass. After 11 days on a high fat diet, *brain-derived neurotrophic factor* was decreased in the ventral hippocampus of high-fat fed adult male rats. These data suggest that the hippocampal transcript profile of rats on a high fat diet changes over time with continued exposure to the diet. Perhaps initially on a high fat diet, learning and memory functions are promoted and protected, suggested by increased *galanin* and *brain-derived neurotrophic factor* in the dorsal hippocampus. If synaptic plasticity in the dorsal hippocampus is enhanced, it could render the animal better able to remember the location and context of this high-calorie food source, an outcome that should promote survival. Over time and with continued consumption, however, high fat diet becomes detrimental, contributing to metabolic disease. In this state, plasticity in the ventral hippocampus is reduced, suggested by the decreased *brain-derived neurotrophic factor* in the ventral

hippocampus of subjects in the conditioned taste aversion experiment, and this region becomes less well suited to respond appropriately to visceral signals, contributing to the notion presented in Chapter 2 that inappropriate genetic reprogramming in the brain contributes to and is a product of obesity. **Figure 6.1** simplifies our initial model in **Figure 2.1** and summarizes these ideas. To test this hypothesis and to pinpoint the turning point at which dietary fats become detrimental to the brain, future studies should measure transcript and protein levels of neurotrophic and neuroprotective factors over a time course of high fat diet consumption, beginning as early as within one hour of first exposure.

It remains to be determined what aspects of high fat diet are driving these genetic changes in the hippocampus. A recent study showed that the number of macrophages/microglia was increased in the hypothalamus and nodose ganglion after only 24 hours on a high fat diet (Waise et al, 2015). During neuroinflammation, activated microglia initiate the release of cytokines. Some of these pro- and anti-inflammatory factors, such as nuclear factor kappa B (NFkB) are themselves transcription factors and others, such as interleukins, directly activate transcription factors like those of the STAT family (Hennighausen and Robinson 2008, Zhang et al, 2008). It has been suggested that cytokine signaling cascades may modulate memory networks during an initial or acute inflammatory event, but that epigenetic reprogramming, as suggested in our Chapter 2, may be necessary to mediate the long-lasting effects of inflammation on memory processes in the brain (Donzis and Tronson 2014).

In the preceding chapter we showed that high fat diet consumed by a pregnant female also impacts the hippocampal transcription profiles of her offspring. Exposure to

high fat diet during gestation and prior to weaning increased *glucocorticoid receptor* transcript in the dorsal hippocampus of male mouse offspring. Different effects of high fat diet exposure are observed two weeks after weaning and without continued exposure to the obesogenic diet. At 35 days of age, weaned offspring of high fat-fed dams have decreased dorsal hippocampal *glucocorticoid receptor* (males and females) and decreased dorsal hippocampal *brain-derived neurotrophic factor* (males only). As discussed in Chapter 5, misregulation of these two transcripts in the high fat diet-exposed young brain likely contributes to the changes in hippocampal neurogenesis and behavior observed in other studies (Gould et al., 1991, Page et al., 2014, Daskalakis et al., 2015, Lepinay et al., 2015). In addition to establishing a more detailed time course of gene expression and protein profiling in the hippocampi high fat diet-exposed animals, additional data should be gathered exploring the impact of high fat diet on chromatin modifications in this region. As discussed, the hippocampal epigenome appears to be exceptionally responsive to environmental stimuli. Not only would epigenetic studies help to elucidate the mechanism behind diet-induced changes to the brain, but identification of new and unique pharmaceutical targets could aid in the prevention of obesity and associated neurodegenerative disease.

In the behavioral study presented in Chapter 4, we uncovered a novel effect of brief (<2 hours) high fat diet exposure to prevent binge-like consumption of an alternate palatable food source and to sustain this effect over an 11-day experimental paradigm. In addition, high-fat fed animals did not readily extinguish a conditioned taste aversion, an observation that may be attributed to a deficit in ventral hippocampal neurotrophic factor. We did not find that peripheral inhibition of the chromatin modifying enzyme histone

deacetylase had any impact on these parameters. Future experiments that aim to uncover the epigenetic mechanisms by which we and other animals learn about and respond to diet may wish to target the hippocampus or other brain regions or circuits directly.

It should be noted that the diets used in all of our studies were formulated to contain an excess of fat and that the singularity of this focus likely does not fully represent the human experience. Typically the foods with which we treat ourselves during our indulgent holidays and vacations are also high in sugar and/or salt, making them particularly palatable. Some research studies attempt to address this issue by providing their research model animals with high energy or Western diets (formulated to be high in sugar and fat) or Cafeteria diets (where animals are offered human foods). Importantly, differences in feeding behavior and metabolic measures have been noted with the different diets (Higa 2014). However, when multiple factors, such as both fat and sugar are introduced, it becomes difficult to determine which nutrient is contributing more prominently or to which aspects of the observed behavioral and molecular effects. In addition, the nutritive and non-nutritive components of Cafeteria diets are not well defined. Components of the diet will vary by laboratory and the animal may not consume equal selections of each food item each day. Our choice to provide our treatment groups with an excess of dietary fat alone allowed us to attribute our findings directly to the dietary fat itself and so put us a step ahead in pinpointing the molecular mechanism behind our observations. Both single-nutrient and varied diet studies are valuable and contribute to scientific knowledge regarding feeding behaviors and the consequences of caloric overload and obesity.

In closing, the relationship between dietary fat and the hippocampus is complex and changes over time. It remains to be determined whether or not acute high fat diet consumption and its associated weight gain leave as lasting an impact on the brain as it does the waistline. In the future we will learn more about how the dietary environment influences gene expression through chromatin modifications. We will study the persistence and the instability of these epigenetic marks to determine their ultimate contributions to obesity-related neurodegenerative diseases. We will target these gatekeepers of the genetic code in an effort to assist individuals battling weight gain and disordered eating behaviors. Understanding the swiftness with which our learning and memory circuitry responds to the dietary environment may afford us the power to intercept detrimental neuronal reprogramming and prevent the slippery slope on which many find themselves sliding into a state of obesity and metabolic disease.

References

Daskalakis NP, De Kloet ER, Yehuda R, Malaspina D, Kranz TM (2015) Early Life Stress Effects on Glucocorticoid-BDNF Interplay in the Hippocampus. Front Mol Neurosci 8:68.

Donzis EJ, Tronson NC (2014) Modulation of learning and memory by cytokines: signaling mechanisms and long term consequences. Neurobiol Learn Mem 115:68-77.

Gould E, Woolley CS, McEwen BS (1991) The hippocampal formation: morphological changes induced by thyroid, gonadal and adrenal hormones. Psychoneuroendocrinology 16:67-84.

Hennighausen L, Robinson GW (2008) Interpretation of cytokine signaling through the transcription factors STAT5A and STAT5B. Genes & Dev 22:711-721.

Higa TS, Spinola AV, Fonseca-Alaniz MH, Evangelista FS (2014) Comparison between cafeteria and high-fat diets in the induction of metabolic dysfunction in mice. Int J Physiol Pathophysiol Pharmacol 6(1):47-54.

Lepinay AL, Larrieu T, Joffre C, Acar N, Garate I, Castanon N, Ferreira G, Langelier B, Guesnet P, Bretillon L, Parnet P, Laye S, Darnaudery M (2015) Perinatal high-fat diet increases hippocampal vulnerability to the adverse effects of subsequent high-fat feeding. Psychoneuroendocrinology 53:82-93.

Page KC, Jones EK, Anday EK (2014) Maternal and postweaning high-fat diets disturb hippocampal gene expression, learning, and memory function. Am J Physiol Regul Integr Comp Physiol 306:R527-537.

Zaved Waise TM, Toshinai K, Naznin F, NamKoong C, Moin ASM, Sakoda H, Nakazato M (2015) One-day high-fat diet induces inflammation in the nodose ganglion and hypothalamus of mice. Biochem Biophys Res Commun 464:1157-1162.

Zhang X, Zhang G, Zhang H, Karin M, Bai H,Cai D (2008) Hypothalamic Ikkb/NFkB and ER stress link overnutrition to energy imbalance and obesity. Cell 135:61-73.

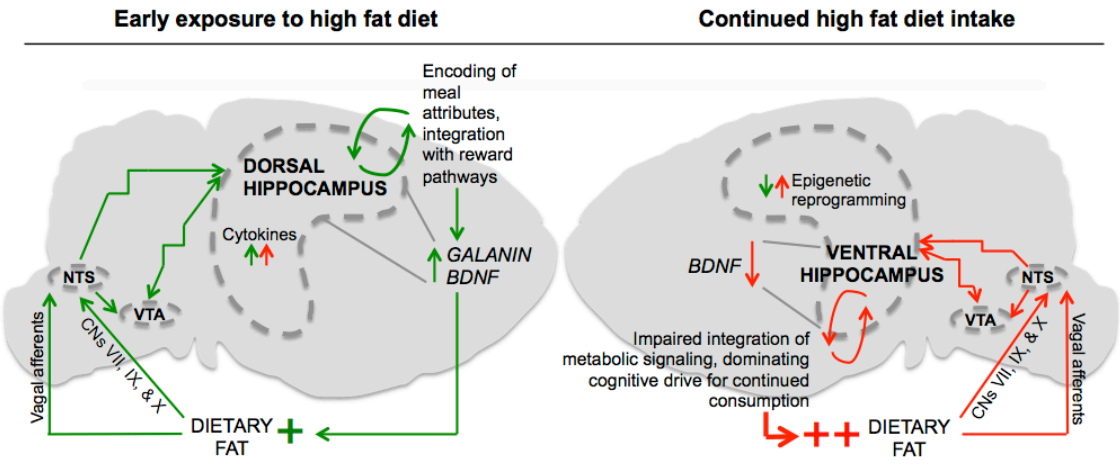


Figure 6.1. Hippocampal response to high fat diet intake: acute exposure versus continued consumption. Upon initial exposure to a high fat diet (left), learning and memory functions are promoted and protected, suggested by increased *galanin* and *brain-derived neurotrophic factor* in the dorsal hippocampus, promoting retention of the location and context of this high-calorie food source, as well as its rewarding properties. Over time and with continued consumption (right), plasticity in the ventral hippocampus is reduced, suggested by the decreased *brain-derived neurotrophic factor* in the ventral hippocampus and this region becomes less well suited to respond appropriately to visceral signals and the cognitive drive to continue consumption of the rewarding high fat food drives further intake. In both of these systems, taste information via cranial nerves (CNs) VII, IX, and X is first sensed in the nucleus of the solitary tract (NTS) in the brainstem and this information is processed in other brain regions before being received in the hippocampus. In addition, signals about nutrient intake are sensed by the gut and sent to the brain via the vagus nerve. Importantly, the ventral tegmental area (VTA) can be directly activated by taste and impact the release of dopamine output in the forebrain. Both the dorsal and the ventral hippocampus also relay information regarding the

rewarding properties of the diet with the VTA to create a positive memory surrounding the eating event. Upon initial diet exposure, both pro- and anti-inflammatory cytokines increase in many brain regions, including the hippocampus, and may help to modulate genetic output. Over the long term, epigenetic reprogramming likely mediates some of the cognitive deficits associated with chronic high fat diet intake.