The Effects of High-Fat Feeding on Synaptic Function in Female Rats and Their Offspring

by
Isabelle Lorraine Messa-Hamidi

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of my thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The prevalence of obesity has been increasing across the globe at an unprecedented rate, and obesity is associated with a number of diseases, including diabetes, hypertension, and certain types of cancer. In recent years, increasing evidence has shown that obesity can also cause cognitive impairment, and research is increasingly aimed at elucidating both the nature of, and the mechanisms behind, these impairments in the brain. Because the hippocampus has been shown to be particularly vulnerable to these effects, both of the studies presented in this thesis aimed to investigate the effects of high-fat diet-induced obesity on hippocampal synaptic plasticity. In study 1, female rats were placed on either a high-fat diet (HFD; 45% saturated fat) or a control diet (CD; 10% fat) for 16 weeks, after which a subset of females were sacrificed, with the remainder being bred to investigate the transgenerational effects of HFD exposure. Body weights, food weights, and oral glucose tolerance were measured throughout the feeding period. All offspring were weaned onto a CD, and were sacrificed at PND 56. In both the maternal and offspring generations, various organ weights were collected at sacrifice, and long-term potentiation (LTP) was measured in the CA1 dendritic field of acutely prepared hippocampal slices. Results showed impaired blood glucose tolerance as early as one month on the HFD in the maternal generation, as well as significantly heavier retroperitoneal fat pads, with no differences in body weight. LTP was also significantly impaired in HFD animals in the maternal generation. HFD offspring were trending towards increased body weight, with no differences in retroperitoneal fat pad weight or oral glucose tolerance. There were also no diet-induced differences in LTP in the offspring. Study 2 had a similar design, although the animals began feeding in adolescence (PND 28), as opposed to young adulthood (PND 56, as in study 1), to investigate the effects of critical periods of exposure. The feeding period was also shortened to 10 weeks, and the CD was changed so as to eliminate its refined sugar content. Finally, because of the longitudinal nature of the transgenerational portion of study 2, the timing was such that only data from the maternal generation is presented here. In study 2, the HFD group was significantly heavier after only one week on the diet, and had increased adiposity at sacrifice. Differences in oral glucose tolerance, however, were not apparent until after 2 months on the diet, and there were no diet-induced differences in LTP. This lack of significant electrophysiological findings is consistent with the lack of differences seen by another student in the Morris Water Maze, and suggests that the shortened feeding period in study 2 may not have allowed sufficient time for the HFD to impair synaptic function.

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List of Abbreviations

aCSF: Artificial Cerebrospinal Fluid

AUC: Area Under the Curve

BDNF: Brain-Derived Neurotrophic Factor

BMI: Body Mass Index

BW: Body Weight

CD: Control Diet

CNS: Central Nervous System

DOHaD: Developmental Origins of Health and Disease

ER: Endoplasmic Reticulum

fEPSP: Field Excitatory Postsynaptic Potential

GABA: Gamma-Amino Butyric Acid

GFAP: Glial Fibrillary Acidic Protein

GIP: Glucose-Dependent Insulinotropic Peptide

GLP-1: Glucagon-Like Peptide-1

HFD: High-Fat Diet

HFS: High-Frequency Stimulation

IL-1β: Interleukin-1 beta

IL-1ra: Interleukin-1 Receptor Antagonist

IL-6: Interleukin-6

IL-10: Interleukin-10

IO: Input-Output

LTD: Long-Term Depression

LTP: Long-Term Potentiation

MWM: Morris Water Maze

NMDA: N-methyl-D-aspartate

OGTT: Oral Glucose Tolerance Test

PND: Post-Natal Day

TLR4: Toll-Like Receptor 4

1.0 Overview

Obesity is a health condition wherein there is an accumulation of body fat to the point of negative health outcomes. In Western societies, the increasing abundance of food and, specifically, the availability of low quality foods as well as the rise of sedentary lifestyles, are ravaging the health of populations. Today, roughly one quarter of the Canadian population is obese (National Center for Health Statistics, 2011), which is an especially staggering proportion when the physical, social, and economic costs of the condition are considered. In fact, a recent estimate of the economic burden of obesity on the Canadian economy reported a total of $4.3 billion in annual expenditures as a result of direct and indirect costs associated with obesity. Unfortunately, even this colossal figure is thought to be an underestimate, as it takes into account only a limited scope of the sequelae associated with overweight and obesity (Public Health Agency of Canada, 2012).

As is well known, the growing prevalence of obesity is by no means restricted to Canada, and countries across the developed and developing world have as much as one third of their populations classified as obese (National Center for Health Statistics, 2011). What is particularly alarming is that, with the globalization of Western diets (diets high in refined sugars and saturated fats), obesity is becoming an epidemic, reaching even underdeveloped nations where populations are still struggling with poverty and malnutrition. In some areas, finding malnutrition coexisting with obesity, even in the same household, is not uncommon (WHO, 2013). According to the World Health Organisation, obesity has doubled worldwide since 1980, and in 2008, 35% of adults aged 20 and over were overweight, while 11%

were obese; this amounts to over 1.4 billion overweight, and 500 million obese adults. However, perhaps the most shocking statistic from this report is that, globally, over 40 million children under the age of 5 were considered overweight in 2011 (WHO, 2013).

Of course, genetic factors do play a role in the development of obesity, and a proportion of individuals will indeed be more susceptible to the effects of a sedentary lifestyle and overeating than others; this cannot, however, explain the extreme and rapid worldwide increase in obesity. The cause of the current obesity epidemic is principally a problem of energy regulation imbalance: the overconsumption of calories without a parallel increase in energy expenditure (Kanoski and Davidson, 2011). In addition, obesity may lead to alterations in neurohormonal signalling pathways that ultimately result in the disruption of feeding regulation and the overconsumption of food, leading to a vicious cycle of cognitive disruption and energy overconsumption (Kanoski and Davidson, 2011).

2.0 Obesity and its Associated Disorders

Obesity is generally measured by Body Mass Index (BMI), which is calculated as body weight divided by the square of an individual's height. Health risks increase with increasing BMI, and an individual with a BMI ≥30 kg/m^2 is considered obese. The condition is associated with a multitude of negative health outcomes, including, but not limited to, Type II diabetes, cardiovascular disease, osteoarthritis, and certain forms of cancer (Balisteri et al, 2010). Obesity is also a principal component

of the metabolic syndrome, which includes dyslipidemia, hyperglycemia, insulin resistance, and hypertension (Vickers, 2011).

Although most individuals seem to be at least vaguely familiar with the cardiovascular and other physiological effects of surplus weight, the effects of obesity on the central nervous system (CNS) remain largely ignored by the general population. Indeed, obesity has been associated with two types of CNS disturbance, the first being disrupted regulation of signalling pathways involved in appetite and energy homeostasis, and the second being cognitive disruption. The current study will focus on the second set of phenomena, namely the cognitive deficits observed with obesity, and, more specifically, on hippocampal functioning, which has been shown to be particularly susceptible to the effects of obesity.

Epidemiological studies show that obese individuals, as well as those who consume diets high in saturated fats, experience an increased risk of dementia in old age (Kanoski and Davidson, 2011), as well as disruptions in cognitive performance throughout the lifespan (Elias et al, 2003). The observed deficits are particularly concerning given the ageing of our population and the concomitant increase in the incidence of Alzheimer's disease and related dementias (Kanoski and Davidson, 2011). If obesity does indeed cause, or exacerbate age-related neurodegenerative disease, then we are only seeing the beginning of the social and economic repercussions of the current obesity epidemic (Balisteri et al, 2010).

Evidence from human populations has led to the development of animal models of obesity in an attempt to better elucidate its effects on a range of

physiological systems. Here I will focus on those studies that relate to the disruption of brain function, as well as the mechanisms behind these effects. These efforts are in hope of both better understanding the condition, the global state of which seems to be rapidly worsening before our eyes, as well as developing potential interventions that might attenuate the effects of obesity on the brain.

2.1 Obesity-related deficits: evidence from animal models

Whereas the majority of animal research traditionally focused on the general metabolic effects of obesity, increasingly more work is being done with regards to its effects on cognition. Particularly, the hippocampus, a bilateral brain structure located in the medial temporal lobe (please see section 4.0), has become an area of focus, in large part because of its role in certain forms of learning and memory.

2.1.1 Cognitive Deficits

A number of studies in rodent models have examined the effects of different components of a Western diet on performance in a variety of behavioral tasks. Impairments in relational learning flexibility (Boitard et al, 2012), spatial reference and working memory (Kanoski and Davidson, 2010), contextual fear conditioning (Hwang et al, 2010), spontaneous alternation (McNay et al., 2010), and conditioned place preference (Privitera et al, 2011), have all been observed following various lengths of high-fat feeding, or else feeding of a diet high in both fat and refined carbohydrates. However, results are not consistent across studies; for example, Mielke et al (2006) showed no difference on Morris water maze (MWM) performance (a test of hippocampal-dependent spatial memory) in mice that had

been maintained on a high-fat diet (HFD) for 10 months, although a possible explanation for this is that the animals in both groups were relatively old when tested, perhaps leading to a floor effect. Comparison between studies, however, is complicated by heterogeneity of experimental protocols (e.g., rodent species, strain, age, length of feeding, constituents of experimental and control diets). There does, however, seem to be compelling evidence pointing to impairment of different aspects of hippocampal-dependent function, most notably spatial learning (Greenwood and Winocur, 1990; Greenwood and Winocur, 2001; Kanoski and Davidson, 2010; Kanoski and Davidson, 2011; Murray et al., 2009). The mechanisms behind the hippocampal disruption are not entirely clear, although impaired glucoregulation and insulin signalling, increases in neuroinflammation and lipid peroxidation, as well as disruptions in neurotrophins leading to decreased neurogenesis and synaptic plasticity, have all been proposed as potential culprits.

2.1.2 Insulin

The primary, and most well-recognized, role of insulin is that of glucoregulation, through the stimulation of glucose uptake into cells of the liver, skeletal muscle, and adipose tissue. The presence of insulin also inhibits proteolysis and lipolysis in muscle and adipose tissue, as well as gluconeogenesis in the liver. Insulin resistance is a characteristic of the metabolic syndrome, and is exceedingly common in obese individuals. Indeed, approximately 80% of obese individuals suffer from insulin resistance, leading to perpetually increased levels of insulin in the bloodstream (Martyn et al., 2008).

Peripheral insulin resistance has recently been suggested as a mediator of the cognitive deficits observed in individuals with the metabolic syndrome, and, more specifically, of hippocampal-related memory impairment. For example, in a study by Greenwood et al. (2003), poor glycemic control was associated with impaired delayed, but not immediate, verbal memory, with post-prandial elevations in blood glucose levels acutely exacerbating the impairment in adults with type 2 diabetes. As well, in a study of individuals with early stage type 2 diabetes (less than 10 years after initial diagnosis) Gold et al. (2007) reported deficits in hippocampal-based memory performance with no seeming effect on other cognitive domains. The study also showed reduced hippocampal volumes in diabetic individuals, the magnitude of which was inversely correlated with glycemic control. Importantly, BMI, hypertension, and dyslipidemia did not contribute independently to the variance in hippocampal volume, suggesting that the reduction in hippocampal volume was due in large part to impaired glucoregulation resulting from insulin resistance.

Evidence in animal models also points to a role of insulin resistance in cognitive impairment. Mielke et al (2005) showed that nutritionally-induced insulin resistance in hamsters significantly affected the neural insulin signalling pathway, as well as hippocampal synaptic plasticity, and Pathan et al (2008) showed that administration of rosiglitazone (an insulin sensitizer) attenuated the impairments in performance of the MWM in high fat diet-fed rats, so that their performance was similar to control rats fed a standard diet. Together, these studies show that there are indeed alterations in insulin signalling in the hippocampus of diet-induced

insulin-resistant animals, that these alterations resulted in an attenuation of insulin-induced synaptic plasticity, and that insulin sensitization improves performance on hippocampal-dependent memory tasks.

2.1.3 Leptin

Along with insulin resistance, a large proportion of obese individuals are also resistant to the hormone leptin (Walker, 2008). Leptin is secreted primarily from white adipose tissue, and blood leptin levels vary proportionately with levels of white adipose tissue in the body. Leptin is able to cross the blood-brain barrier, and its primary role is in the regulation of appetite via its effects on the hypothalamus (Harvey et al, 2006). Two types of leptin-associated genetic mutations give rise to rodent models of obesity and type 2 diabetes: those in the hormone (ob/ob), and those in its receptor (db/db).

Recently, a role for leptin outside of its role in hypothalamic energy regulation has been acknowledged. Indeed, leptin receptors are expressed in areas of the brain that are not normally associated with energy balance, including the hippocampus, thalamus, brain stem, and cerebellum (Harvey et al, 2006). It has been posited that leptin may modulate neuronal excitability and synaptic function, and leptin arising from peripheral tissues is thought to modulate hippocampal function in particular (Harvey et al, 2006). For example, intraperitoneal injection of leptin has been shown to influence hippocampal glucocorticoid expression (Harvey et al, 2006), and fluctuations in glucocorticoids have been shown to influence synaptic plasticity (Boitard et al, 2012). Leptin also seems to influence NMDA

receptor expression and functioning, and the NMDA receptor is known to play an important role in hippocampal synaptic plasticity (Harvey et al., 2006; Walker et al, 2008).

2.1.4 Neurogenesis

Although it was once thought that new neurons did not arise in the adult brain, a select few brain regions do indeed continue the process of neurogenesis, including the dentate gyrus of the hippocampus. Hippocampal neurogenesis participates in hippocampal-dependent memory, particularly flexible memory expression (Boitard et al, 2012). Memory flexibility that relies on a relational representation of previously acquired separate experiences is mediated by the hippocampus in mice, and, interestingly, represents a model of human declarative memory, which is particularly affected in overweight and obese adults (Nilsson and Nilsson, 2009). This point is of particular interest given the fact that HFD-fed mice have been shown to be impaired in memory flexibility, and have also been shown to have decreased hippocampal neurogenesis, as indicated by a decrease of immature neurons in the dentate gyrus (Boitard et al, 2012), suggesting a causal mechanism. Theories have been put forward regarding the mechanistic link between HFD consumption and decreased neurogenesis, including elevated levels of neuroinflammation and oxidative stress, both of which have been shown to be induced by HFD feeding (Boitard et al, 2012; Lindqvist et al., 2006; Pistell et al, 2010; Tozuka et al, 2009).

2.1.5 Brain-Derived Neurotrophic Factor (BDNF)

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays an important role in the growth, maintenance, and survival of many types of neurons (Kanoski and Davidson, 2011). BDNF is present in the hippocampus, and disruptions in the neurotrophin, or its receptor, can reduce hippocampal synaptic plasticity and neurogenesis, both of which have been postulated to underlie hippocampal-dependent learning and memory (Kanoski and Davidson, 2011). In effect, research has shown that consumption of diets high in fat and refined sugars decreased the level of BDNF in the hippocampus of rats, and that this decrease was accompanied by decreased synaptic plasticity, as well as decreased performance in tests of hippocampal-dependent memory (Molteni et al, 2002; Stranahan et al, 2008). Molteni et al (2004) have also shown that the decrease in hippocampal-dependent memory performance as a result of HFD could be prevented by voluntary exercise, and that this improved performance was associated with an increase in BDNF and its downstream signalling molecules relative to animals that did not exercise. Thus, BNDF does appear to play a modulating role in the relationship between the consumption of a Western diet, and resulting cognitive deficits.

3.0 Maternal Obesity

In parallel with the increase in obesity worldwide has been an increase in the number of obese women of reproductive age. Indeed, in 2012, roughly 45% of Canadian women over the age of 18 were classified as overweight, or obese (Statistics Canada, 2013). In developed countries, over 60% of women of

childbearing age are overweight (Rodriguez et al, 2012). Obesity is associated with an increase in pregnancy-related complications, including gestational diabetes and preterm delivery, increased complications during labor and delivery, as well as difficulties for both the mother and offspring postpartum (Hanson et al, 2012). Animal studies corroborate human data, and indicate that maternal obesity can indeed have a significant negative impact on offspring development, both at the level of peripheral physiology, as well as the central nervous system. Importantly, in both the F_0 and F_1 generations, the cognitive effects of obesity have been predominantly studied in male animals, and, given that the effects do seem to be sex-specific, we currently have a relative dearth of information concerning effects on the female brain.

3.1 Developmental Origins of Health and Disease (DOHaD)

The idea that the maternal environment can lead to disruptions in the offspring phenotype is commonly referred to as the Developmental Origins of Health and Disease Theory (DOHaD), and its origins are most commonly associated with work done by Barker and colleagues after a series of epidemiological studies of birth and death records that revealed a high geographic correlation between rates of infant mortality and certain classes of later adult deaths, as well as an association between birthweight and rates of adult death from ischemic heart disease (Wadwha et al., 2009). Notions of the theory were articulated even earlier, however, by Forsdhal, who used Norwegian statistical data to show that early-life poverty

followed by prosperity was associated with increased risk of death from coronary heart disease (Brenseke et al, 2013).

The DOHaD theory proposes that the fetus uses the maternal environment as a proxy for the environment into which it will be born, and "adapts" its physiology to best be able to thrive in its predicted environment. Problems arise, however, when the post-natal environment is not well matched to what was predicted, and irreversible physiological adaptations made by the fetus in pregnancy predispose to disease. Much work has since been done to elucidate the influence of maternal nutritional environment on offspring development.

Although the bulk of work concerning DOHaD was originally focused on maternal undernutrition, the current global nutritional environment has led to a shift in focus to overnutrition. Paradoxically, some of the same characteristics of the metabolic syndrome seen in offspring born to undernourished mothers occur in those born to obese mothers, suggesting a U-shaped curve for optimal maternal energy environment.

3.2 Metabolic effects in offspring

Children of obese women have increased BMI, body fat percentage, and insulin resistance, and high-fat-fed dams produce offspring with insulin resistance, increased fat deposition, and elevated circulating leptin at birth (Bilbo and Tsang, 2010). The offspring are thus primed to develop the metabolic syndrome and its associated complications, a particularly concerning phenomenon considering the obesogenic environments in which we live, and the potential negative effects that

obesity can have on the developing organism. In addition to the increased risk of somatic sequelae, maternal overnutrition has been shown to induce changes in central energy balance and appetite regulation, principally via disruptions of the hypothalamic neurocircuitry that regulate feeding behavior (Bouret, 2009). An increased risk of metabolic complications combined with a propensity towards energy dysregulation and a lack of appetitive control represents a "double hit" for these individuals, who will then struggle with weight regulation despite even the best intentions.

3.3 Effects on Offspring Cognition

In both human and animal studies, maternal obesity, as well as obesity in childhood (given that the offspring of these mothers are more likely to be obese themselves), has also been associated with cognitive disruption. For example, a prospective clinical study of maternal obesity outcomes reported high inattention scores and a two-fold increase in risk of difficulties with emotional regulation in 5-year-old children (Rodriguez et al, 2012). Another study showed that obese children had lower BDNF levels (El-Garbway et al, 2006), and performed worse on D2 and Wisconsin tests (tests of attention and mental flexibility, respectively) (Cserjesi et al, 2007).

In animal models, maternal obesity causes developmental abnormalities in both hypothalamic and hippocampal brain regions, as well as disruptions in the serotonergic, dopaminergic, and opioid systems, which result in increased anxiety, impairment in learning and memory, and desensitization of reward systems

(Rodriguez et al, 2012). For example, Peleg-Raibstein et al (2012), showed that maternal exposure to a high-fat diet for 9 weeks resulted in offspring that displayed increased anxiety-like behaviors, as indicated by performance in the elevated plus maze and food neophobia, while conditioned fear response and exploratory behavior remained unaffected. The same study showed that such changes were associated with changes in GABAergic, and serotonergic receptors, as well as BDNF levels, in the hippocampus of offspring of high fat diet-fed dams. Another group also showed increased anxiety in HFD pups, although these animals also showed facilitated acquisition of a MWM task with respect to controls (Bilbo and Tsang, 2010). The observations were accompanied by increased pro-inflammatory cytokines and levels of microglial activation in the hippocampus. Interestingly, Rodriguez et al (2012) showed that offspring born to high-fat diet-fed dams had decreased anxiety in an open field test, but found no group differences in an elevated plus maze. This group also showed learning impairment in the HFD group in a test of operant conditioning, and this effect was not prevented by switching dams to a control diet prior to gestation and lactation.

As in studies of obesity in adulthood, the interpretation and comparison across studies is complicated by the heterogeneity of experimental protocols, although it seems clear that developmental disruption does indeed occur at the level of the hippocampus in offspring born to HFD-fed mothers, and thus that an obese maternal environment is able to adversely affect this brain region.

4.0 The Hippocampus

The hippocampus is a limbic structure that is well known for its role in learning and memory, and is located in the medial temporal lobe of both hemispheres. The structure plays a role in the consolidation of memories, as well as in spatial navigation and learning. The morphology of the hippocampus is well defined, and consists of the dentate gyrus, Ammon's horn, and the subiculum; the structure's highly organized nature, as well as its relatively conserved and straightforward anatomy, has allowed for its extensive use in the study of neurophysiological phenomena. The hippocampus has been shown to be a brain region particularly susceptible to a variety of insults and is, in fact, one of the first brain structures to be affected in Alzheimer's disease and other dementias (Kanoski and Davidson, 2011). As we have seen, the hippocampus is also a prime target of obesity-related disturbances in the brain.

4.1 Long-Term Potentiation

Long-Term Potentiation (LTP) is a cellular mechanism underlying learning and memory, and has been particularly well-studied in the hippocampus. The phenomenon can be described as an increase in synaptic efficiency following a brief train of high frequency stimulation (HFS) (Gerges et al, 2003), and many of the molecular events that occur with LTP have also been shown to occur with learning and memory (for a review, see Lynch, 2004). Perhaps some of the strongest evidence for the role of LTP in learning and memory, however, comes from studies showing LTP-like changes in the behaving animal subsequent to learning. Indeed, studies using implanted electrodes in area CA1 of the hippocampus have shown that

the acquisition of hippocampal-dependent tasks is accompanied by the potentiation of synaptic responses that last for several hours (Gruart et al, 2006; Whitlock et al, 2006). Like LTP, this potentiation was shown to be NMDA-receptor-depedent (Gruart et al, 2006), and was occluded by HFS-induced potentiation (Whitlock et al, 2006), providing strong evidence for the occurrence of LTP in the behaving animal. Moreover, inhibition of LTP has been shown to disrupt hippocampal-dependent learning and retention (Morris et al, 1986). LTP in the CA1 hippocampal region has been shown to be attenuated in genetic animal models of obesity (Gerges et al, 2003; Li et al, 2002), as well as in nutritionally-induced models of obesity (Hwang et al, 2010; Porter et al, 2011; Stranahan et al, 2008). Conversely, Mielke et al (2006) did not see differences in hippocampal LTP in mice fed a HFD for one year, or in fructose-fed hamsters (Mielke et al, 2005, 2006).

Interestingly, Porter et al (2011) showed that the deleterious effects of HFD on LTP in the CA1 hippocampal region could be reversed by administration of glucose dependent insulinotropic peptide (GIP), which improves glucoregulation, suggesting that glucose homeostasis plays a role in this phenomenon. As well, Farr et al (2008), have shown that triglycerides, which are normally elevated in the serum of obese individuals, can impair maintenance of the NMDA component of hippocampal long term potentiation. Only one study has looked at sex-differences, and showed decreased hippocampal LTP in male, but not female mice (Hwang et al, 2010), which is consistent with the literature, as male animals do seem to be more susceptible to the deleterious effects of a high-fat diet than female ones.

Additionally, to our knowledge, LTP in the hippocampus of offspring born to HFD-fed mothers has not yet been explored.

5.0 Study 1

Our first study explored the effects of a four-month feeding protocol on synaptic function in adult female rats and their offspring. We measured body weight, food consumption, and oral glucose tolerance throughout the feeding period. Upon sacrifice, a series of terminal biometrics were collected, including retroperitoneal fat pad weight as a measure of adiposity. Finally, LTP was measured in the CA1 region of hippocampal slices acutely prepared from animals in each group.

5.1 Methods

5.1.1 Animals and Diets

Female, non-sibling Sprague-Dawley rats were received at post-natal day (PND) 21 and housed in polypropylene cages with woodchip bedding and stainless steel wire lids. Animals were fed Harlan Teklad rodent diet ad libitum with free access to water until PND56, at which point they were randomly assigned to HFD and control diet (CD) groups. Rats were placed on either a HFD (20% protein, 35% carbohydrate, 45% fat; Research Diets D12451), or CD (20% protein, 70% carbohydrate, 10% fat; Research Diets D12450B) ad libitum for 16 weeks (See Appendix for full details of diet composition). Animals were kept on a 12-hour dark/light cycle in groups of 3 rats per cage. Food and body weights were collected

weekly throughout the 16-week feeding period. Food consumption was recorded as mass (in grams) of food consumed by each group of rats, weekly.

5.1.2 Oral Glucose Tolerance Testing

Oral glucose tolerance tests (OGTTs) were performed at 4, 8, 12, and 16 weeks on the diet. Animals were fasted for 10-12 hours prior to being gavaged with a 50% glucose solution (4 g/kg). Blood glucose measurements were taken at baseline, and at 30, 60, 90, and 120 minutes after glucose administration via a tail poke, using a standard glucose meter. Area under the curve (AUC) was then calculated for each animal to give an overall measure of glucose tolerance.

5.1.3 Terminal Biometrics

Two of the three animals from each cage were decapitated following a 12-hour fast and anaesthesia induced with CO_2. Fasting blood glucose was measured from trunk blood using a standard glucose meter. Spleen, liver, adrenal glands, and retroperitoneal fat pads were excised and weighed. Liver volume was also measured.

5.1.4 Electrophysiology

5.1.4.1 Hippocampal Extraction and Slice Preparation

After sacrifice, each brain was quickly removed and placed in chilled oxygenated (95% O_2:5% CO_2) artificial cerebrospinal fluid (aCSF; ≤4°C; composition 127.0 mM NaCl, 2.0 mM KCl, 1.2 mM KH_2PO_4, 26.0 mM $NaHCO_3$, 2.0 mM $MgSO_4$, 2.0 mM $CaCl_2$, 10.0 mM glucose; pH 7.37-7.43; osmolality 300-320 mOsm). One

hippocampus was extracted (the right hippocampus was preferred) and cut with a McIlwain tissue chopper into 350 μm thick slices. Slices were then incubated on a microfilter and allowed to recover at interface for a minimum of 1 hour in a chamber with warm aCSF (35°C) and flowing carbogen (95% O_2:5% CO_2) prior to the start of experiments.

5.1.4.2 Field Potential Recording

Field excitatory postsynaptic potentials (fEPSPs) were recorded by placing the recovered hippocampal slice onto a 8 x 8 multi-electrode array probe (electrode size: 50 x 50 μm, and inter-electrode distance: 100 μm). The fEPSPs were sampled using the MED64 system (Alpha MED Scientific Inc., Osaka). Slices were placed on a probe, immersed in warmed aCSF, and immobilized by a mesh and anchor. The probe was connected to a perfusion system running at 1.6 mL aCSF/min. After 20 minutes of stabilization, points for Schaffer collateral stimulation were selected, with the recording point always located in the CA1 dendritic field. Figure 1 shows a slice placed on the micro-electrode array.

5.1.4.3 I-O curve

An input-output curve (IO) was made to determine the stimulation intensity needed to evoke a response eliciting 30-50% of the maximal fEPSP amplitude. To measure the input-output relationship, fEPSP amplitudes were recorded against increasing stimulation intensities at increments of 5 μA, with each biphasic pulse lasting 0.2 ms. Maximum fEPSP amplitude was defined as occurring immediately before the waveform showed contamination from other sources (i.e., a population spike). Because of the variation in the degree of contact between the probe and each

individual slice, as well as the responsiveness of each individual slice, slices tend to show variation in the minimum intensity at which they display a response. For analysis purposes, the point at which each slice shows first shows a response was denoted point 1, and each subsequent point is 5 μA greater than the previous point.

5.1.4.4 Long-Term Potentiation

A control period of baseline activity was recorded for a minimum of 20 minutes before application of a tetanus (two trains of 1 s, 100 Hz, 20 s apart). Field potentials were recorded for 30 minutes post-HFS, and potentiation was measured as an average of the last 5 minutes of recording, taken as a percentage of the baseline average.

5.1.5 Breeding Protocol

At the end of the 16-week feeding protocol, the third rat from each cage was bred with a male Sprague-Dawley rat. Pregnant dams continued to be fed their respective diets (CD, or HFD) throughout gestation and lactation. Litters were kept between 4-8 pups in order to control for the variability in nutrition and maternal care received by each pup in litters of varying sizes. Litters with more than 8 pups were culled to 8, and litters with less than 4 pups were not included in the analyses. At PND 21, all pups were weaned onto CD, and male and female animals from each litter were separated and housed in groups of 2-4 animals per cage. Body weights were monitored once weekly. Pups were maintained on the CD until young adulthood (PND 56), at which point they were sacrificed for analyses. Figure 2 shows a schematic of the general experimental timeline.

5.1.5.1 Oral Glucose Tolerance Testing

Pup OGTTs were performed once at preadolescence (PND 28), and once at young adulthood (PND 56), as described for the maternal generation (Section 5.1.2).

5.1.5.2 Terminal Biometrics

Animals were sacrificed at young adulthood (PND 56-70) in the same manner as the maternal generation, and the same terminal biometric measurements were completed (see section 5.1.3).

5.1.5.3 Electrophysiological recordings

Hippocampal slice preparation and experimental protocols for electrophysiological measures were conducted exactly as described for the maternal generation (see section 5.1.4).

5.1.6 Data Analysis

All data were analyzed via unpaired, two-tailed t-tests using GraphPad Prism software, except for the slice ratio data, which were analyzed with a chi square test. Suspected outliers were subjected to the Grubb's test, and removed if deemed to be significant.

*Note: Study 1 was conducted in two rounds, over a period of two years. I arrived in the lab prior to the commencement of the second round of the study, and thus was only involved in part of the data collection. I did, however, perform the data extraction and analysis for both sets of data. The data presented here are a combination of both cohorts (as the studies were exact replicates of one another), in order to allow for an increased sample size.

5.2 Results

5.2.1 Maternal Generation

5.2.1.1 Body Weight and Food Consumption

Weight tracking data revealed no differences in body weight between the CD and HFD groups over the duration of the feeding period (Table 1; Figure 4). As well, beginning from the second week on the diet, HFD animals consumed significantly less food by weight (weeks 2-12, $P<0.01$; weeks 13-15, $P<0.05$; Figure 5), but more food by energy (weeks 1, 8, 11, and 14, $P<0.05$; Figure 6), than CD animals .

5.2.1.2 Oral Glucose Tolerance

Results showed statistically significant differences between HFD and CD groups as early as one month on the diet (CD AUC: 341.0 ± 21.76, N=19; HFD AUC: 424.0 ± 23.48, N=17; P = 0.0138, t=2.60, df=34), and at each subsequent timepoint until sacrifice (**8 weeks** CD AUC: 279.1 ± 15.67, N=20; HFD AUC: 359.7 ± 16.58, N=19; P=0.0011, t=3.54, df=37; **12 weeks** CD AUC: 268.1 ± 11.28, N=20; HFD AUC: 350.8 ± 20.87, N=20; P=0.0013, t=3.48, df=38; **16 weeks** CD AUC: 234.7 ± 11.02, N=20; HFD AUC: 290.9 ± 15.7, N=20; P=0.0057, t=2.93, df=38). Figure 7 shows a summary of the OGTT data across the feeding period. Data are mean ± SEM.

5.2.1.3 Terminal Biometrics

Significant differences were found only for retroperitoneal fat pad weight (CD: 0.4776 ± 0.02224; HFD: 0.6273 ± 0.03751; P=0.0015, t=3.43, df=38). Results showed no differences between groups in fasting blood glucose, spleen weight, liver

weight, liver volume, or adrenal gland weight (Table 2). Figure 8 shows a graphical representation of the fat pad weight data.

5.2.1.4 I-O Curve

Baseline synaptic transmission, as measured by an input-output curve, did not differ between groups (Figure 9).

5.2.1.5 Long Term Potentiation

Results showed a significant impairment in potentiation in the HFD group (CD: 138.6% +/- 3%; HFD: 127.6% +/- 1.5%; P=0.0064, t=3.03, df=21; Figure 10). The same data are presented in Figure 11a as a bar graph, along with the corresponding results for slope (Figure 11b), which followed the same pattern of impairment in HFD animals (CD: 149.6% ± 6.4%; HFD: 132.3% ± 3.9%; P=0.045, t=2.14, df=21). Data are mean ± SEM. Figure 12 shows a set of representative traces both before and after HFS, for both diet groups.

5.2.1.6 Ratio of Slices Displaying Potentiation

Given that we set a 20% threshold for potentiation (i.e., slices that did not show a minimum of 20% potentiation above baseline were not included), we report the ratio of the number of slices that showed potentiation to the total number of slices that displayed a stable baseline (i.e., slices exhibiting robust baseline synaptic transmission). There were no diet-induced differences in the proportion of slices displaying potentiation versus those with a stable baseline (P=0.667, χ^2=0.186, df=1). The data is shown in table 3.

5.2.2 Pup Generation

5.2.2.1 Offspring Body Weight

Female HFD offspring were significantly heavier than their CD counterparts at PND 35 (CD: 117.4g ± 1.7g; HFD: 127.1g ± 1.4g; P=0.0048, t=4.35, df =6) and PND 42 (CD: 150.1g ± 1.4g; HFD: 161.4g ± 2.7g; P=0.0104, t =3.67, df=6), and the trend remained until sacrifice at PND 56 (Figure 13b). There were no significant differences in body weight in male offspring at any time point (Figure 13a). Data are mean ± SEM.

5.2.2.2 Oral Glucose Tolerance

There were no diet-induced differences in oral glucose tolerance in the offspring of either sex, at any time point (Figure 14).

5.2.2.3 Terminal Biometrics

There were no significant differences in FBG, spleen weight, liver weight, liver volume, adrenal gland weight, or retroperitoneal fat pad weight in CD versus HFD offspring of either sex (Tables 4 and 5).

5.2.2.4 I-O Curves

There were no diet-induced differences in baseline synaptic transmission, as measured by input-output curves, in offspring of either sex (Figure 15).

5.2.2.5 Long-Term Potentiation

There were no diet-induced differences in potentiation of either amplitude, or slope in male offspring (Figure 16). Figure 10 shows a summary of the magnitude of potentiation of both amplitude (17a) and slope (17b) in both diet groups.

5.2.2.6 Successful Slice Ratio

Although there were no significant diet-induced differences in the successful slice ratio in offspring of either gender (males: P=0.886, χ^2=.0204, df=1; females: P=0.201, χ^2=1.636, df=1), the female HFD offspring had a considerably lower success ratio than their CD counterparts (CD: 62.5%; HFD 14.3%; Table 7). The success ratio in this group was so low that, after the exclusion of all slices that did not meet the threshold for potentiation there was insufficient data to run statistical comparisons between the diet groups in the female offspring. LTP data are therefore only presented for male offspring.

5.3 Discussion

Maternal Generation

To my knowledge, this is the first study to show decreased hippocampal LTP in female animals as a result of HFD-feeding. Several other studies have shown a similar reduction in male animals (Farr et al, 2008; Hwang et al, 2010; Lennox et al, 2014; Porter et al, 2011; Stranahan et al, 2008), although this is not always the case (Mielke et al, 2006). Mielke et al (2006) fed male mice the same diet used in the present study for a period of one year and did not report changes in LTP; the difference may be an age effect, for LTP is known to decrease with age (Blau et al, 2012) and the mice were considerably older than our rats (one year vs. six months of age, respectively). As well, Hwang et al (2010) were the only other group to examine the effect of sex, and they did not find differences in LTP in female mice as a result of HFD-feeding, although they did find a difference in male animals. Notably, these mice were approximately the same age, and of the same strain as those used by Mielke et al (2006), and both groups fed their animals for similar time periods (Mielke et al (2006) fed for one year, and Hwang et al (2010) fed for 9 months to one year). It is difficult to speculate about the source of the differences in the findings because Hwang et al (2010) do not report the composition of their HFD. The possibility therefore exists that the concentration of saturated fats and/or refined sugars was elevated with respect to the diet used by Mielke et al (2006), and that more drastic impairments were seen as a result. Nevertheless, the lack of an effect in female animals in the study by Hwang et al (2010) may indeed be the result of a floor effect due to the advanced age of the animals, given that spatial memory

performance (which is tightly associated with hippocampal LTP; Malenka and Nicoll, 1999) is known to differ between sexes across the lifespan (Bucci et al, 1995). We may therefore have been able to detect an effect because we sampled the animals earlier in the lifespan, although differences in species and diet composition cannot be discounted.

Interestingly, the impairments we saw in hippocampal LTP were not accompanied by differences in body weight, although we did see increased adiposity and impaired glucose tolerance in the animals. The lack of a difference in body weight may be due, in part, to the fact that female animals are generally less susceptible to the metabolic effects of a HFD (Hwang et al, 2010). Another reason may be the composition of our control diet, which, being relatively high in refined sugars (35% sucrose by energy in the CD, whereas this proportion was only 17% in the HFD), may have masked our ability to detect significant differences in body weight. The presence of moderately elevated concentrations of refined sugars in the control diet, and the presence of a diet-related difference, potentially speaks to the particularly detrimental effects of high concentrations of dietary saturated fats.

Indeed, Farr et al (2008) specifically investigated the effects of fats on spatial memory by demonstrating that daily administration of gemfibrozil, a drug that lowers triglycerides, for two weeks, rescued the performance of the HFD mice on a T-maze foot shock avoidance task. To confirm the specific effects of triglycerides, Farr et al (2008) directly administered the triglyceride triolein into the 3rd ventricle and found that this impaired performance on the T-maze. The authors further

extended the investigation of the effects of triglycerides on hippocampal function by showing that direct application of triglycerides to hippocampal slices abolished both the induction and maintenance of LTP, and did so by reversibly inhibiting the NMDA component of the fEPSP.

Although we did not measure plasma triglycerides, hypertriglyceridemia is a component of the metabolic syndrome that is often seen with increased adiposity and insulin resistance (Subramanian and Chait, 2012), and has been previously seen in rodents with diet-induced obesity (Banks, 2004). Hypertriglyceridemia therefore represents a potential mechanism for the impairments observed. The reversibility of the effects seen by Farr et al (2008) are also in agreement with the biochemical data from our study (completed by another student), which showed no differences in hippocampal NMDA receptor subunit expression between groups (Pavlov, 2013); a transient effect on NMDA receptor function would not likely manifest as a difference in subunit expression, but instead as a functional change (e.g., phosphorylation).

Saturated fatty acids have also been shown to disrupt brain function via central inflammatory processes (Milanski et al, 2009). Indeed, obesity is recognized as a state of chronic, low-grade inflammation, which is thought to be, at least in part, responsible for the insulin and leptin resistance seen in these individuals (Miller and Spencer, 2014). Milanski et al (2009) show that, in the hypothalamus, saturated fatty acids are able to bind to Toll-like receptor 4 (TLR4), a component of the innate immune system expressed primarily on microglia in the brain. The binding activates

microglia, causing them to secrete pro-inflammatory cytokines, which promote endoplasmic reticulum stress (ER stress) (Milanski et al, 2009). Both inflammation and ER stress promote oxidative stress, which, in turn, feeds back to promote inflammation in a vicious positive feedback loop (Miller and Spencer, 2014). The possibility exists that the saturated fatty acids in a HFD activate the TLR4 in the hippocampus as well, leading to similar consequences, and ultimately cognitive disruption. Indeed, increased microglial activation and TLR4 expression in the hippocampus have been seen as a result of HFD feeding (Bilbo and Tsang, 2010), and HFD-induced central inflammation has been suggested to impair cognition (Pistell et al, 2011), although both of these cited studies used a higher concentration of saturated fat than was present in our HFD (60% vs 45%, respectively). The high saturated fat content of our HFD may have, nonetheless, provoked central inflammatory processes and oxidative stress in the animals, leading to impairments in hippocampal function. A schematic summarizing this proposed mechanistic pathway is presented in figure 3.

In agreement with this mechanistic pathway, Lennox et al (2014) showed that the LTP impairment they observed as a result of HFD-feeding was restored by an intervention that reduced markers of central inflammation and oxidative stress (i.e., administration of a GLP-1 receptor agonist for 20 days), and that this was accompanied by improved performance on an object recognition task, which is hippocampal-dependent. Previous studies have also shown that both behavioral and pharmacological interventions that reduce central oxidative stress (Molteni et al 2004; Wu et al, 2004) and inflammation (Jeon et al, 2012; Lu et al, 2011) in the

context of HFD-feeding also restore hippocampal-dependent memory. Moreover, the results by Lennox et al (2014) were seen without the normalization of body weight (i.e., the HFD animals were heavier than their CD counterparts even after treatment, when their hippocampal function was no longer impaired), providing further evidence that differences in body weight alone are not directly responsible for differences in hippocampal function between animals.

Erion et al (2014) further demonstrate the importance of adiposity (as opposed to body weight) on brain function by demonstrating that impairments in both cognition and hippocampal LTP in leptin-insensitive mice (i.e., db/db mice; a genetic model of obesity) are reversed by interventions that reduce adipose tissue, and that this is mediated by IL-1β, a pro-inflammatory cytokine that is released by adipose tissue in obese individuals. The relevance of these findings is twofold. First, they support the notion that differences in adiposity may be a more sensitive predictor of obesity-related cognitive impairment than differences in body weight. Second, they suggest that obesity-related cognitive deficits are a function of inflammatory processes at least partly driven by excess adipose tissue, and not of defective leptin-receptor signalling per se, given that cognition was rescued in these animals by decreasing adiposity without restoring leptin receptor function (Erion et al, 2014). Therefore, because we saw increased adiposity in our animals, peripheral inflammation may also have driven and/or contributed to the disruptions in hippocampal function seen. In addition, blood-brain barrier integrity is compromised in the obese state (Kanoski et al, 2010), and this may allow increased access of peripheral cytokines into the brain, further propagating central

inflammatory stress in a process of cytokine-mediated cytokine release (Miller and Spencer, 2014).

The mechanisms whereby inflammation can affect brain function have not yet been fully elucidated, although both direct and indirect mechanisms have been posited. Elevated concentrations of pro-inflammatory cytokines in the brain can directly promote neurodegeneration and beta-amyloid formation, one of the hallmarks of Alzheimer's disease (Miller and Spencer, 2014), and are also likely to affect neurogenesis and synaptic plasticity, at least in part through their actions on neurotrophins that mediate these processes.

One such neurotrophin is BDNF, which is known to regulate synaptic efficacy via its actions on proteins that regulate synaptic function (Molteni et al, 2002). BDNF plays a role in the conversion of electrical activity into changes in synaptic strength (Panja and Bramham, 2014; Poo, 2001; Schnider and Poo, 2000) and has been shown to have a facilitative role in LTP (Panja and Bramham, 2013). For example, BDNF knockout mice are significantly impaired in hippocampal CA1 LTP and recombinant-BDNF treatment of slices from these mice returned LTP to wild-type levels (Patterson et al, 1996). HFD has consistently been shown to downregulate hippocampal BDNF (Kanoski et al, 2007; Molteni et al, 2002; Park et al, 2010), and this has been seen concomitantly with increased oxidative stress (Molteni et al, 2004) and inflammation (Pistell et al, 2011). Stranahan et al (2008) also showed that HFD-induced impairments in hippocampal LTP were accompanied by reductions in both hippocampal BDNF, as well as dendritic spine density.

Moreover, interventions that prevent the diet-induced decreases in BDNF and its downstream effectors have been shown to prevent spatial memory impairments in the MWM (Molteni et al, 2004).

Taken together, the evidence suggests that, in our animals, a high-fat diet led to increased inflammation and oxidative stress, which may have ultimately reduced hippocampal BDNF and downstream synaptic plasticity, leading to the observed impairments in LTP. Figure 3 shows a summary schematic of the hypothesized mechanistic pathway. We cannot be sure of this mechanism, however, given that we did not include measures of central inflammation, oxidative stress, or neurotrophins in our study. Moreover, although our results reached significance, the magnitude of the reduction in LTP that we observed was modest, and our lack of behavioral measures prevents us from making conclusions about the biological significance of the results (i.e., whether they would be accompanied by impairments in hippocampal-dependent memory). Future studies should aim to include measures of each of these factors in the same study to tease apart the mechanisms underlying HFD-induced cognitive impairment. In addition, the studies cited above vary considerably in important ways from both each other and the current study. Differences in species, strain, sex, diet composition, and feeding length all compromise the generalizability of the studies, and the ability to compare between them. Future research attempt to control for these factors in order to more systematically assess the effects of obesity on cognition.

Offspring Generation

The second portion of our study involved the offspring generation, which had only been exposed to the HFD through their mothers (i.e., 3 weeks of gestation and 3 weeks of lactation). An important point is that siblings from the same litter were not counted as individual data points, and thus, even with 100% breeding success, the maximum possible sample size for the offspring generation would have been N = 10 litters per diet condition (5 from each round of the study). Given the limited success we had with breeding, however, the actual sample sizes were lower.

Although offspring born to HFD dams tended to be heavier than their CD counterparts, we did not see any significant differences in glucose tolerance, or retroperitoneal fat pad weight. The apparent lack of a metabolic difference is contrary to what is found in the literature, which suggests that maternal obesity induces obesity and related metabolic impairments in offspring (for a review, see Li et al, 2011). Because the maternal generation did exhibit significant metabolic alterations as a result of HFD feeding, the lack of a significant difference between members of the offspring generation is likely the result of insufficient sample size. Indeed, the results from our OGTT procedure are inherently variable, and thus a larger sample size is required. Moreover, despite our small sample size (N=4), we did observe trends towards increased body weight in offspring of both sexes, suggesting that differences are indeed present, and that increasing the sample size would serve to emphasize these differences.

To my knowledge, this is the first study to investigate hippocampal LTP in the offspring of HFD-fed mothers, although previous studies do suggest that hippocampal function is disrupted in offspring as a result of maternal obesity (Bilbo and Tsang, 2010; Niculescu and Lupu, 2009; Page et al, 2014; Peleg-Raibstein et al, 2012; Sasaki et al, 2013, 2014; Tozuka et al, 2009, 2010; Walker, 2008; White et al 2009). Specifically, previous studies have shown impaired spatial memory performance (Page et al, 2014; Tozuka et al, 2010; White et al, 2009), increased hippocampal inflammation (Bilbo and Tsang, 2010; Sasaki et al, 2014) and oxidative stress (Tozuka et al, 2009, 2010), as well as decreased neurogenesis and plasticity (Niculescu and Lupu, 2009; Tozuka et al, 2009, 2010). As with the parental generation, however, considerable variability between experimental protocols makes comparing across studies difficult, and interferes with the ability to make any definitive statements about the effects of maternal obesity on offspring brain function.

Taken together, the literature suggests that LTP might also be impaired in the offspring of obese mothers. Despite this, we found no differences in potentiation between groups after excluding slices that did not meet our 120% potentiation threshold for LTP. Again, our small sample size likely led to a level of power insufficient to detect significant differences, particularly given the relatively modest effect size seen in the maternal generation. Of potential significance, however, the ratio of successfully potentiated slices in female offspring was considerably reduced in the HFD group (Table 8). The possibility exists that this was a result of the maternal diet almost completely abolishing LTP in these animals, although this

possibility should be interpreted with caution given the limited sample size and lack of behavioral data measuring memory in the intact animal.

Overall, drawing firm conclusions with respect to the offspring generation is difficult due to the limited sample size. Future iterations of the study should seek to optimize the breeding environment to increase breeding success, as well as incorporate behavioral measures of hippocampal memory (e.g., Morris Water Maze) in order to test the biological significance of any conclusions drawn from LTP data.

6.0 Study 2

The purpose of study 2 was to extend upon the findings of study 1, as well as to explore some of the mechanistic questions that arose. Although the electrophysiological assays remained the same, behavioral measures and biochemical measures of inflammation were performed by other students. The design was similar to that of study 1, although some important changes were made to the feeding protocol:

1) As opposed to starting the animals on their diets at young adulthood (PND56, as per study 1), animals were randomly assigned to either HFD or CD at PND28, an age at which rodents are still considered to be in adolescence.
2) The composition of the CD was changed such that the carbohydrate source was primarily complex carbohydrate as opposed to simple sugars (0% sucrose in study 2 versus 35% sucrose in study 1; see appendix for the exact composition of the diets in each study).
3) The feeding protocol was shortened from 16 weeks to 10 weeks.

The decision to put the animals on their respective diets at an earlier timepoint was based on data from the literature, suggesting that younger animals are more vulnerable to the effects of HFD feeding than their adult counterparts (Boitard et al, 2012, 2014; Hwang et al, 2010), which seems intuitively plausible, given that disturbances to the physiological milieu are more likely to be disruptive while development is still underway than after the majority of development has taken place. Moreover, the shift lent ecological relevance to the study, as it is likely that

obese individuals are not exclusively exposed to their obesogenic diets in adulthood, but over the course of their early lives as well. We hypothesized that, despite the shorter exposure to the diet, we would see more dramatic changes (particularly in body weight, where we saw no significant changes in study 1) in the HFD animals.

The decision to shorten the feeding period was both a practical one, and one that was supported by the literature, suggesting that similarly brief feeding periods affected both metabolic and cognitive function when the diet was administered during development (Boitard et al, 2012). Moreover, we suspected that eliminating the refined carbohydrate content from the CD would further serve to emphasize the impairments due to HFD exposure.

Notably, although study 2 also had a breeding component incorporated into the study design, the timing of the experiments were such that the data collection for the offspring generation of study 2 would not be complete in time for inclusion in my thesis document. As a result, only the data for the maternal generation of study 2 are presented here.

6.1 Methods

6.1.1 Animals and Diets

Female, non-sibling Sprague-Dawley rats were received at PND 21 and housed as described in study 1. The animals were permitted to acclimatize for one week while being fed the Haran Teklad rodent diet ad libitum, after which (PND 28) they were separated into the HFD and CD groups. The HFD was the same as in study

1 whereas the CD diet was replaced with a control diet similar in all respects, except that the sucrose was replaced by complex carbohydrate. The diets were fed ad libitum for 10 weeks, with body weights and food consumption collected as described for study 1.

6.1.2 Oral Glucose Tolerance Tests

Oral glucose tolerance tests (OGTTs) were performed after 4 and 8 weeks on the diet as described for the animals in study 1.

6.1.3 Terminal Biometrics

Spleen, liver, adrenal glands, and retroperitoneal fat pads were excised and weighed as described for study 1, with the exception of the 12-hour fast. The animals in this study were not fasted prior to sacrifice, in order to reduce stress.

6.1.4 Electrophysiology

Slice preparation and electrophysiological recordings were performed exactly as described in study 1, except that the I-O curves increased at intervals of 2 μA, as opposed to 5 μA.

6.2 Results

6.2.1 Body Weight and Food Consumption

HFD animals were heavier than CD animals as early as one week on the respective diets (weeks 1 and 2, $P<0.01$; weeks 3, 4, 6 and 8, $P<0.05$; Figure 18), and tended to eat significantly more food by energy (weeks 1, 4, 8, and 9, $P<0.01$; weeks

3, 5, 6, and 7, $P<0.05$; Figure 19). As in study 1, however, the HFD animals generally ate significantly less than the CD animals by mass (weeks 1, 3, 5, 6, 8, and 9; $P<0.01$; Figure 20).

6.2.2 Oral Glucose Tolerance

There were no differences in AUC values after one month on the diet (Figure 21). After two months on the diet, however, there was a trend towards impaired glucose tolerance in the HFD group (CD: 233.9 ± 15.62; HFD: 286.4 ± 21.75; $P=0.0582$, $t=1.96$, $df = 34$). Notably, in month two there was one value in the HFD group that was uncharacteristically low, but was not a significant outlier. As a result, the value was included in the analysis; without this data point, the difference reaches statistical significance (see discussion).

6.2.3 Terminal Biometrics

HFD animals had significantly heavier fat pads than CD animals (%BW: CD, 0.4206 ± 0.02429; HFD, 0.5245 ± 0.03108; $P=0.0136$, $t=2.63$, $df=28$; Figure 22), although there were no significant differences in spleen weight, liver weight, or adrenal gland weight (Table 8).

6.2.4 I-O Curves

There were no differences in baseline synaptic transmission, as measured by an IO curve (Figure 23).

6.2.5 Long-Term Potentiation

In study 2 there were no differences in potentiation between HFD and CD animals (Figure 24). Moreover, there were no differences in the successful slice ratio (P=0.928, χ^2=.00818, df=1; Table 9). Figure 25 provides of summary of the data for both amplitude (25a) and slope (25b).

6.3 Discussion

As we expected, exposing the animals to the HFD at a younger age caused the animals to become significantly heavier than their CD counterparts over the duration of the feeding period. Surprisingly, however, given the increased susceptibility to weight gain, there were no significant differences in glucose tolerance after one and two months on the diet, although there were trends towards impaired glucose tolerance in the HFD group. Notably, at the second timepoint (i.e., after 8 weeks on the diet) there was one AUC value in the HFD group that was uncharacteristically low (greater than 2 standard deviations below the mean), although it did not reach significance as an outlier, and was therefore not excluded from the analysis; this type of low value can occur if the baseline value is either authentically, or erroneously high, or if the post-bolus values are authentically, or erroneously low. Either scenario causes the value to fall below the baseline value (at which the y-axis of the AUC graph is set to 0), resulting in the subtraction of a portion of the graph from the overall AUC. Normally, when an inaccurate reading is suspected, the animal is re-sampled in order to verify the value. The single uncharacteristically low value for AUC in the animal may have been the result of an experimental oversight, in that it was not immediately obvious that a second blood draw needed to be done to confirm the blood glucose reading. Given that the value is indeed much lower than even normal control values for AUC, this was likely the case. If one excludes this data point, the data reaches significance for month 2 ($P=0.0133$), and it is therefore not unreasonable to posit that the HFD animals were impaired in their glucose tolerance after 2 months on the diet.

There were, however, no significant differences in glucose tolerance after one month on the diet, which is in contrast to study one, where differences were seen at this timepoint. The results seem to suggest that the younger animals were in fact less vulnerable to the effects of the diet on glucose metabolism, which is contrary to what is suggested in the literature (Boitard et al, 2012), and may be due to a compensatory effect that is more robust in younger animals. The HFD group did, however, have significantly heavier fat pads at the time of sacrifice (i.e., after 10 weeks on the diet), which suggests that the shortened feeding period was indeed sufficient to increase adiposity when the animals were exposed to the diet during development. Unfortunately, we cannot say whether a similar increase in adiposity would have been seen in the older animals from study 1 after only 10 weeks on the diet, given that fat pad excision is a terminal procedure and therefore can only be done at the time of sacrifice. The literature would suggest, however, that insulin resistance is, in part, due to inflammation from excessive adipose tissue (Miller and Spencer, 2014), and given that the dams in study 1 were impaired in their glucose tolerance at both 8 and 12 weeks on the diet, it is not unreasonable to suggest that their fat stores were elevated with respect to CD at 10 weeks. Overall, with the exception of significant increases in body weight in the animals in study 2, beginning feeding at a younger age did not result in exaggerated metabolic effects of the HFD relative to study 1.

We also saw no differences in hippocampal LTP between HFD and CD animals in study 2, and the slice ratio did not seem to suggest a difference between the groups that might have been missed as a result of our LTP threshold (54.5% and

56.7% success in potentiation, respectively). A possible explanation for this is insufficient length of exposure to the diet. Indeed, insofar as peripheral glucose intolerance is caused by inflammatory processes (Miller and Spencer, 2014), the lack of a significant impairment in metabolic control after one month on the diet suggests that there may not have been sufficient inflammation present to provoke these changes. Although the impairments in glucoregulation were apparent after 2 months, terminal measures taken after only 10 weeks of diet exposure may not have allowed sufficient time for these changes to result in impairments in hippocampal function. Consistent with this possibility, preliminary findings from another student in our lab showed no diet-induced differences in protein levels of hippocampal IL-1β, IL-6, IL-1ra, IL-10, suggesting no differences in either inflammatory, or anti-inflammatory cytokines. In effect, all other studies showing HFD diet-induced impairments in LTP had considerably longer feeding periods (Erion et al, 2014; Farr et al, 2008; Hwang et al, 2010; Lennox et al, 2014; Porter et al, 2010; Stranahan et al, 2008), the shortest of which was 5 months (Lennox et al, 2014). The shorter feeding here may therefore not have allowed sufficient time for inflammation to affect hippocampal function. Our biochemical data did show a decrease in hippocampal GFAP, a marker of astrocytic activation, in the HFD animals, which may be the beginning of a compensatory change in response to inflammation in the periphery or in other parts of the brain that was not yet sufficient to evoke inflammatory changes in the hippocampus. Moreover, a trend towards a decrease in total Akt was also seen, with no difference in phospho-Akt. Akt is part of a number of important signalling pathways, including insulin, leptin, and BDNF, all of which have been

shown to play a role in synaptic plasticity (Brunet, 2001; McNay and Recknagel, 2011; Morrsion, 2009). These differences may therefore be indicative of preliminary changes in the Akt pathway, which, over time, could manifest in impaired synaptic plasticity, learning, and memory. Another possibility is that the HFD-induced changes must occur concomitantly with other changes that occur as a result of aging, that were not yet present in our younger animals.

Another student in our lab conducted behavioral experiments to examine whether the diet had any effects on hippocampal-dependent spatial memory, as measured by the MWM. In accordance with the rest of our findings, there were no diet-induced differences in either acquisition, or retention of the task. Given that deficits in spatial learning performance in the MWM are often accompanied by impairments in LTP (Malenka and Nicoll, 1999), these results are consistent with a lack of diet-induced impairments in LTP in these animals. Overall, it would seem that shifting the feeding period to cover adolescence did not have the expected exaggerated effects on physiology in these animals, perhaps both because of age-related compensatory effects, as well as insufficient timing of diet exposure.

One important possibility, however, is that there may have been more subtle deficits in our animals that we may have missed with our battery of tests. Indeed, Boitard et al (2014) ran a similar study with 3 week-old male Wistar rats, using the same HFD that we used here. After 8 weeks on the diet, Boitard et al (2014) showed no differences in acquisition of a MWM, or on a short-term probe test (consistent with our results). When they performed a second probe test 4 days after the final

training day however (i.e., a long-term probe), the CD animals still showed a preference for the target quadrant, whereas the HFD animals did not, suggesting impaired long-term memory, or memory consolidation, in the HFD animals (Boitard et al, 2014). The HFD animals were also impaired on a reversal task, suggesting impaired mental flexibility (Boitard et al, 2014). These results suggest that, because we did not conduct a long-term probe or reversal task, we may have missed more subtle deficits in hippocampal function that may have been present in our animals. In line with this, recent evidence suggests that hippocampal long-term depression (LTD), an activity-dependent decrease in synaptic strength, is more important than LTP for both consolidation of spatial memory (Ge et al, 2010) and performance of the reversal task (Dong et al, 2013), both of which were shown to be impaired by Boitard et al (2014). The possibility exists, then, that measuring hippocampal LTD may have yielded diet-induced differences in our study. Indeed, hippocampal LTD has previously been shown to be impaired in the context of HFD-feeding (Hwang et al, 2010).

7.0 Limitations and Future Directions

Overall, considering the data from both studies, drawing any firm conclusions regarding the effects of HFD on female animals and their offspring is difficult. Although we did see impairments in LTP in the maternal generation in study 1, the same effects were neither present in the offspring, nor in the maternal generation in study 2. Moreover, the offspring in study 1 did not show metabolic alterations as a result of maternal HFD exposure, which is contrary to what is suggested in the literature (Li et al, 2011). The HFD offspring were, however, trending towards

increased body weight, suggesting that our limited sample size may not have allowed for the detection of significant metabolic differences. Future studies should therefore seek to maximize the number of successful litters in order to have sufficient power to detect an effect. As well, all studies investigating the effects of HFD on hippocampal function should attempt to incorporate behavioral tasks into the study design in order to assess the biological relevance of any electrophysiological and/or biochemical data.

The lack of significant findings for LTP in the maternal generation of study 2 was also unexpected, given the literature suggesting the increased vulnerability of younger animals to the effects of HFD (Boitard et al, 2012), as well as our data from study 1. Future studies should investigate the relative importance of age, diet duration, and diet composition on hippocampal function. As well, species, strain, and sex differences may add to the variability in findings between studies, and so the effects of these variables should be controlled for. Moreover, attention should be paid to the subtleties of behavioral and electrophysiological tasks, and as wide an array of measures should be incorporated as possible, in order to fully characterize the extent of cognitive deficits that may be present in these animals.

Ultimately, it would seem that HFD feeding has the potential to disrupt brain function, but that this effect is dependent on a number of factors, including animal age, sex, duration of exposure to the diet, as well as the nature of the assessment being used. Given the current obesity surge, it is of paramount importance that the

contribution of each of these factors, the precise nature of the impairments, and the mechanisms underlying them be elucidated.

8.0 Tables and Figures

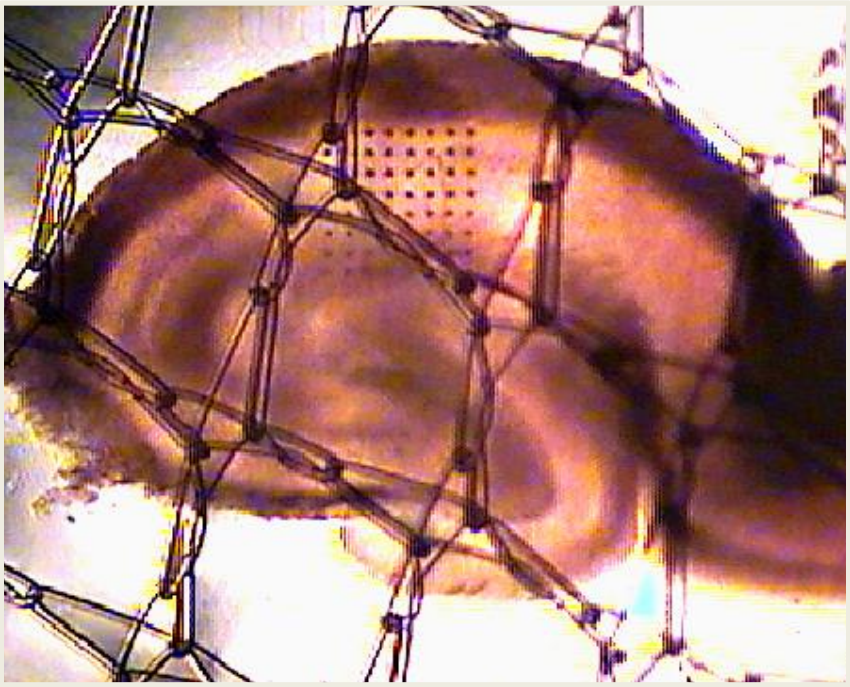


Figure 1 - Image of slice on MED 64-point probe: 8 x 8 microelectrode array surrounded by four reference electrodes. The electrodes are placed so as to encompass the dendritic field of area CA1.

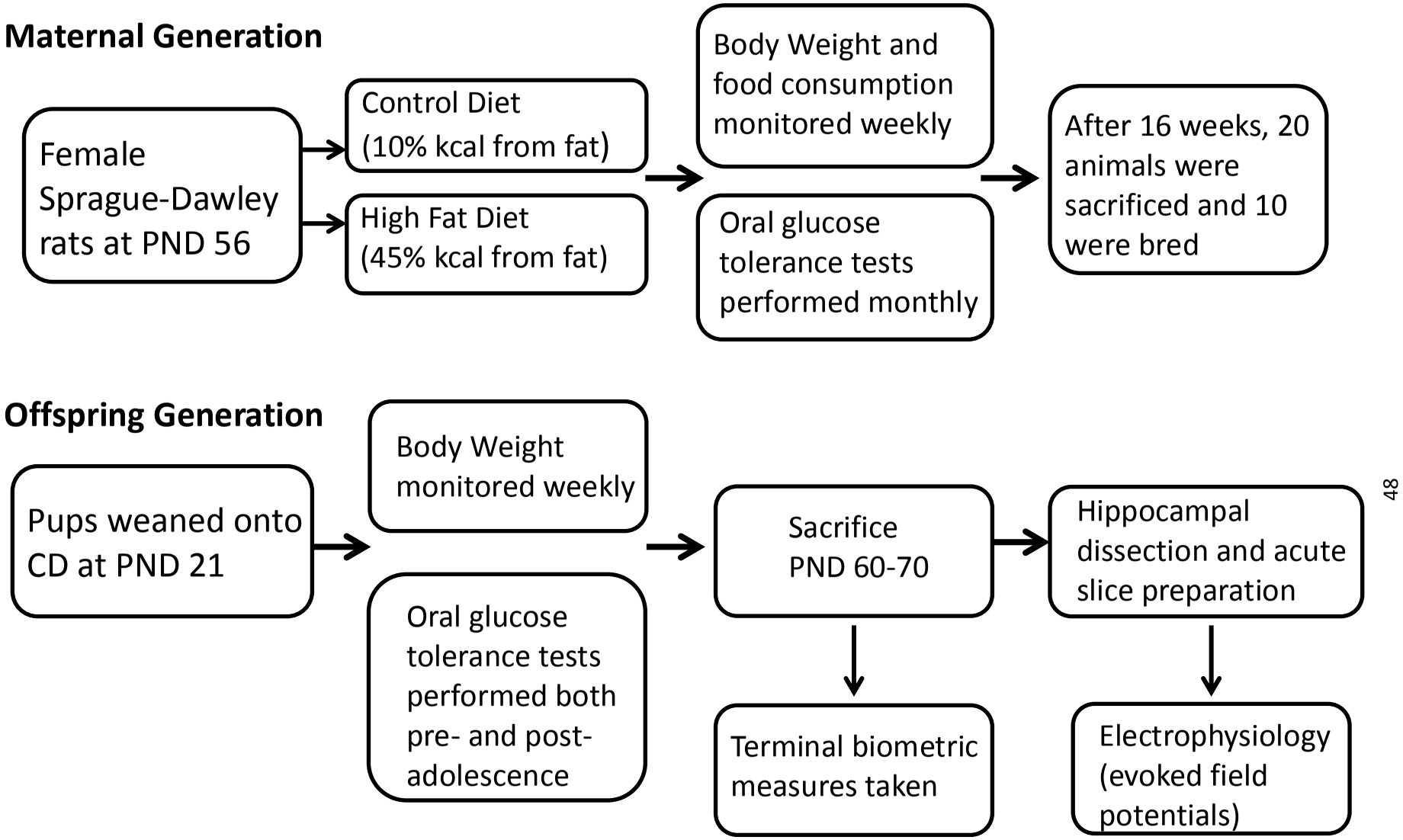


Figure 2- Generalized experimental protocol for study 1.

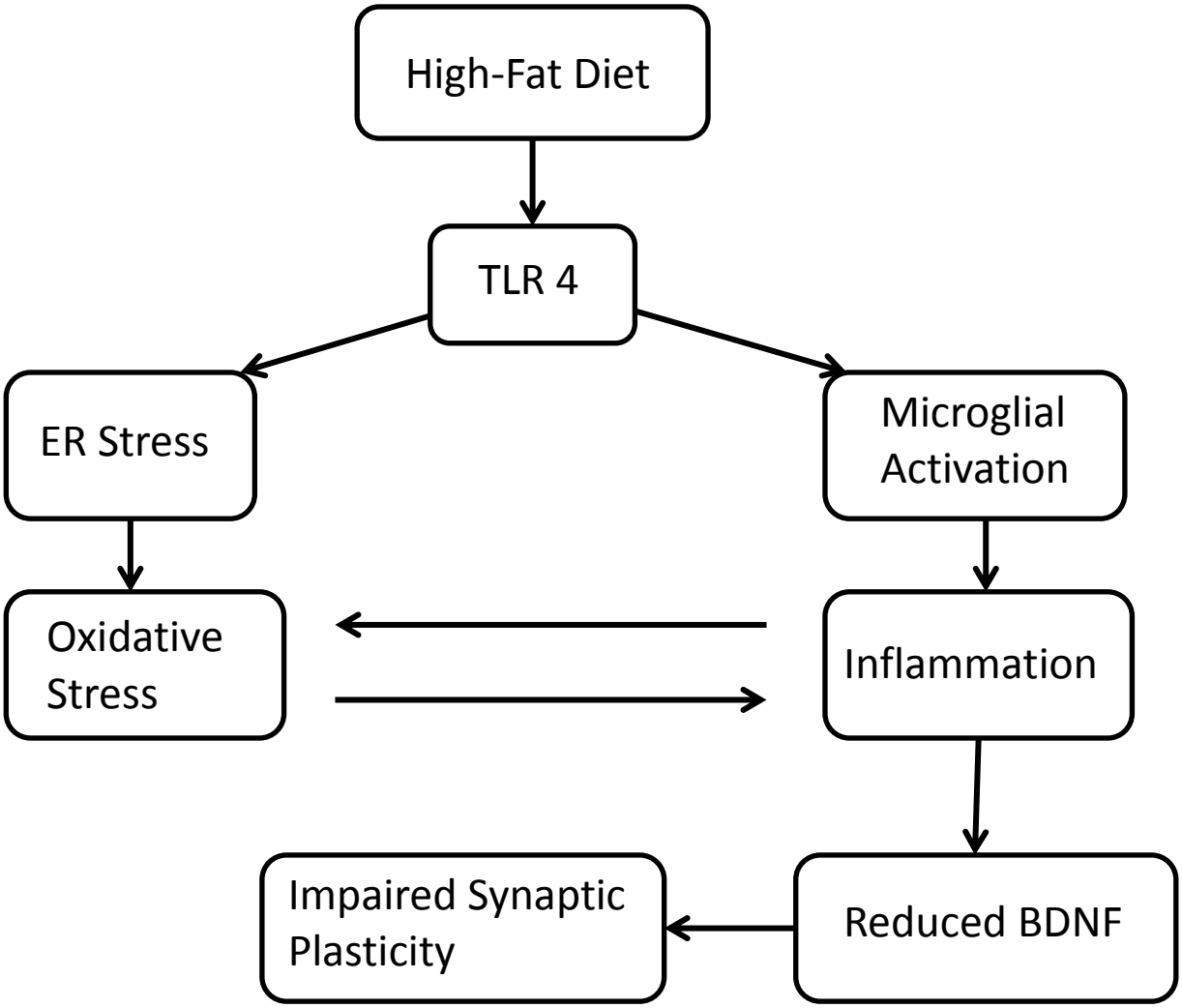


Figure 3 – Schematic of proposed mechanistic pathway of impairment of hippocampal synaptic plasticity.

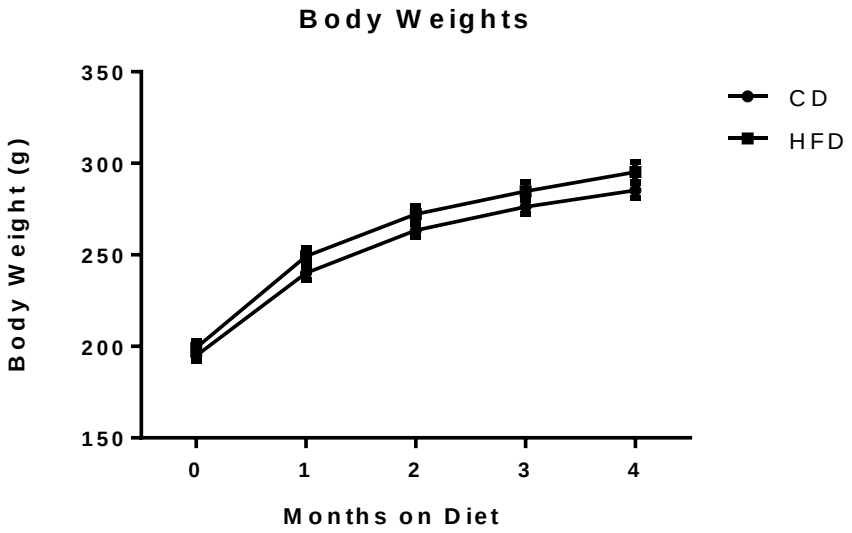


Figure 4 - Body weights at baseline, and at the end of each month on the diet. Data are mean +/- SEM. N=20 for both groups.

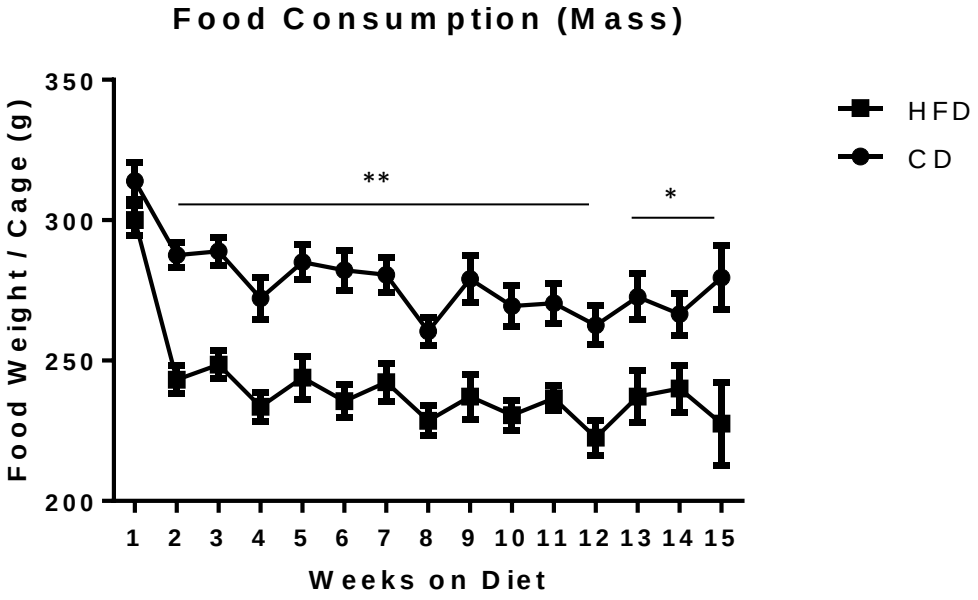


Figure 5 - Food consumption as measured in grams of food consumed per cage of 3 rats over the duration of the feeding protocol. Data are mean +/- SEM. *P<0.05. **P<0.01. N=10 for both groups.

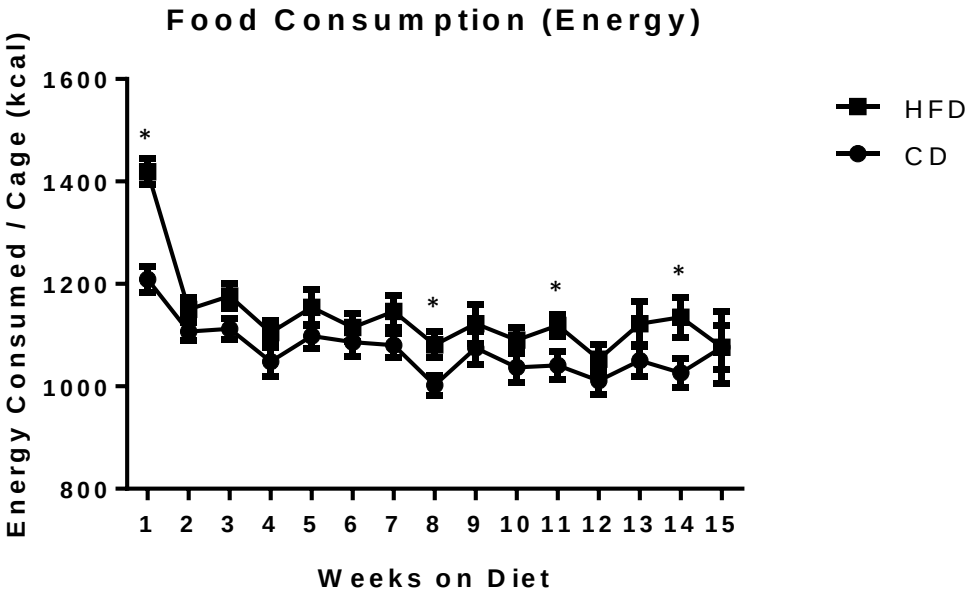


Figure 6 - Food consumption as measured in kilocalories of food consumed per cage of 3 rats over the duration of the feeding protocol. Data are mean +/- SEM. *P<0.05. N=10 for both groups.

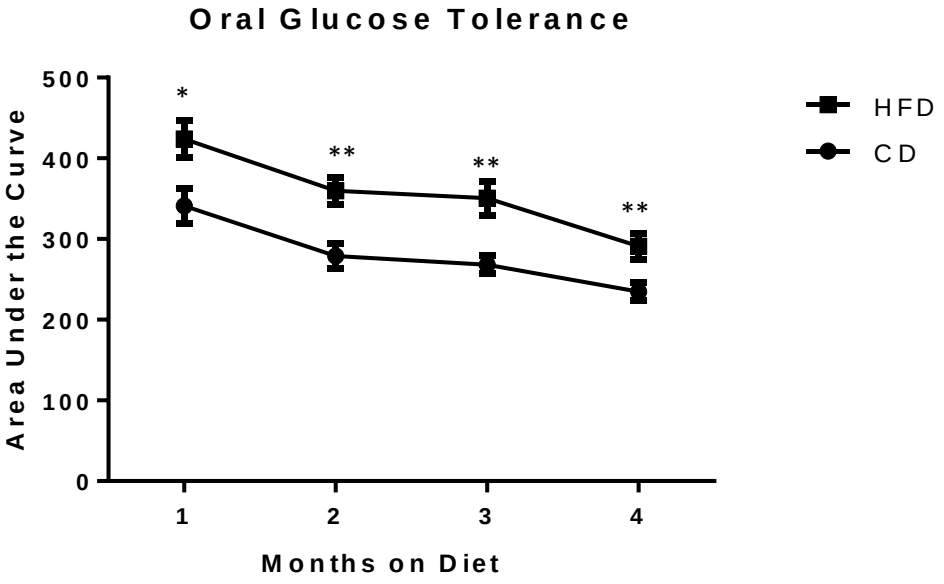


Figure 7 - Oral glucose tolerance tests were performed on all animals at 1, 2, 3, and 4 months on the respective diets. Data are mean +/- SEM. *P<0.05, **P < 0.01. N=20.

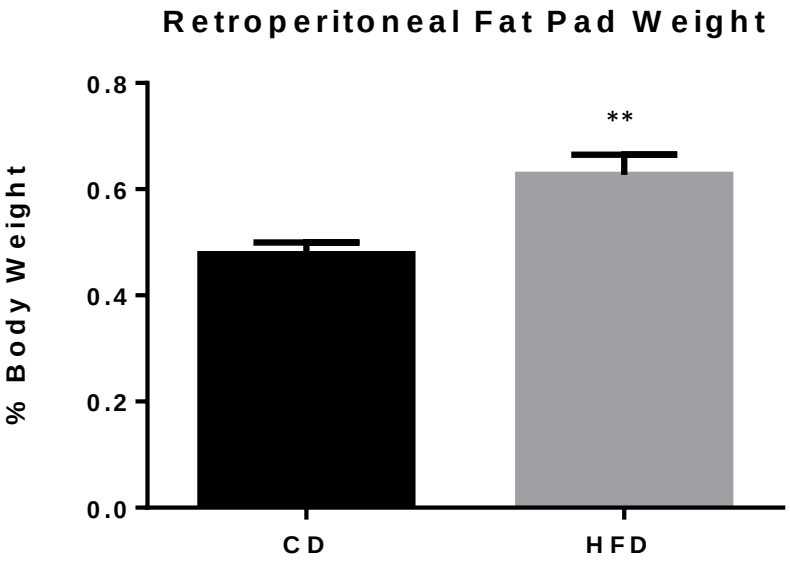


Figure 8 – Retroperitoneal fat pad mass, shown as a percentage of body weight (% BW). Data are mean +/- SEM. **P ≤ 0.01. N=20.

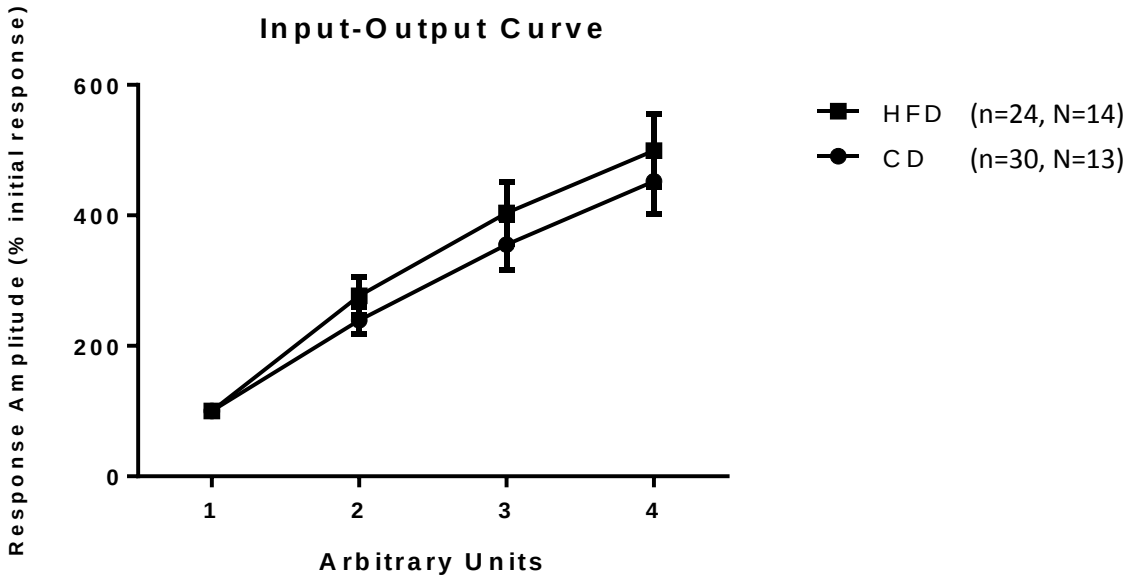


Figure 9 – Input-output curves. Response amplitude for stimulations of increasing intensity (at intervals of 5 μA). Shown as a percentage of initial response. n=24-30 slices from N=13-14 animals.

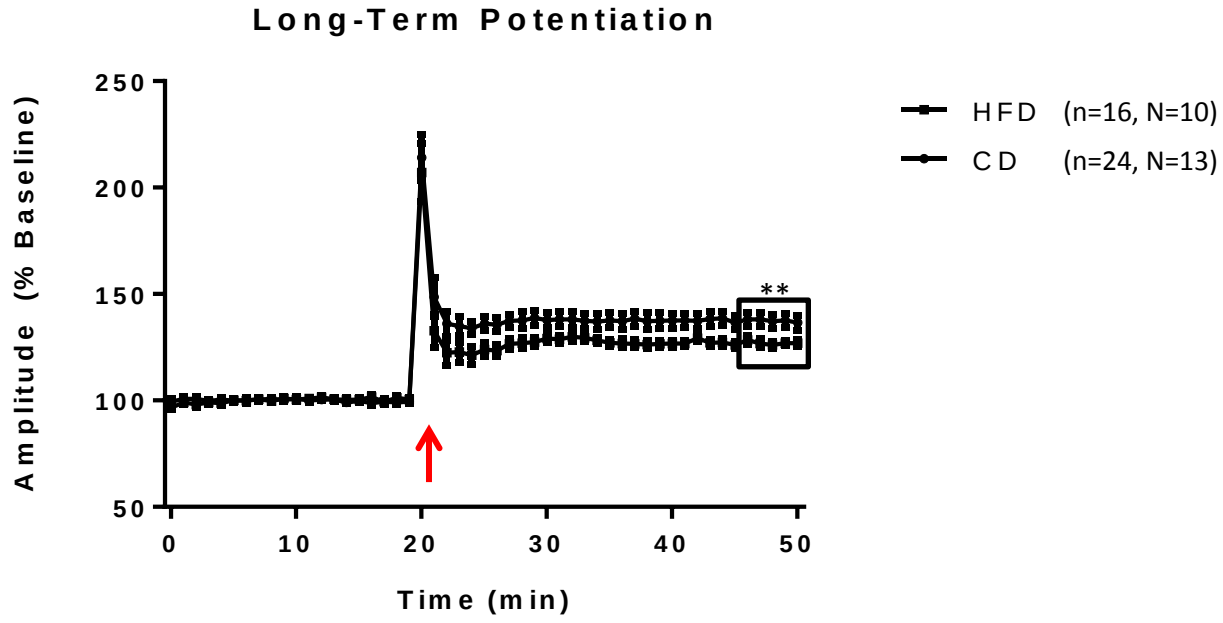


Figure 10 – fEPSP amplitude, shown as a percentage of baseline. Comparisons were performed for the last five minutes of post-HFS recording denoted by boxed area. Data are mean +/- SEM. **P ≤ 0.01. Arrow denotes high-frequency stimulation.

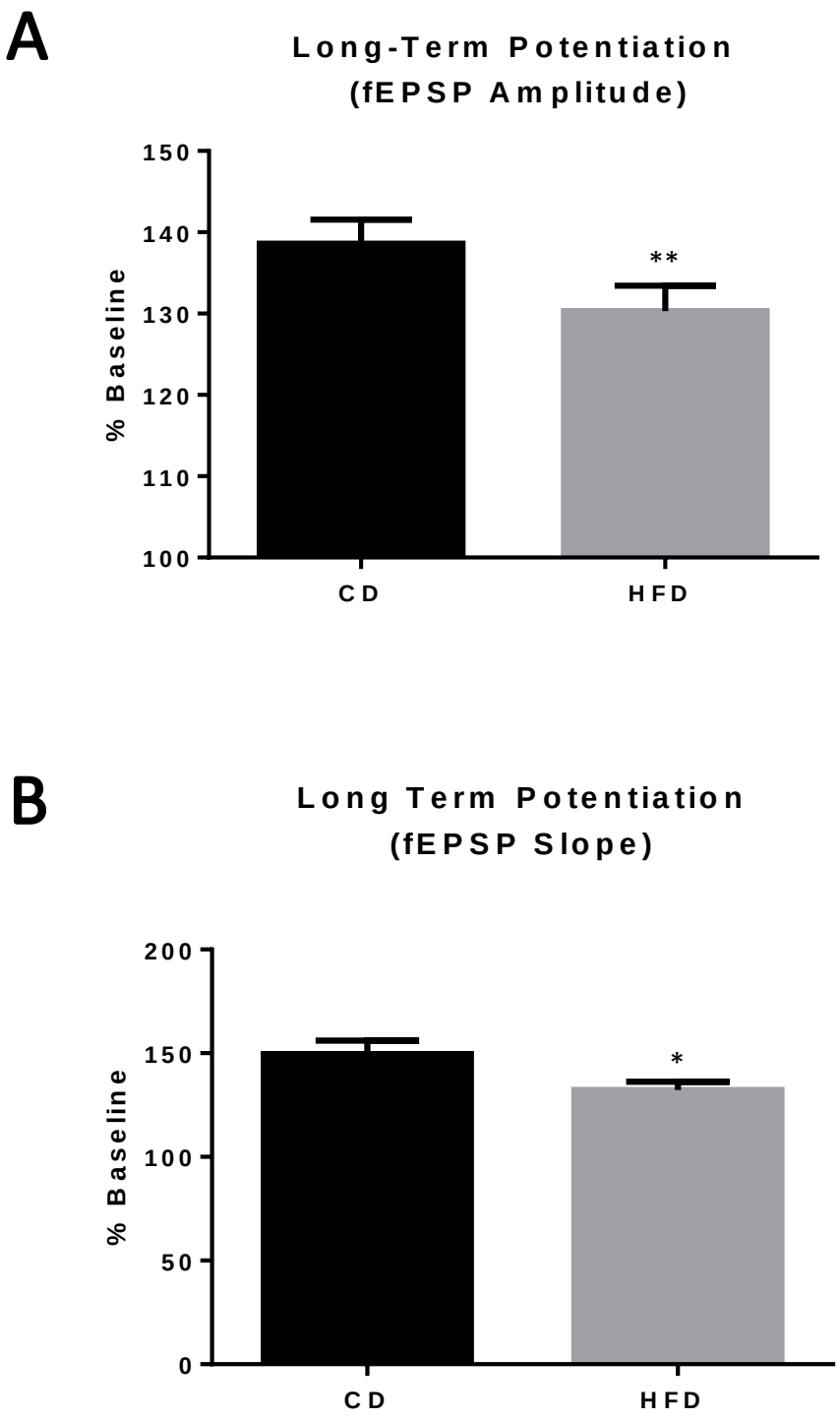


Figure 11 – A) fEPSP amplitude, shown as a percentage of baseline. B) fEPSP Slope, shown as a percentage of baseline. Data are mean +/- SEM. *P<0.05, **P < 0.01.

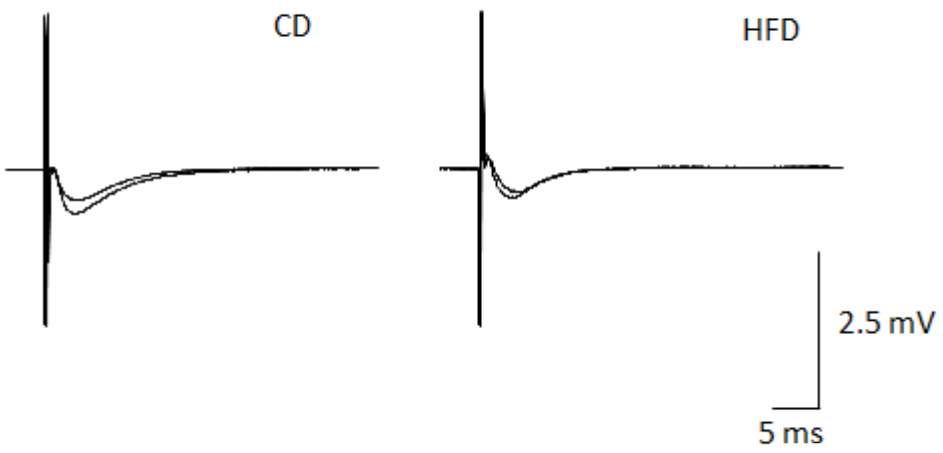
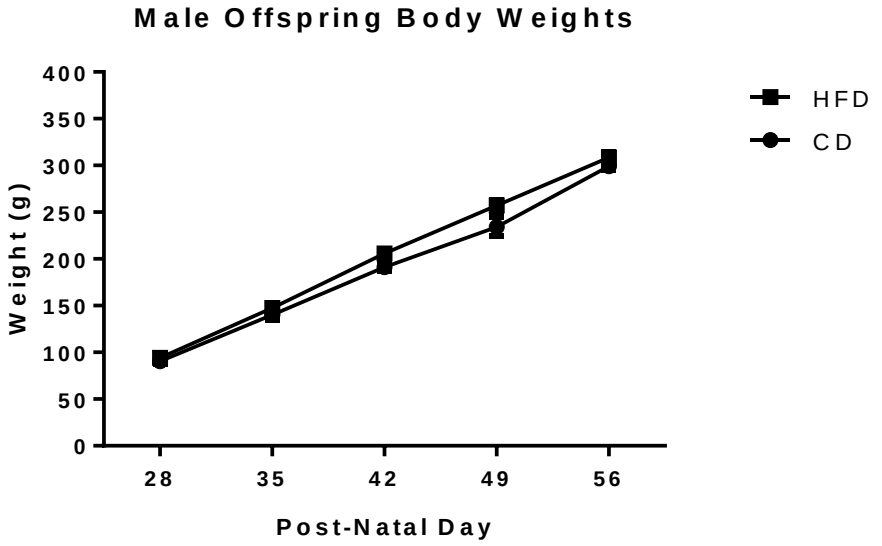


Figure 12 – Representative traces pre- and post- HFS overlaid on one another for both the CD and HFD groups. The bottom trace in each image is the trace post-HFS.

A



B

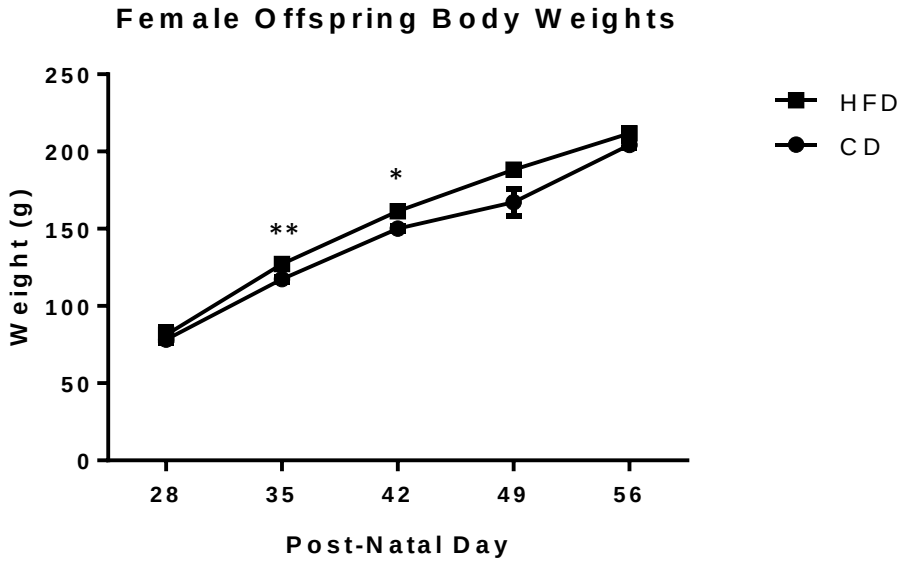


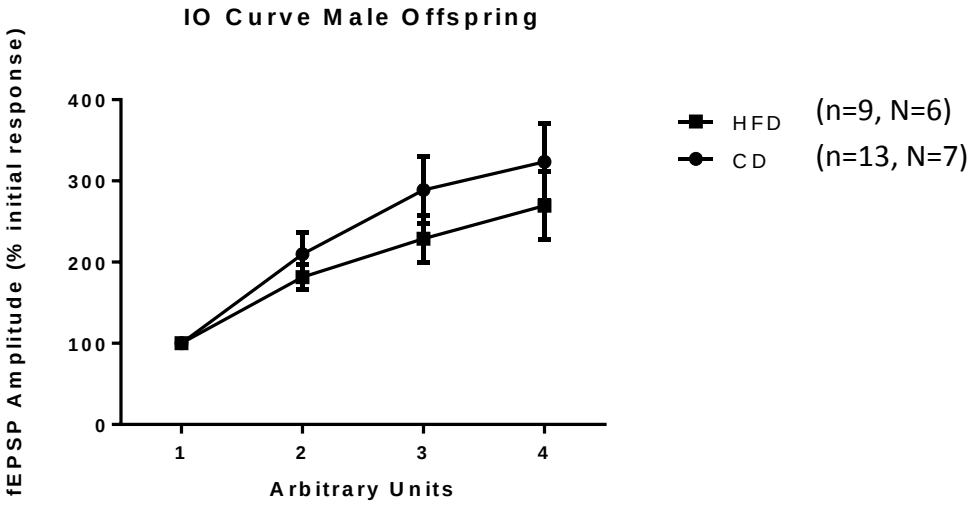
Figure 13 – A) male and B) Female offspring body weights. Data are mean +/- SEM. *P < 0.05, **P < 0.01. N=4 for both CD and HFD.

A

B

Figure 14- Oral glucose tolerance tests were performed on A) male and B) female offspring at PND 28 (adolescence) and PND 56 (young adulthood). Data are mean +/- SEM. N=4 for both groups.

A



B

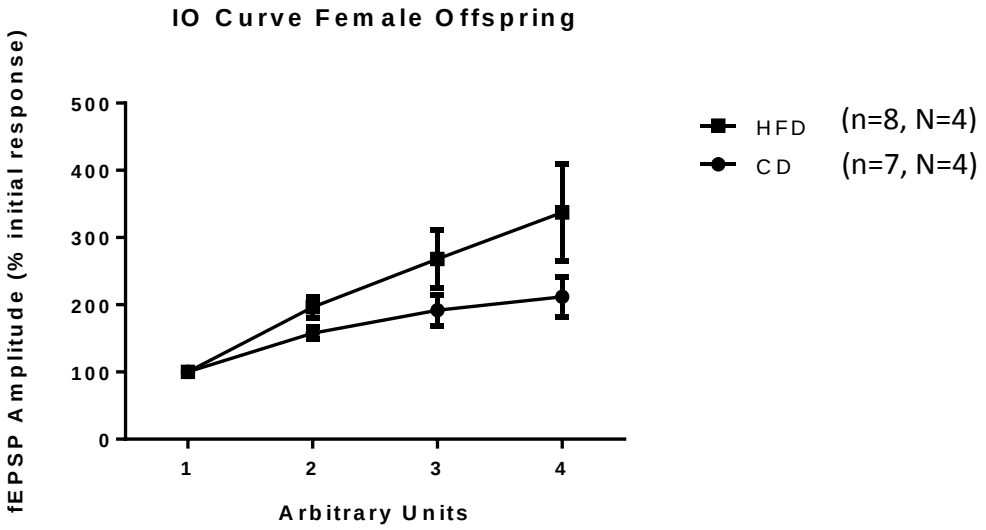


Figure 15 – Input-output curves in A) male and B) female offspring born to HFD, or CD mothers. Response amplitude for stimulations of increasing intensity (at intervals of 5 μA). Shown as a percentage of initial response. Data are mean +/- SEM.

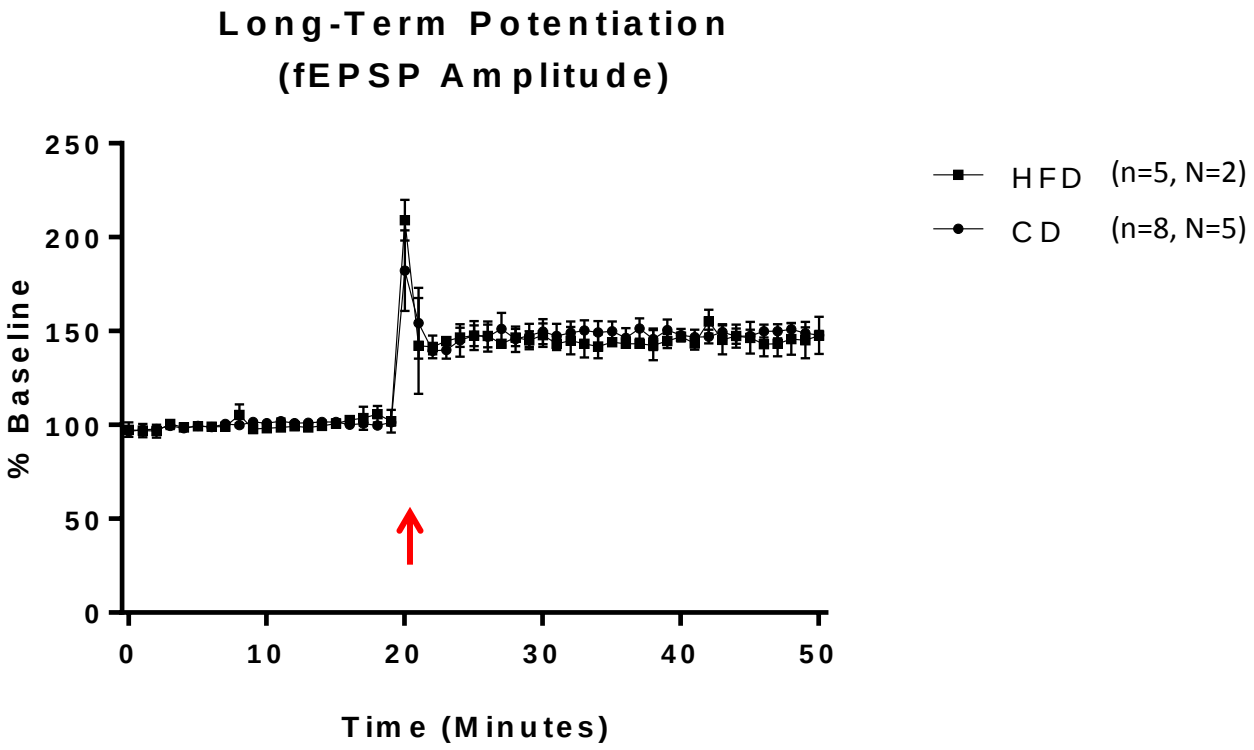
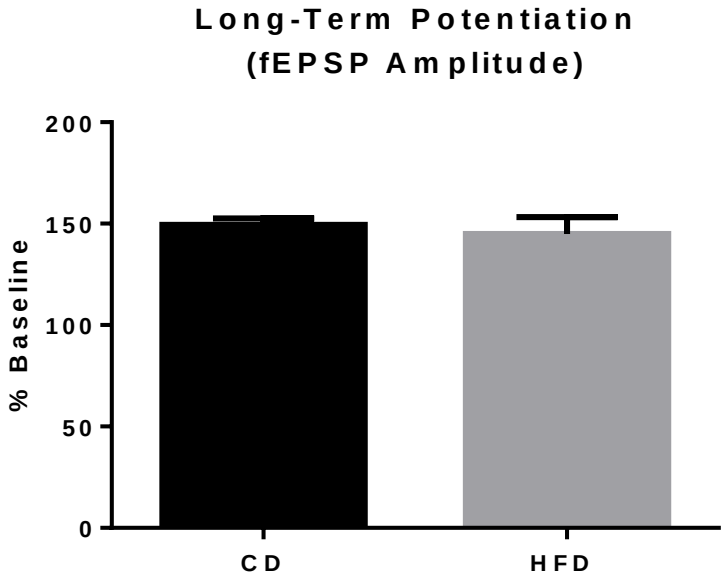


Figure 16 – fEPSP amplitude, shown as a percentage of baseline. Comparisons were performed for the last five minutes of post-HFS recording. Data are mean +/- SEM. Arrow denotes high-frequency stimulation.

A



B

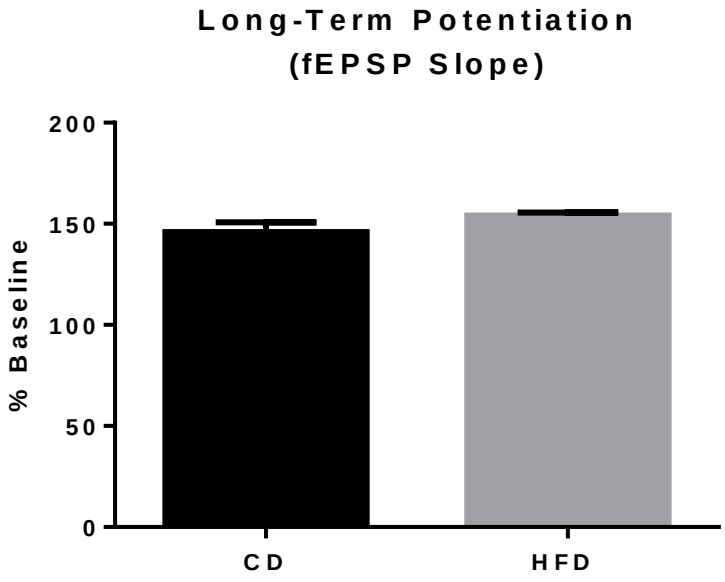


Figure 17 – fEPSP A) amplitude and B) slope, shown as a percentage of baseline for male offspring. Comparisons were performed for the last five minutes of post-HFS recording. Data are mean +/- SEM.

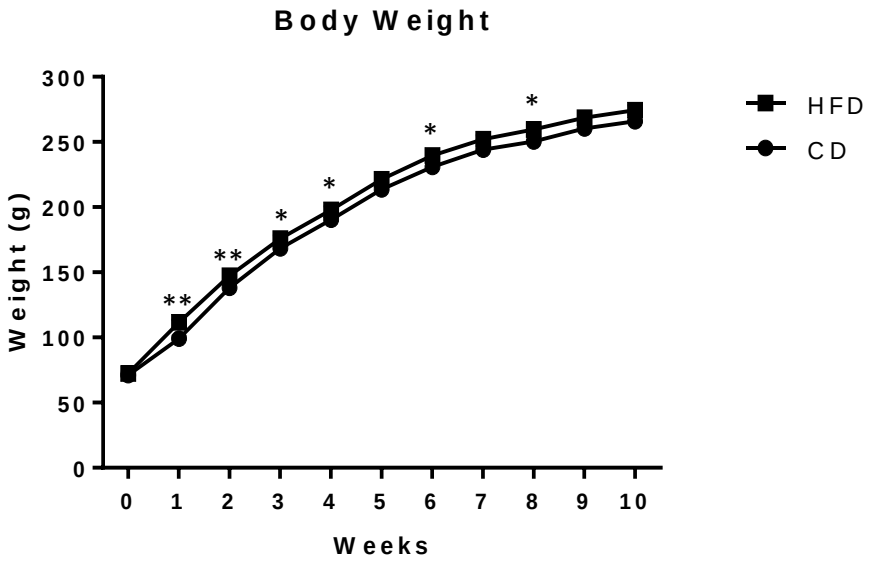


Figure 18 - Body weights at baseline, and over the course of 10 weeks on the diets. *P<0.05, **P<0.01. Data are mean +/- SEM. N=30 for both CD and HFD groups.

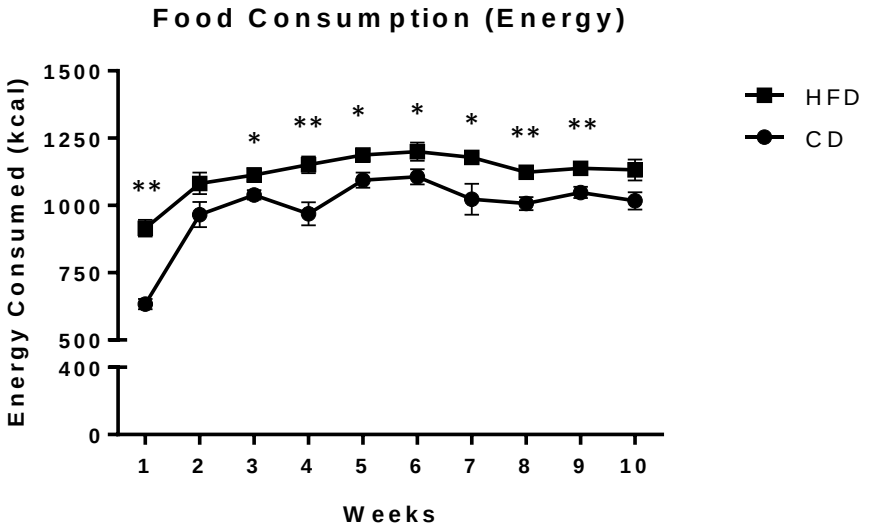


Figure 19 - Food consumption as measured by kilocalories of food consumed per cage of 3 rats over the duration of the feeding protocol. Data are mean +/- SEM. *P<0.05, **P<0.01. N=10 for both groups.

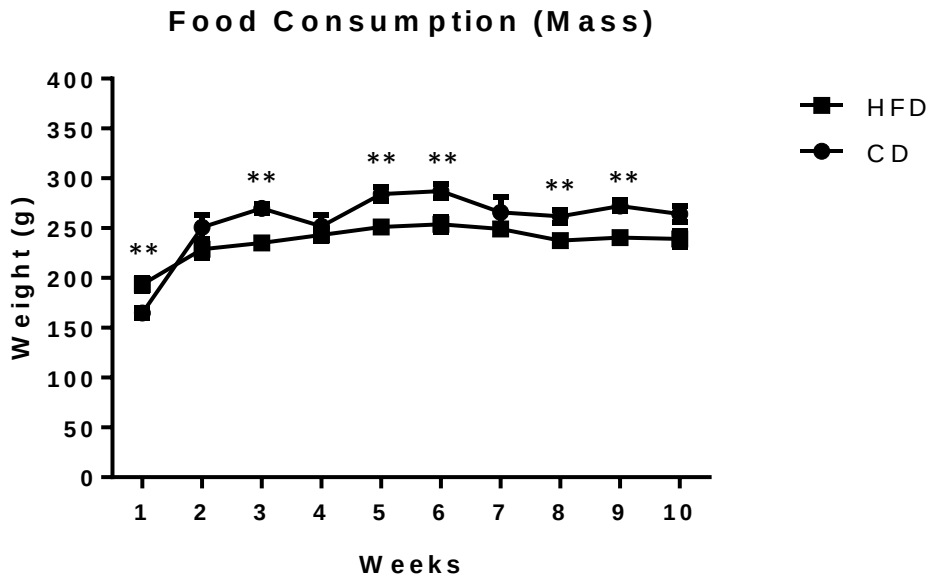


Figure 20 - Food consumption as measured by grams of food consumed per cage of 3 rats over the duration of the feeding protocol. Data are mean +/- SEM. **P<0.01. N=10 for both groups.

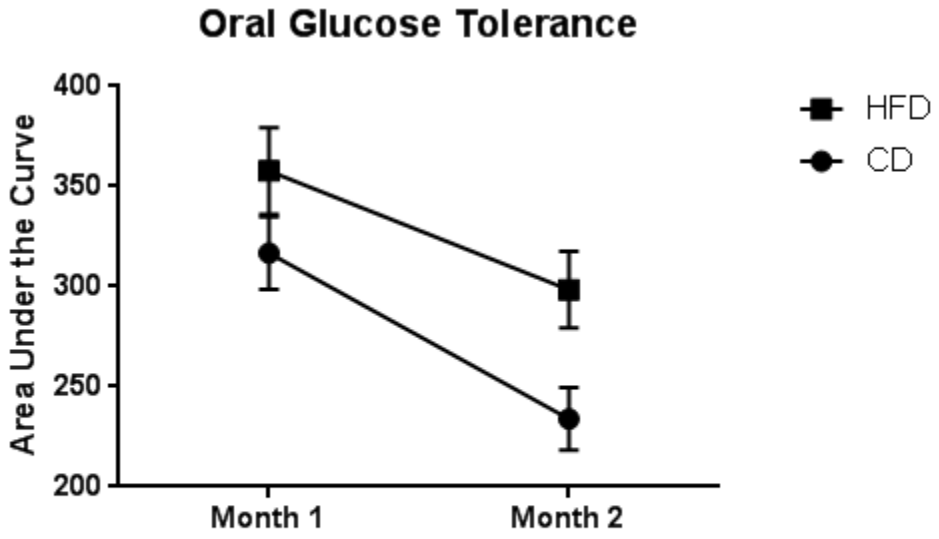


Figure 21 - Oral glucose tolerance tests were performed on the animals after 1 and 2 months on their respective diets. Data are mean +/- SEM. N=18 for both groups.

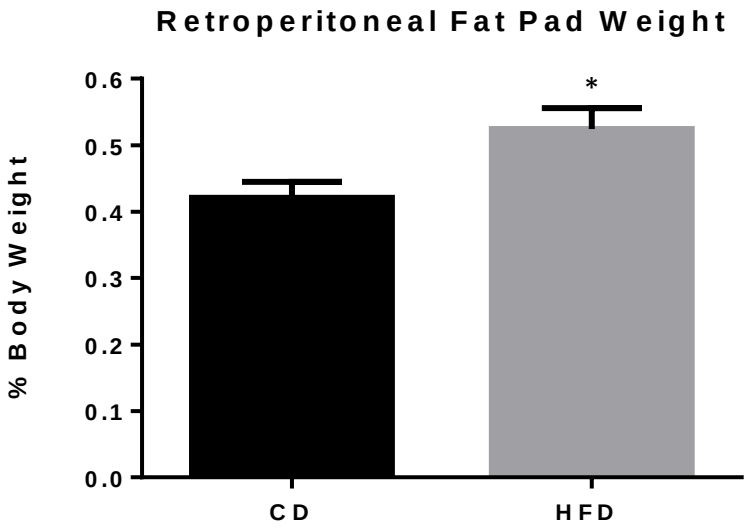


Figure 22 – Retroperitoneal fat pad mass, shown as a percentage of body weight (%BW). Data are mean +/- SEM. *P ≤ 0.05. N=15 for both groups.

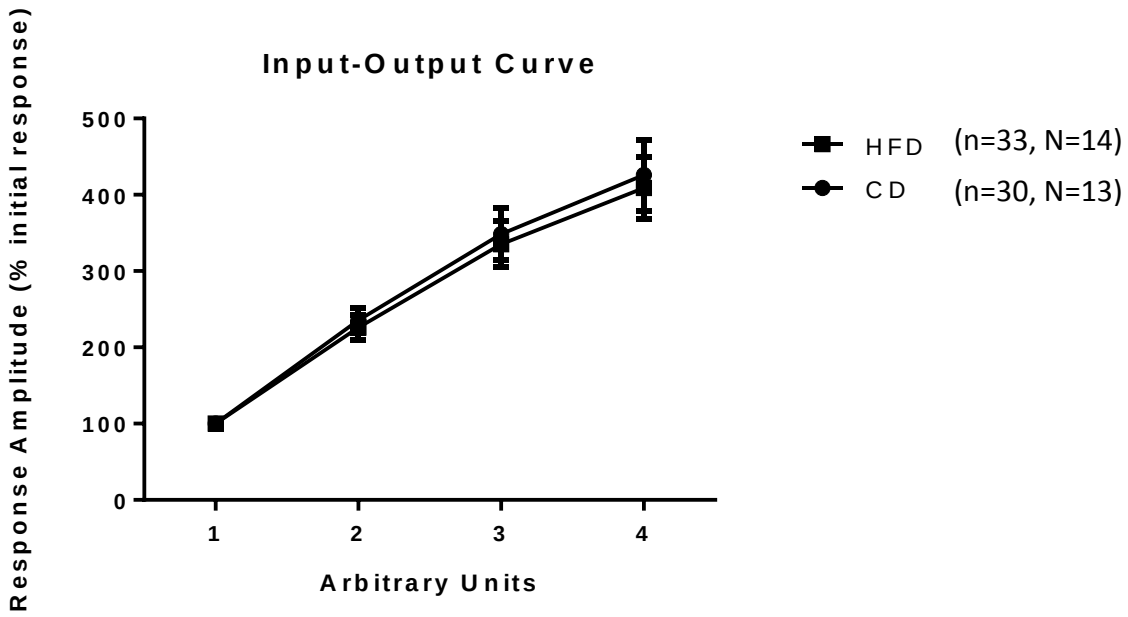


Figure 23 – Input-output curves in female animals. Response amplitude for stimulations of increasing intensity (at intervals of 2 μA). Shown as a percentage of initial response. Data are mean +/- SEM.

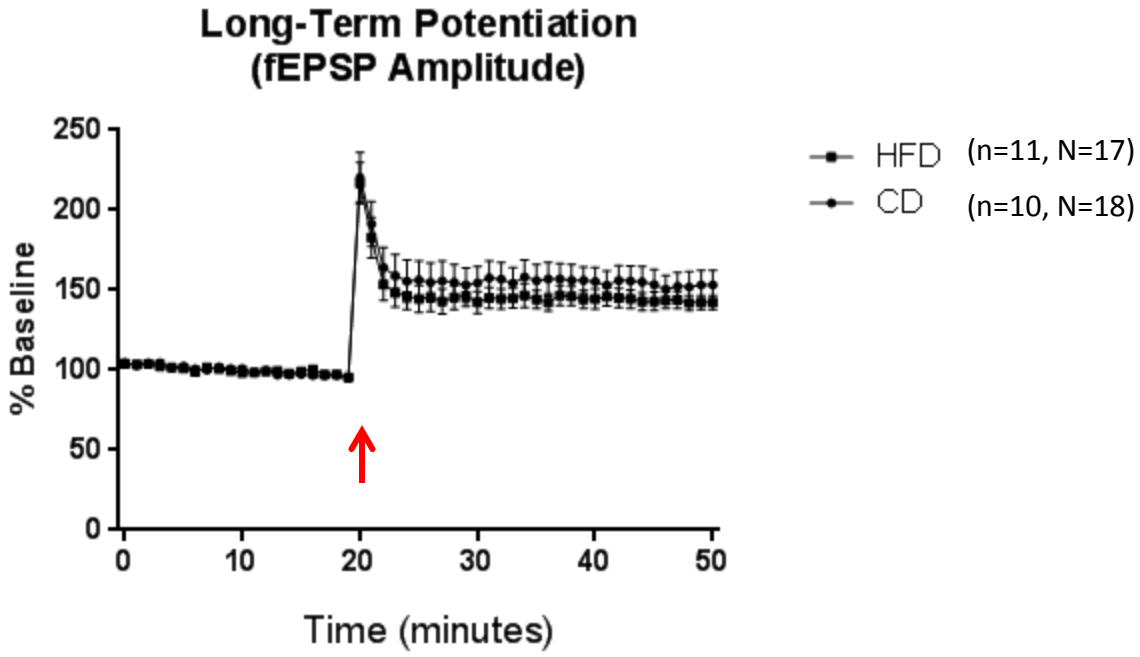


Figure 24 – fEPSP amplitude, shown as a percentage of baseline. Comparisons were performed for the last five minutes of post-HFS recording. Data are mean +/- SEM. Arrow denotes high-frequency stimulation.

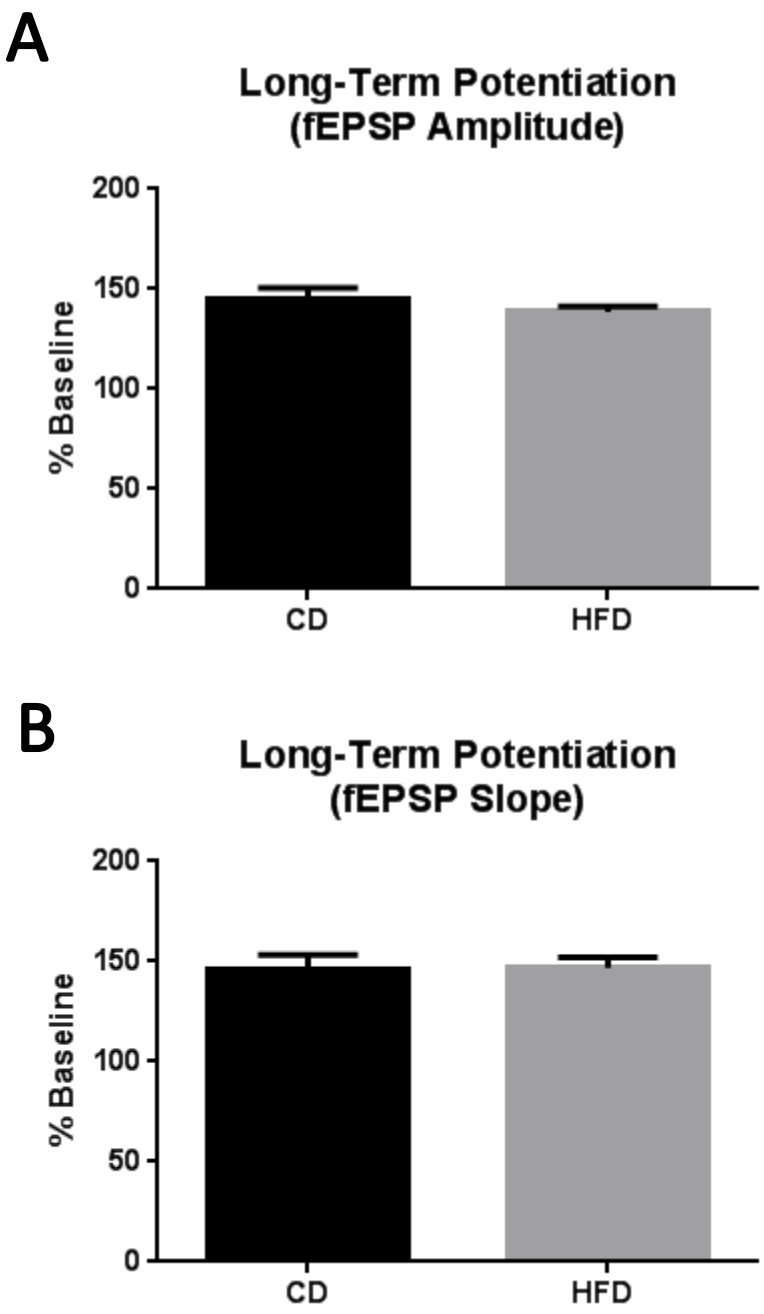


Figure 25 – fEPSP A) amplitude and B) slope, shown as a percentage of baseline. Comparisons were performed for the last five minutes of post-HFS recording. Data are mean +/- SEM.

	CD (g)	HFD (g)	P-value	t-value	df
Baseline	194.9 ± 3.06	199.2 ± 3.65	0.3692	0.9087	38
Month 1	240.0 ± 3.88	249.2 ± 4.47	0.1291	1.551	38
Month 2	263.4 ± 3.49	272.2 ± 4.80	0.1439	1.492	38
Month 3	276.2 ± 3.74	284.8 ± 4.91	0.1709	1.396	38
Month 4	285.2 ± 4.03	295.3 ± 5.65	0.1531	1.458	38

Table 1 – Body weight was monitored for all animals throughout the feeding period, and is shown at baseline, 1, 2, 3, and 4 months on the respective diets. Data are mean +/- SEM. N=20 for both CD and HFD groups.

	CD	HFD	P-value	t-value	df
FBG (mmol/L)	6.4 ± 0.27	6.4 ± 0.19	0.8578	0.01804	38
Spleen (%BW)	0.210 ± 0.00438	0.219 ± 0.00385	0.1386	1.517	34
Liver Weight (%BW)	2.30 ± 0.036	2.23 ± 0.047	0.2649	1.132	38
Liver Volume (mL/g)	0.0214 ± 0.000463	0.0210 ± 0.000403	0.4750	0.7215	38
Adrenal Glands (%BW)	0.0220 ± 0.000610	0.0233 ± 0.000790	0.1721	1.392	38
Retro-Peritoneal Fat Pads (%BW)**	0.478 ± 0.0222	0.627 ± 0.0375	0.0015	3.433	38

Table 2 – Summary table of terminal biometric measures. Data are mean +/- SEM. N=20 for all measures except for spleen weights, for which N=18. **P<0.01.

	CD	HFD
Total Slices With a Stable Baseline	30	24
Total Slices Displaying LTP Above Threshold	24	16
Percent Potentiated ((LTP Slices/Stable Slices)*100)	80%	66.67%

Table 3 – Ratio of slices displaying potentiation to slices exhibiting normal baseline synaptic transmission in the maternal generation.

Male Offspring	CD	HFD	P-value	t-value	df
FBG (mmol/L)	5.8 ± 0.56	6.0 ± 0.48	0.7776	0.2895	11
Spleen (%BW)	0.231 ± 0.0102	0.223 ± 0.00682	0.5141	0.6742	11
Liver Weight (%BW)	3.12 ± 0.137	3.10 ± 0.060	0.9326	0.08659	11
Liver Volume (mL/g)	0.0276 ± 0.00223	0.0255 ± 0.00212	0.5303	0.6500	10
Adrenal Glands (%BW)	0.0161 ± 0.000697	0.0155 ± 0.00125	0.6814	0.4216	11
Retroperitoneal Fat pads (%BW)	0.315 ± 0.0351	0.437 ± 0.0608	0.0992	1.801	11

Table 4 – Summary of terminal biometrics for male offspring. Data are mean +/- SEM. CD: N=7 and HFD: N=6.

Female Offspring	CD	HFD	P-value	t-value	df
FBG (mmol/L)	6.3 ± 0.54	6.1 ± 0.57	0.7861	0.2781	11
Spleen (%BW)	0.260 ± 0.00730	0.259 ± 0.0160	0.9436	0.07243	11
Liver weight (%BW)	2.91 ± 0.108	2.86 ± 0.0837	0.6968	0.4011	10
Liver volume (mL/g)	0.0290 ± 0.00102	0.0282 ± 0.000909	0.5858	0.5678	8
Adrenals Glands (%BW)	0.0271 ± 0.000968	0.0285 ± 0.00173	0.4742	0.7410	11
Retroperitoneal Fat pads (%BW)	0.314 ± 0.0397	0.379 ± 0.0281	0.2201	0.2201	11

Table 5 – Summary of terminal biometrics for female offspring. Data are mean +/- SEM. CD: N=7 and HFD: N=6.

Male Offspring	CD	HFD
Total Slices With a Stable Baseline	13	9
Total Slices Displaying LTP Above Threshold	8	5
Percent	61.5%	55.6%
Final Sample Size	5	2

Table 6 – Ratio of slices displaying potentiation to slices exhibiting normal baseline synaptic transmission in male animals.

Female Offspring	CD	HFD
Total Slices With a Stable Baseline	8	7
Total Slices Displaying LTP Above Threshold	5	1
Percent	62.5%	14.3%
Final Sample Size	3	1

Table 7 – Ratio of slices displaying potentiation to slices exhibiting normal baseline synaptic transmission in female animals.

	CD	HFD	P-value	t-value	df
Spleen (%BW)	0.255 ± 0.00932	0.240 ± 0.00381	0.1306	1.558	28
Liver Weight (%BW)	3.00 ± 0.071	3.01 ± 0.0364	0.8948	0.1335	28
Adrenal Glands (%BW)	0.0240 ± 0.000425	0.0243 ± 0.000570	0.7157	0.3679	28
Retro-Peritoneal Fat Pads (%BW)*	0.421 ± 0.0243	0.525 ± 0.0311	0.0136	2.633	28

Table 8 – Summary table of terminal biometric measures. Data are mean +/- SEM. N=15. *P<0.05.

	CD	HFD
Total Slices With a Stable Baseline	33	30
Total Slices Displaying LTP Above Threshold	18	17
Percent	54.5%	56.7%

Table 9 – Ratio of slices displaying potentiation to slices exhibiting normal baseline synaptic transmission.

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Appendix – Composition of Diets Used in Studies 1 and 2

	HFD, Studies 1 and 2		CD, Study 1		CD, Study 2	
Ingredient	mg	kcal	mg	kcal	Mg	kcal
Casein, 30 Mesh	200	800	0	0	0	0
Casein, 80 Mesh	0	0	200	800	200	800
L-Cystine	3	12	3	12	3	12
Corn Starch	72.8	291	315	1260	550	2200
Maltodextrin 10	100	400	35	140	150	600
Sucrose	172.8	691	350	1400	0	0
Cellulose, BW200	50	0	50	0	50	0
Soybean Oil	25	225	25	225	25	225
Lard	177.5	1598	20	180	20	180
Mineral Mix S10026	10	0	10	0	10	0
DiCalcium Phosphate	13	0	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0	5.5	0
Potassium Citrate, 1H2O	16.5	0	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40	10	40
Choline Bitartrate	2	0	2	0	2	0
FD&C Red Dye #40	0.05	0	0	0	0.025	0
FD&C Yellow Dye #5	0	0	0.05	0	0	0
FD&C Blue Dye #1	0	0	0	0	0.025	0