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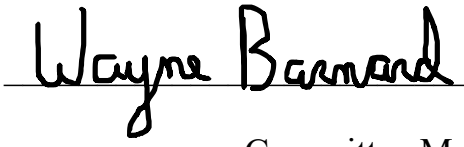

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Sleep Deprivation and High-Fat Diet during Adolescence Protect Stress Effects on Object Memory during Adulthood

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1. Introduction - Human Studies

The adolescent period is crucial for normal brain development. The abnormalities that may occur during this period may have a profound effect on behavior into adulthood (Blakemore & Choudhury, 2006; Anderson, 2003; Crews, He, & Hodge, 2007). Stressful situations are prevalent in all age groups, but teens report higher rates of stress compared to adults (*Stress in America: Are Teens Adopting Adults' Stress Habits?,* 2014). Stress has been shown to cause changes in brain function, during which the adolescent period is especially susceptible (Romeo & McEwen, 2006). Currently, teenagers are under greater stress and the levels continue to increase with each year (Leonard et al., 2015). Whereas college preparation once began during junior year of high school, it now begins in middle school, with the incorporation of pre-standardized testing and pre-college classes (Kouzma & Kennedy, 2002). These effects and the pressure of extracurricular activities have contributed to many behavioral dysfunctions, including sleep deprivation and poorer eating habits (Chaput & Dutil, 2016). Additionally, many teens are overstimulated due to light exposure, caffeine intake, and give little priority to sleep, so they sacrifice their sleep to accomplish everything on their plate (Chaput & Dutil, 2016). Therefore, with the changing adolescent landscape, there is a critical need to investigate how changes in normal eating and sleeping habits potentially contribute to long-term brain functioning and behavior (Eagan et al., 2016; Natvig, Albrektsen, Anderssen, & Qvarnstrøm, 1999).

1.1 Human Development

The stage from childhood into adulthood (i.e. the adolescent period) occurs between the ages of ten and nineteen (Brenhouse & Andersen, 2011). This period has many characteristic biological and cognitive changes. In humans, the brain's size changes very little after the age of five. The changes that occur after this age involve an increase of white matter and a decrease of

gray matter (Yurgelun-Tood, 2007). White matter consists of myelinated axons, while gray matter is made up of the cell bodies and dendrites of the neurons. The increase of white matter usually indicates an increase of myelination, which is the material that insulates a neuron's axon. Impulses travel more quickly along the neuron due to myelination; therefore, the increase in myelination provides improved cognitive functioning and faster, more efficient information sharing throughout the brain (Yurgelun-Tood, 2007; Paus, 2005). The impact of gray matter loss is not yet known.

Adolescence is also an important time for prefrontal cortex (PFC) maturation, which does not stop developing until the mid-twenties. The changes in the PFC indicate changes in controlled attention and behavior during adolescence; individuals also develop the ability to read social and emotional cues in others and are better able to control their risk impulses. PFC changes also improve executive functions, such as abstract thoughts, organization, and planning (Yurgelun-Tood, 2007; Brenhouse & Andersen, 2011). As the frontal lobe is not yet developed to control decision making pathways during this period, adolescents make decisions based on emotion, determined by the limbic system: the hippocampus, the amygdala, and the hypothalamus (Arain et al., 2013).

1.2 Eating Habits

Obesity is a rising concern in American healthcare. Forty-two percent of adult Americans are obese, and it has been found that obesity increases the prevalence of heart disease, stroke, and type two diabetes. Not only does obesity affect an individual's health, it also is costly to treat. Obese individuals have $1,400 more expensive medical care compared to individuals of normal weight ("Overweight & Obesity: Adult Obesity Facts", 2020). Additionally, children and adolescents have also experienced an increase in obesity. There are an estimated 18.5% or 13.7 million obese children and adolescents in the United States ("Overweight & Obesity: Childhood

Obesity Facts", 2019). Childhood obesity exacerbates health concerns in adulthood and the eating habits and sedentary lifestyle persist into adult life (Daniels, 2006).

Due to the often-hectic nature of adolescence, many teens partake in ease and shorter relief of diets high in fat and processed sugar (US Department of Health and Human Services, 2017). Unhealthy eating habits during adolescence, especially high-fat diets (HFD), increase the risk of adult obesity, cardiovascular issues, and mental illness (Eisenmann, Wickel, Welk, & Blair, 2005; Guo, Wu, Chumlea, & Roche, 2002; Jacka et al., 2011). The impact of adolescent diet choices on adult life has yet to be investigated.

1.3 Sleep

Sleep is an important aspect of life in both adolescence and adulthood. It is necessary for memory consolidation, reparations throughout the body, and an enhanced immune system. Adolescents are recommended to sleep between 8 and 10 hours per night; however, 45% of adolescents have been shown to sleep less than 8 hours on school nights and 43% of teens sleep less than 8 hours on weekends (*Summary of findings: 2006 Teens and Sleep,* 2006). The importance of sleep during this life stage is shown through the increased production of melatonin, a hormone that induces sleep and increases the amount of sleep required to feel rested. (Arain, et al., 2013). Lack of sleep also shortens the amount of non-rapid eye movement (NREM) sleep, which then causes a decrease in encoding and learning in the hippocampus. The reverse is found when NREM is increased. Lack of sleep not only causes acute problems, but it has also been found to impact the progression of ageing and dementia (Krause et al, 2017).

Not getting enough sleep has been shown to increase aggressive behavior, substance abuse, and the risk of obesity. It also decreases learning ability and concentration ("Teens and sleep", n.d.). Inadequate sleep also indicates an increase in depression, poor academic

performance, risk taking behaviors, and disruption of cognitive functions: mood, concentration, memory, and reaction time (Chaput & Dutil, 2016). Additionally, longer awake times decrease levels of the feeding hormone, leptin, and increase levels of the stomach hormone, ghrelin, both of which serve to stimulate appetite. Increased appetite, more hours awake, and a decreased metabolism can lead to weight gain (Knutson, Spiegel, Penev, & Van Cauter, 2007).

1.4 Sleep + Eating Habits

Sleep deprivation also leads to unhealthy food choices and more frequent and larger meals ("Sleep in America Poll", 2003). The average meal for an adolescent consists of 40% empty calories from sugars and solid fat, while a healthy diet limits these calorie sources to 10% (US Department of Health and Human Services, 2017). Unhealthy food choices during adolescence have been found to increase the risk of diabetes, high blood pressure, cholesterol, and asthma, and decrease overall health status ("Nutrition and the Health of Young People", 2008). Longer sleep times during adolescence imply lower fat indications, better emotional regulation, and better well-being, indicating that sleep plays a significant role in overall health (Chaput & Dutil, 2016). Nonetheless, society downplays the importance of sleep and it is usually one of the first things forfeited in everyday life.

1.5 Stress

The period of adolescence in itself is a stressful period. During this period, many teens are exposed to a variety of stressors: bodily changes, school, family life, and relationships, to name a few (LaRue & Herrman, 2008). If this chronic stress is not processed in a healthy manner, it may cause an increase in high-risk behaviors (Romeo, 2017). An unhealthy way of processing stress may include eating food high in sugar or fat; to this effect research has shown that stressful periods in life induce high fat seeking behaviors. The body stores more fat during stages of stress than

during stages of calm, indicating that stress influences food choices (Björntorp, 2001). Sleep deprivation, on the other hand, has been found to intensify stress, causing insomnia in many individuals. Not being able to sleep then becomes a stressor and thus further increases stress levels ("Stress and Insomnia", n.d.). In addition, humans with obesity and sleep deprivation are shown to be more susceptible to aversive life events or stress (Rosmond, Dallman, & Björntorp, 1998; McEwen, 2006), signifying that unhealthy food choices and poor sleeping habits may make an individual more prone to stress and other health difficulties.

One psychological process that is disrupted by stress is learning and memory. Stress has been shown to have a negative impact on working, or short-term memory (Oei, Everaerd, Elzinga, van Well, & Bermond, 2006). In addition, even after information has been encoded and consolidated, stress disrupts the ability to retrieve learned information (Kuhlmann, Piel, & Wolf, 2005). Thus, if adolescent poor eating and sleeping habits produce a sensitized stress response, then these behaviors can serve to exacerbate the negative effects of stress on cognitive function.

1.6 Closing

Food and sleep are two aspects of life that are present for the duration of life. Humans need both to survive and establishing healthy habits in both areas is pertinent. Many of the habits formed during the teen years tend to remain with a person throughout his or her life span. Therefore, it is critical that more research is conducted in both HFD and sleep deprivation (SD), independently. The interrelated relationship between food and sleep should also be examined, as people do not live compartmentalized lives. Diet and sleep both have influence on each other. A decision made in one aspect of life can have profound effects in another area. Therefore, this study hopes to examine the effects of HFD and SD during adolescence on how stress impacts adult memory and neuronal activation.

2. Rodent Study

This study will use a rodent model due to its longitudinal nature. Rodent models make it possible to complete a longitudinal study in only four months, because the same developmental period in rodents occurs over months instead of years as in humans (Eiland & Romeo, 2013). Complete experimental control can be utilized in a rodent model, as well. Rodents are a comparable model to humans because they are behaviorally and biologically similar, allowing for potential translation of any effects into the human condition.

2.1 Development

The adolescent period in rodents is between 35 and 60 postnatal days (pnd) (Brenhouse & Andersen, 2011). This period was determined by comparing rodent brain changes to human brain changes—myelination and white matter growth continues throughout a rodent's adolescence, just as in humans (Semple et al., 2013; Baloch et al., 2009). During this period, rodents also start to show increased sociability and more cognitive control, both comparable to human development (Semple et al., 2013; Sturman & Muoghaddam, 2011).

2.2 Eating Habits

The obesity model in rodents has been exhibited by providing HFD chow to induce the development of obesity (Hariri & Thibault, 2010). HFD has been found to impair spatial learning and memory tasks (Abbott et al., 2019). Interestingly, HFD was found to protect rodents from some chronic social stress, such as anxiety-like and depressive-like behavior (Finger, Dinan, & Cryan, 2011). Furthermore, feeding adolescent mice HFD chow has been shown to decrease memory extinction of learned fear and spatial perception (Del Rio, Morales, Ruiz-Gayo, & Del Olma, 2016; Baker & Reichelt, 2016; Valladolid-Acebes et al., 2013). When HFD was induced after adolescence (during adulthood), the effects were less robust with little reliability between

studies. Although it has been suggested that the continuation of HFD into adulthood compared to the cessation of HFD after adolescence has a greater effect on behavior (Boitard et al., 2016), there has been insufficient research relative to the long-term effects into adulthood of HFD during only adolescence. One goal of the present study is to better understand how HFD during adolescence can contribute to long-lasting effects on the brain and behavior into middle adulthood.

2.3 Sleep

Rodents are nocturnal animals, awake during dark periods and restful during light periods. Sleep deprivation (SD) in rodents is often induced by housing them on a slowly rotating ball, preventing sleep throughout their non-active hours; however, this produces complete sleep loss instead of more moderate effects on sleep as seen in human adolescents. More recently, light deprivation has been used to model 24-hour light effects in humans, producing abnormal sleep-wake patterns in experimental rodents, producing cell death of sleep-wake neurons, and shifting the circadian rhythm (González & Aston-Jones, 2006). Light deprivation during adulthood in rodents has been shown to decrease learning (Zhou et al., 2018).

SD reduces protein synthesis, which is associated with neuroplasticity and neurogenesis in the hippocampus. Increased wake times also cause an extracellular buildup of adenosine, which then affects plasticity as well. High adenosine levels cause a decrease in cAMP signaling, which then impacts the AMPA and NMDA receptor signaling in the hippocampus. These all lead to a decrease in long term potentiation (LTP), which is a form of synaptic plasticity that increases synaptic activity between neurons (Bliss & Lømo, 1973; Krause et al., 2017). Adequate sleep lengths provide a solution to high adenosine levels, as proper sleep clears adenosine. The lack of emphasis on the importance of sleep during adolescence can lead to complications during adolescence and adulthood. It is of outmost importance to understand the full effects of sleep and

how it influences both biological and psychological changes. However, no studies have investigated the specific effects of light deprivation on rodents during the adolescent developmental period, and no studies have examined the long-term effects of light deprivation on behavior and memory.

2.4 Object Memory Testing

Many different types of memory tests have been developed for rodent studies. Most require multiple weeks of training in order to determine effects of experimental manipulations on learning and retention. Because the effects of HFD and sleep disturbance have been shown to be sensitive to long delays in time, one-trial memory tests were used to avoid this confound. The object recognition and object location memory tests are both one-trial tests that measure two forms of memory: spatial and non-spatial (Barker & Warburton, 2011). These two memory tests are dependent on two different brain regions, namely the hippocampus (object location memory) and perirhinal cortex (object recognition memory; Barker & Warburton, 2011). In both tests, rats learn two objects by exploring their presence in an open field. After a period of time for memory consolidation, rats are put back into the open field where one object has been changed, either because it is replaced by a new object, or moved to a new location. Memory is typically quantified by rats preferring to explore either the new object (object recognition memory) or moved object (object location memory).

2.5 Stress

Stress has been shown to decrease LTP throughout the brain (Foy et al., 1987). Within the hippocampus, stress was found to decrease LTP and limit plasticity and neurogenesis, as well as dendritic spines (Kim & Diamond, 2002; McEwen, 2000; Gould et al., 1997), suggesting that stress can interfere with learning and memory of rodents. In obesity studies of rats, memory deficits are

often found only following an acute stressor, suggesting that obesity may not affect baseline memory processing, but more so affects the normal physiological stress response in rats (Morris, Beilharz, Maniam, Reichelt, & Westbrook, 2015). However, acute one-hour long restraint stress has been shown to have little impact on object recognition or object location memory in naïve rats. Both, non-stressed and stressed rodents, explored the new object or new location more than the familiar object or location, indicating that acute stress did not impact the object recognition and object location memory tests (Li et al., 2012). Therefore, to test if adolescent HFD and SD affect stress processes in adulthood, it was tested whether restraint stress would preferentially impact object memory in HFD and SD rats, compared to rats with normal eating and sleeping schedules.

2.6 Conclusion

Both HFD and sleep disturbance through light deprivation have individually shown to have negative short-term effects. Long-term effects of either have not been studied fully and there has been no research conducted on the compound effects of sleep deprivation and HFD during adolescence and the consequences in adulthood. Therefore, the purpose of this study was to test the long-term effects of HFD during the adolescence period, and whether the combination of HFD and sleep disturbance due to light deprivation exacerbated the effects into adulthood compared to HFD and control rodents. It was expected that rodents with the combination of HFD and SD would have worse memory performance than the HFD rodents and control rodents.

3. Research Methods

3.1 Animals

There were 20 three-week-old male Sprague Dawley rats (*r. norveficus domestica;* Envigo) used in this experiment. They were housed in groups of 3 or 4 for the entirety of the experiment and were given free (*ad libitum*) access to water throughout the study. The weight of all rats was

recorded weekly from 4 weeks old until the end of the experiment (fig. 1). All experiments followed the National Research Council Guidelines for the care and use of laboratory animals and followed procedures dictated by the Belmont Institutional Animal Care and Use Committee.

3.2 Experimental Design

Prior to experimentation, the rats had one week to acclimate to their surroundings. Three different research groups were utilized in this experiment: the control (n=6), the HFD (n=7), and the HFD + SD (n=7). All manipulations occurred during the adolescent period in rats (4-8 weeks old), but brain structure and cognitive function was tested in young adulthood (13-14 weeks old) (fig. 2).

The control group was given *ad libitum* access to a standard laboratory diet where only 13% of the calories came from fat (Laboratory Rodent Diet 5001, LabDiet), and was kept on 12-hour light/dark cycle (lights on from 6am-6pm). The HFD groups were given *ad libitum* access to a high-fat diet, with similar nutrition except that 45% of the calories came from fat (D12451, ResearchDiets; Farley et al., 2003). They were on the same 12-hour light/dark cycle. The HFD + SD rats were on the same diet protocol as the HFD subjects, but they were placed in light deprivation boxes (dark boxes with red light exposure only; Lafayette Instruments) for 24 hours/day during the manipulation period, with the only white light exposure during cage change and food and water refilling. Red light was used because rodents cannot perceive it, and thus it disrupts their circadian rhythms, inducing sleep deprivation (Dauchy, et al., 2015).

After the manipulation period (8 weeks old), all rats remained undisturbed with a meal-fed diet of standard laboratory chow (16g/rat/day) and 12-hour light-dark cycle until 13-weeks old. After this 5-week period, they were tested on the effects of stress on object and spatial memory. The memory experiments took place over 2-weeks, after which the rats were euthanized 50

minutes after object location testing, and their brains were extracted (fig. 2). This timepoint of euthanasia was chosen to measure neuronal activation in the hippocampus during object location retrieval testing.

3.3 Memory Testing

Two memory tests were performed: object recognition (ORT) and object location (OLT) (fig. 3 & 4). Object recognition is dependent on the perirhinal cortex, while object location memory is dependent upon the hippocampus (Barker & Warburton, 2011), therefore both forms of memory were tested to understand how HFD and SD affects memory, dependent on two different brain circuits. Before memory testing, all rats were habituated over the course of 3 days to an open field (40 in x 40 in) that was used for object testing, to make sure that no rats had anxiety-like responses to the arena itself. During the ORT, the rats explored two identical objects during an acquisition period (4 minutes), wherein they needed to explore the objects cumulatively for at least 30 seconds (nose pointed < 1inch from the object). Twenty-four hours later they were placed in the same arena, where one of the objects was replaced with a new object for the test period (3 minutes). The OLT followed the same procedure as the ORT, except that instead of presentation of a novel object in the test phase, one of the objects changed locations within the arena. During the test period of both memory tests, exploration of both objects was manually recorded and a discrimination ratio

$$\frac{(time\ spent\ exploring\ new\ object)-(time\ spent\ exploring\ old\ object)}{total\ time\ spent\ exploring\ objects}$$

was calculated. Because rats naturally explore new objects (novel or moved objects), positive discrimination ratios indicate long-term memory of the state of objects from the acquisition period. All rats were tested for both object recognition and object location memory.

Acute stress has been shown to affect object exploration, sometimes causing rats to show novelty-avoidance, confounding potential memory interpretations. Therefore, to model stress

effects in our rats, just after object acquisition (but not before memory retrieval testing), the rats were exposed to a brief, acute stressor via physical restraint for 30 minutes in a clear plexiglass restraint-tube (Harvard Apparatus) before being returned to their home cage for the remainder of the night.

3.4 Brain Processing

Sixty minutes following the last memory test, rats were euthanized via rapid decapitation to preserve brain tissue to measure hippocampal volume using cresyl violet, adult neurogenesis through analyzing expression of doublecortin (DCX, a protein marker of immature neurons), and neuronal activation through expression of c-fos (a protein transcription factor that is used as a marker of indirect neuronal activation, because it becomes maximally expressed within cell bodies 60 minutes after strong neuronal activity) in the hippocampus (Vann, Brown, Erichsen, & Aggleton, 2000; Barker & Warburton, 2011). After rapid decapitation, the rat's brain was extracted and was fixed in 4% paraformaldehyde (Sigma Aldrich) for analysis. Brains remained in fixative for 24 hours, after which they were transferred into 20% sucrose for at least 48 hours before being sliced, to ensure that brains were sufficiently cryoprotected. 40µm sections throughout the hippocampus were cut using a sliding microtome (American Optical) under dry ice (Jeni's Splendid Ice Cream). Sections were stored in 1N phosphate buffer solution (PBS) until immunohistochemical reactions.

3.5 Cresyl Violet Stain (Zeuthen, Glushchak, Davis, & Schoenfeld, *in review).*

To measure hippocampal volume, a cresyl violet stain was utilized to stain all cell bodies in tissue slices to trace brain areas. Sections were mounted onto SupraFrost slides (ThermoFisher Scientific) and allowed to dry overnight. They were stained with 0.1% cresyl violet (Sigma Aldrich) solution for 4 to 6 minutes. Each slide was then rinsed in water and then placed in 70%

ethanol containing acetic acid for 3 minutes. Then the slide was moved in 95% ethanol for 3 minutes, followed by 100% ethanol for at least 5 minutes. Lastly, the slide was transitioned to xylene (Sigma Aldrich) for 5 minutes before being cover slipped with Permount (ThermoFisher Scientific). To measure the volume of the hippocampus, each slice was placed under an Olympus BX50 light microscope. Individual sections of hippocampus were traced per slice. All slices were summed together and the entire volume of the hippocampus of the brain was then estimated based on ratio of sections (1:12) and thickness of slices (Schoenfeld, McCausland, Morris, Padmanaban, & Cameron, 2017; fig. 5).

3.6 Immunohistochemistry for Doublecortin (Zeuthen, Glushchak, Davis, & Schoenfeld, *in review).*

Doublecortin (DCX) is a protein only expressed in neurons less than 3 weeks old, so it is commonly used as a global marker of neurogenesis in adult brain tissue. Sections containing hippocampal tissue were washed three times for 5 minutes in PBS. Then the sections were placed into a blocking solution (5% tween-20 and normal donkey serum) for 20 minutes. Then the sections were incubated in rabbit anti-DCX primary antibody (1:200; Cell Signalling Technology) for 72 hours. The sections were then washed in PBS three times for 5 minutes and then incubated in a solution with donkey anti-rabbit Alexa 488 fluorescent secondary antibody (1:200; Molecular Probes) in the dark for 1 hour. This fluorescent secondary antibody allows for the visualization of DCX protein under green light conditions. The sections were washed in PBS three times for 5 minutes and they were incubated in Hoechst (1:1000; Sigma Aldrich) for 5 minutes and rinsed in PBS three more times for 5 minutes. Hoechst is a fluorescent Nissl stain that labels all cell bodies fluorescent blue so is used as a geographic marker. Slices were then mounted onto slides and cover slipped with 3:1 glycerol. The tagged neurons were counted via a mercury-bulb epifluorescent

microscope and the total amount of immature neurons was averaged per slice for each brain (fig. 6).

3.7 Immunohistochemistry for C-fos and Analysis

Sections were stained with c-fos through a free-floating reaction. Sections were incubated in 0.01 mM citric acid (pH 6.0) at 90°C for 15 minutes to denature the nucleus membrane. Then, the sections were washed in 1N PBS for 10 minutes, three times. Sections were incubated for 20 minutes in normal blocking solution (containing 5% Tween-20 and normal donkey serum) before a 3-day incubation in c-fos protein tagged with fluorescent dye sensitive to 488nm light (Santa Cruz Biotechnologies). This dyed all neurons that expressed c-fos following memory test a neon green that would become illuminated under fluorescent light. After incubation, all sections were rinsed with Hoescht, which labeled all cell bodies in brain tissue neon blue to serve as a background stain. Sections were mounted onto standard microscope slides and cover-slipped using glycerol to preserve fluorescence. All c-fos+ cells were counted in a 1:12 series of sections throughout the dentate gyrus using an epifluorescent BX-45 microscope (Olympus) and used to estimate total neuronal activation in the hippocampus following memory tests (fig. 7).

3.8 Statistical Analysis

Averages were calculated for weight, exploration times for new and old objects for both memory tests, hippocampal volume, immature neurons, and c-fos activation (number of cells/number of slices). A 3x8 ANOVA was completed for weight, 2x3 ANOVAs were completed for object exploration times, and one-way ANOVAs were conducted for discrimination ratios for both memory tests, hippocampal volume averages, doublecortin cell averages, and c-fos activation averages using SPSS software. A Tukey post-hoc test was used for each ANOVA if the main effect

was significant to determine where significant differences were among control, HFD, and HFD + SD rats.

4. Results

4.1 Weight Distributions

To determine if HFD and SD affect weight gain during adolescence and adulthood, a 3x8 (diet x age) mixed factorial ANOVA was performed. There was a main effect of the diet manipulations ($F(2, 17) = 6.63$, $p < 0.05$) (fig 1). A Tukey post hoc showed that HFD rats were significantly heavier than the control rats ($p < 0.05$) and the HFD + SD rats ($p < 0.05$). The relationship between control rats and sleep deprived + HFD was not significant ($p > 0.05$).

A main effect of age, ($F(8, 136) = 1130.31$, $p < 0.05$) (fig 1), shows that all rats gained weight over time. Lastly, a significant interaction between diet and age ($F(16, 136) = 2.21$, $p < 0.05$) following by simple effects showed that there were no differences between diet manipulations from ages 4-7 weeks ($p > .05$), however starting at 8 weeks of age (4 weeks after being on HFD and sleep deprivation), HFD rats were heavier than the other two groups ($F(2, 17) = 6.81$, $p < 0.05$). This weight enlargement for HFD rats lasted until 11 weeks of age, 3 weeks after returning to a control diet.

4.2 Memory Testing

To measure how HFD and SD affect object recognition memory, a 2x3 (object x manipulation) mixed factorial ANOVA was performed on object exploration times (fig 8.A). There were no main effects of diet manipulation ($F(2, 17) = 1.92$, $p = 0.18$) but a significant main effect of object type ($F(1, 17) = 9.27$, $p < 0.05$), suggesting that overall the novel object was explored more. This main effect is explained by a significant interaction ($F(2, 17) = 3.76$, $p < 0.05$),

showing that HFD + SD rats had increased novel object exploration ($t(6) = 3.46$, $p < .05$), but neither control rats nor HFD rats displayed novel object exploration time differences ($p > .05$).

To measure if the combination of HFD and SD affect object location memory, a 2x3 (location x manipulation) mixed factorial ANOVA was performed (fig 8.B). A significant main effect of object location suggested that within all groups, there was more exploration of the moved object compared to the unmoved object ($F(1, 17) = 8.36$, $p < 0.05$). There was no main effect of diet manipulation ($F(2, 17) = 2.78$, $p = 0.09$) nor interaction ($F(2, 17) = 0.36$, $p = 0.70$).

To compare object discrimination memory between groups, two one-way ANOVAs were performed, one for each memory test (fig 8.C). A main effect was found in the object recognition test ($F(2, 17) = 5.19$, $p < 0.05$). Tukey's post-hoc tests showed that the HFD + SD rats had significantly higher discrimination than control rats ($p < .05$). No differences were found between groups on discrimination ratios for the object location test ($F(2,17) = 0.01$, $p = 0.99$).

4.3 Hippocampal Volume (Zeuthen, Glushchak, Davis, & Schoenfeld, *in review).*

To measure if the combination of HFD and SD affects hippocampal volume, a one-way ANOVA was performed. There was no effect of diet manipulation on adult hippocampal volume ($F(2,16) = 0.20$, $p = 0.82$) (fig. 9).

4.4 Doublecortin Analysis (Zeuthen, Glushchak, Davis, & Schoenfeld, *in review).*

To measure if the combination of HFD and SD affects hippocampal neurogenesis during adulthood, a one-way ANOVA was performed. There was no effect of diet manipulation on the number of immature neurons in the adult hippocampus ($F(2,9) = 0.46$, $p = 0.65$) (fig. 10).

4.5 C-fos Analysis

To measure if the combination of HFD and sleep deprivation decreases c-fos staining, and thus hippocampal neuron activation, during object location testing, a one-way ANOVA was

performed. There was a main effect of c-fos activation ($F(2, 16) = 8.32$, $p < 0.05$) (fig 11). Post hoc comparisons using the Tukey HSD test indicated that the means for the HFD+SD ($M = 4.38$, $SD = 3.77$) and the HFD ($M = 6.08$, $SD = 2.90$) were significantly less than the control ($M = 12.81$, $SD = 2.58$) indicating that HFD + SD rats and HFD rats had decreased activation of hippocampal neurons compared to the control rats.

5. Discussion

To study whether HFD and the conjunction of sleep deprivation during adolescence has an effect on adult memory and brain structure, this study utilized two memory tests, object recognition and object location, hippocampal volume, DCX activation, and c-fos activation. This study found that following acute stress, the HFD + SD rats had higher novel object exploration in the ORT compared to the control rats. All of the rats spent more time exploring that new location more than the familiar location in the OLT. Both the HFD + SD rats had lower c-fos activation compared to the control rats. There were no differences in hippocampal volume and doublecortin activation between groups.

The surprising object recognition results indicate that HFD + SD rats had better memory retrieval than the control rats and one reason for this may be the introduction of stress after acquisition during memory testing. The stress may have influenced memory consolidation in the control rats (Schwabe & Wolf, 2010) as they did not have prior stress exposure such as the HFD + SD rats. Sleep deprivation, in itself, is considered to be a stressor, so exposure to sleep deprivation during adolescence may have provided protective effects in the future stressful situation (McEwen, 2006). This was also shown in the discrimination ratios where the HFD + SD rodents had higher levels of memory in the object recognition task than the control rats.

The recovery time between manipulations and testing may have contributed to the overall trend of greater novel location exploration than old location within all three groups. As stated in the previous paragraph, it has been shown that HFD causes location memory deficits (Beilharz, Maniam, & Morris, 2014) and it has been found that sleep deprivation also impairs spatial memory (Guan, Peng, & Fang, 2004). This study did not find differences between groups, but the control rats did have greater overall exploration times than the HFD and HFD + SD groups, indicating that there may be an effect between groups that needs more investigation, especially in the aspect of recovery time.

The data in ORT and OLT may moreover indicate the HFD + SD rat's inclination to risky behavior. In ORT there are two objects, one that is familiar and one that is novel. A rodent in its natural environment would be cautious around a novel object, as it does not yet know if it is a predator. With this in mind, the control rats were more cautious compared to the HFD + SD rats in ORT. This interpretation correlates with past literature in that SD has been shown to increase risk behaviors (Chaput & Dutil, 2016). In OLT, there was prior familiarity with the objects due to ORT taking place before OLT, the familiar object just changed locations. So, object location did not have the magnitude of risky behavior as object recognition did as the object was known to be safe.

C-fos activation was found to be higher in the controls, who also had higher total exploration times compared to the two other groups. This indicates that activation of cells in the dentate gyrus (DG) is associated with exploration more than memory. The DG has been found to contribute to exploration of novel environments, as interneurons in the DG increase their firing rates in novel environments, be it novel objects or location (Nitz & McNaughton, 2004). Future

research could analyze CA1 region of the hippocampus to see if there was a difference in activation of cells compared to the DG.

Hippocampal volume and hippocampal neurogenesis (DCX activation) remained similar between groups, indicating that the manipulations during adolescence did not have an effect on these measures during adulthood. It has been found that adolescent stress does not induce changes in adult hippocampal volume following adolescent stress (Lupien et al., 2011). Adult hippocampal neurogenesis was found to increase during adulthood compared to adolescent levels after adolescent stress (Hueston, Cryan, & Nolan, 2017), demonstrating that neurogenesis levels normalize during adulthood. Although this data reveals that there are no changes in structure of the hippocampus between groups during adulthood, it is not certain if structural changes occur as a result of diet and sleep manipulations during adolescence. Therefore, it is possible that these null effects reflect recovery of brain structure following adolescent experiences. However, because there are differences in neuronal activation and behavior, this indicates that the circuitry within the hippocampus may have more subtle alterations following manipulations and recovery.

Some limitations of this study included the use of stress via restraint tube after the acquisition period, which provided a way to look memory following stress, but it was not possible to study the effects of no stress on testing (Schwabe & Wolf, 2010). This study used the open field to measure behavior and memory, but the use of the open field also allows for influences, such as light, temperature, and odor to have an effect on the rodent's performance (Tatem et al., 2014). The decision to use only male rodents provided a way to measure memory and behavior devoid of sexual drive that male rats exhibit in the presence of female rats, which limited this study in the sex differences that may be due to variances in adolescent development due to sex (Gleason, Fuxjager, Oyegbile, & Marler, 2009).

Nonetheless, this study was able to provide some insight into the relationship of high fat diet and sleep deprivation during adolescence. This study supported previous research in that HFD increases body weight of rodents more rapidly than regular chow and makes it difficult to treat obesity in the future (McNay & Speakman, 2013). As the HFD rats maintained their heavier weights throughout the study even after the regular diet was administered indicating that it is harder to reverse the progression of obesity. In addition, control diets were forced onto rats, but it is unknown how much more difficult it would be to reduce weight when HFD and control diets are volitional choices. This then pushes for education in the prevention of obesity, which would include diet, sleep habits, and genetics. These results also may indicate that stress during the adolescent period may influence the way learning and memory function in adulthood during stressful conditions. Due to the influence of stress on memory and behavior, the timing and use of stress should be considered in future studies. Due to the recovery time between manipulations and memory testing, it is hard to say what the long-term effects of HFD + SD may be. As it is not certain that there are negative effects of the conjunction of HFD and SD. Future studies should consider not implementing a period of recovery after manipulations prior to conducting research with either HFD or SD to investigate the full effects of HFD and SD. This then may provide support that poor adolescent habits involving food or sleep may not have long-term effects on memory later in life, if these habits are improved in late adolescence/early adulthood. Although the HFD + SD rats performed better than the control rats in recognition memory, there is a need for further research into the long-term effects of high fat diet and sleep deprivation.

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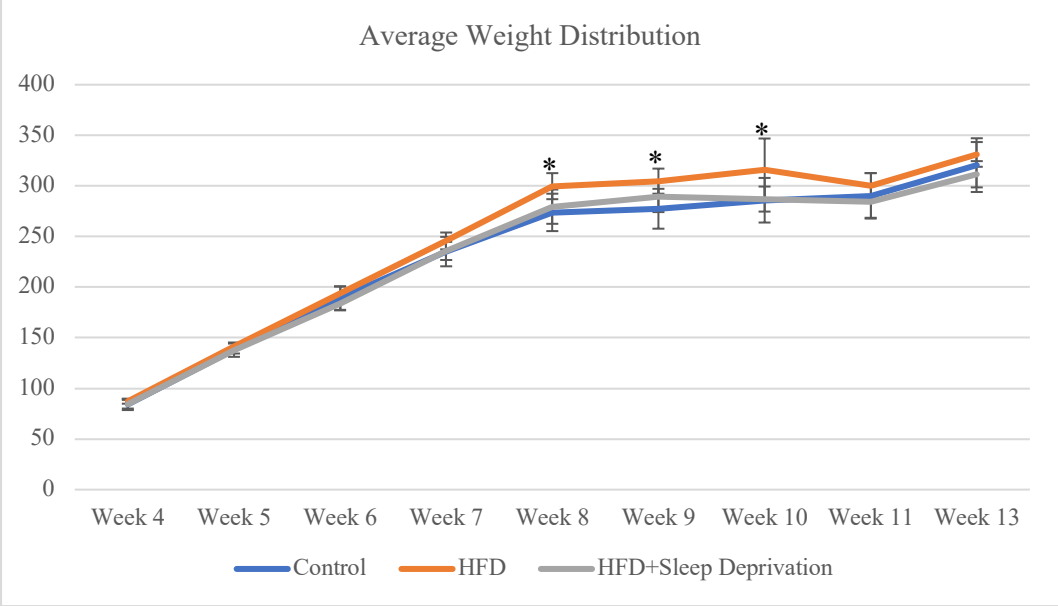
Figures

Figure 1: Average Weight Distribution Over Time



Graph of the average weight of each manipulation group as time progressed.

Figure 2: Timeline of Experiment

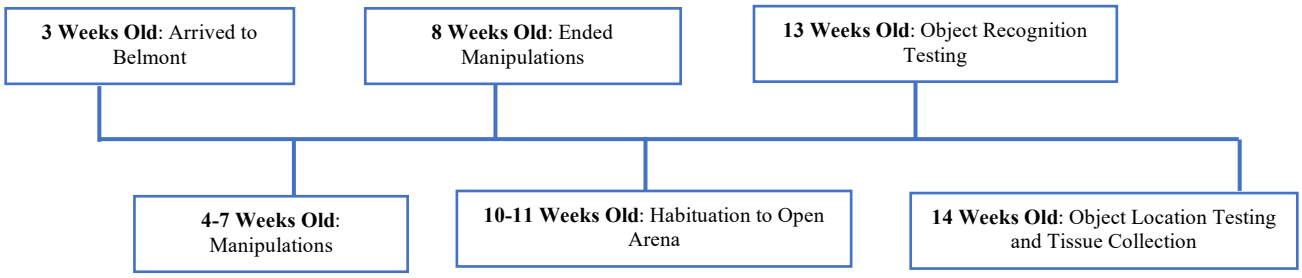


Figure 3: Object Recognition Test

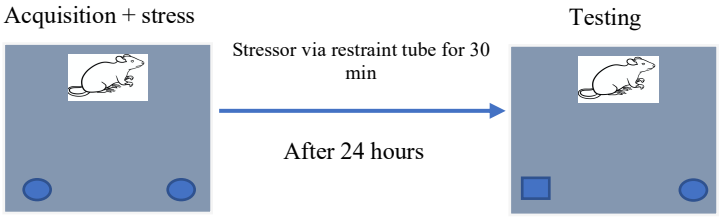


Figure 4: Objection Location Test

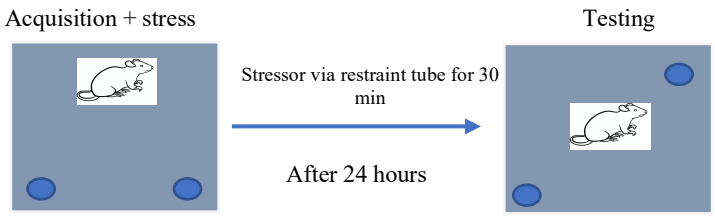
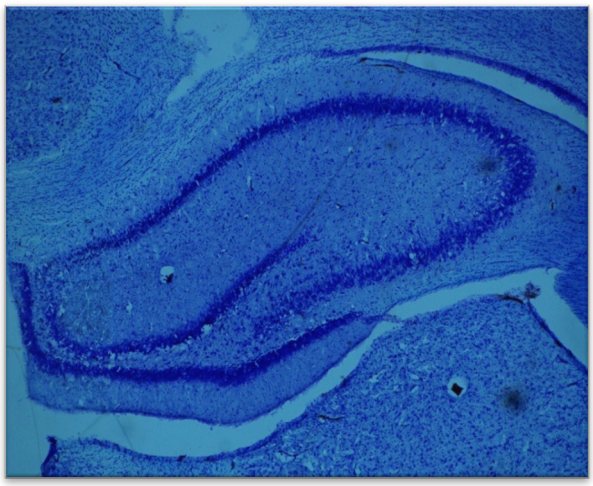


Figure 5: Cresyl Violet Stain



Cresyl Violet stain of a coronal slice of the hippocampus

Figure 6: Doublecortin Stain

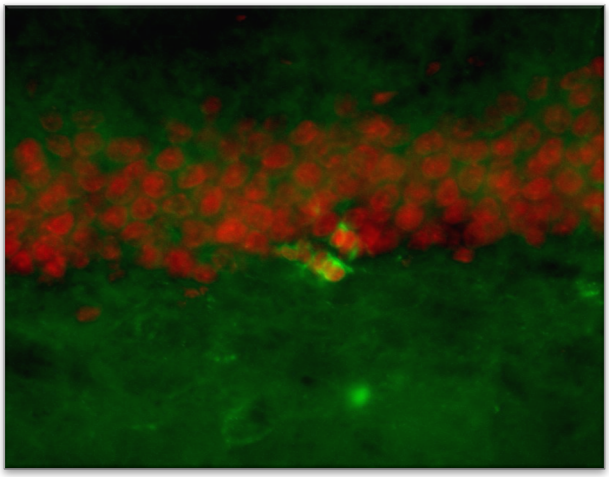
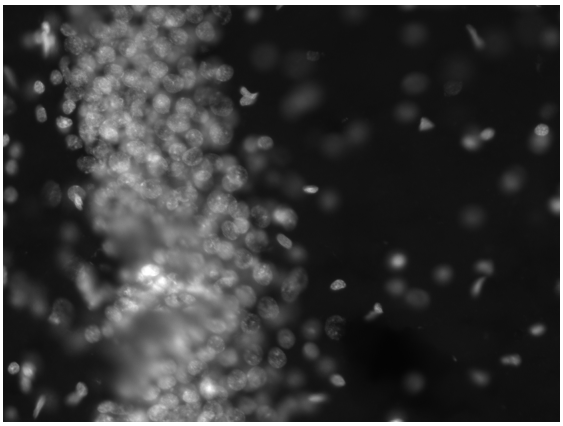
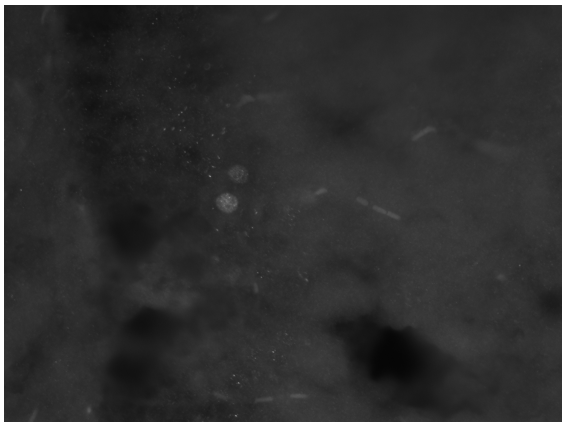


Image of a doublecortin stained cell (in green) in the dentate gyrus, indicating neurogenesis

Figure 7: C-fos

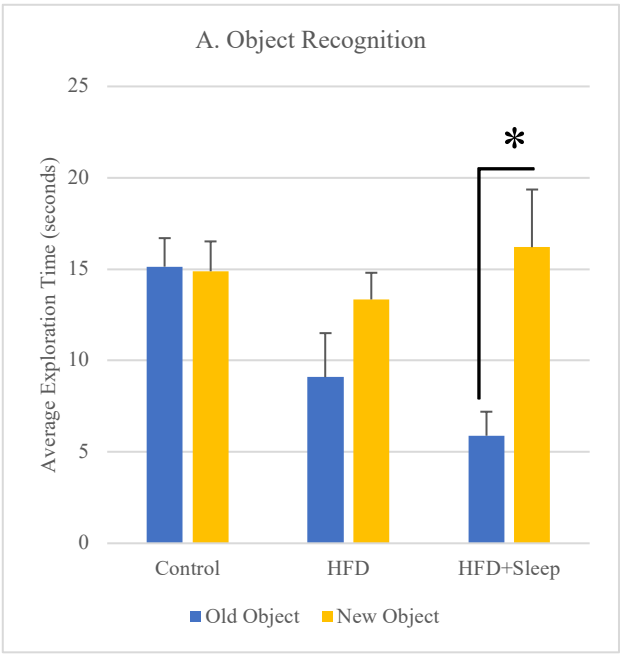


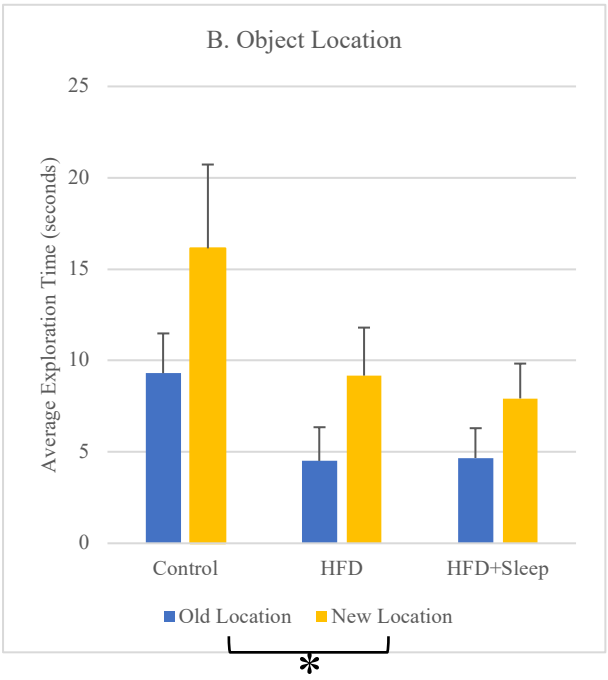
A. Neurons in the DG of the hippocampus

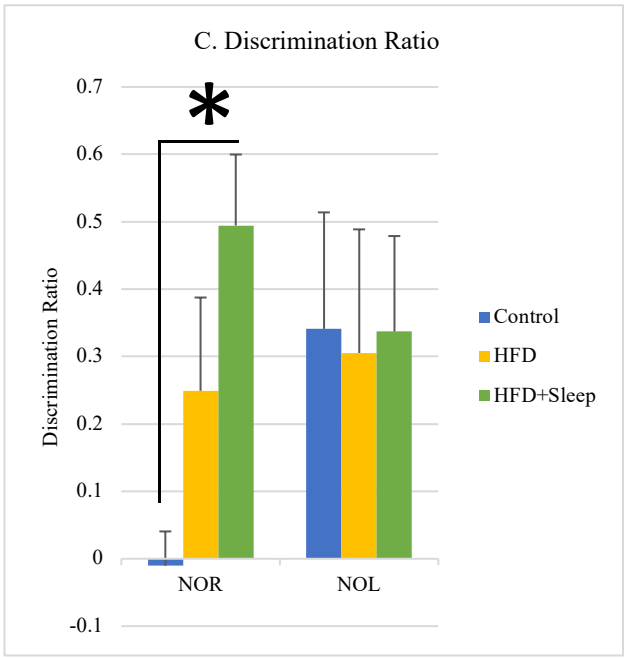


B. Neurons activated by c-fos in the same region as fig. 5A

Figure 8: Memory tests:



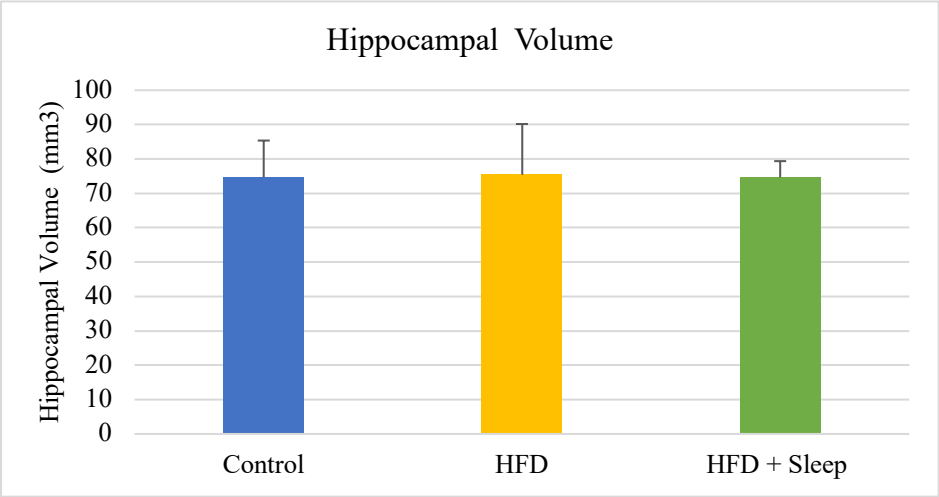




A. Old and novel object exploration averages. The HFD + sleep deprivation rats had significantly greater exploration of novel object compared to old object than the control or HFD groups. B. Old and novel

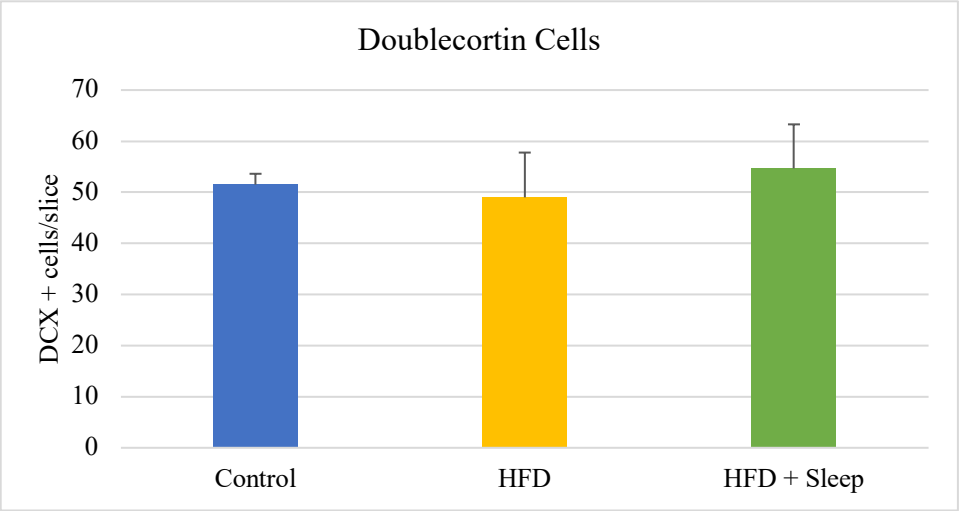
location exploration averages. All groups had greater exploration time of new location compared to old location. C. Average discrimination ratios for both memory tests and each manipulation group. The HFD + sleep deprivation rats had better memory scores than the control rats in the object recognition test. Data are represented as mean + SEM (error bars) and are significant at *p < 0.05.

Figure 9: Hippocampal volume averages



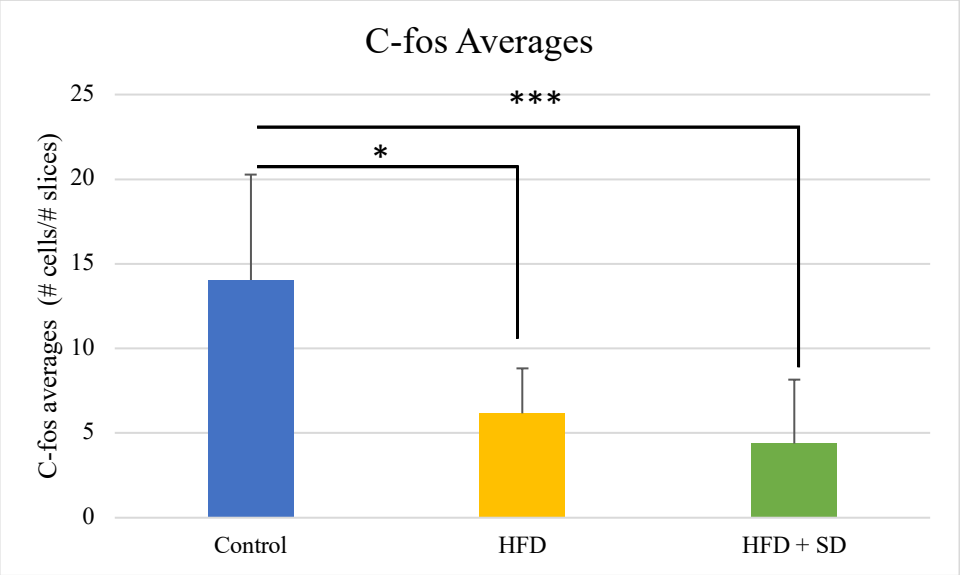
Hippocampal volume averages are shown for all three groups. There was not a significant difference between groups.

Figure 10: Doublecortin averages



DCX averages are shown for all three groups. There was not a significant difference between groups.

Figure 11: C-fos averages



C-fos averages are shown for all three groups. The control group had the highest amount of c-fos activated cells on average compared to both the HFD and HFD + SD groups. Data are represented as mean + SEM (error bars) and are significant at *$p < 0.05$.