

**EFFECTS OF SPORT TRAINING IN ADOLESCENCE ON COGNITION IN  
YOUNG ADULTHOOD**

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## **ABSTRACT**

Brain and behaviour are heavily impacted by our environment. This study examines how a positive experience, exercise, impacts brain development in adolescence and how that relates to brain and behavioural changes in young adulthood. A rat model was used to determine the impact of exercise, specifically complex exercise, such as sport and multi-sports, has on brain development. Adolescent rats were grouped and subjected to either, no exercise, exercise in the form of wheel running, training on one of three single complex motor tasks (“sports”), or training on all three complex motor tasks, for 3 weeks. Following training, rats completed a battery of behavioural tests during young adulthood and their brains examined. Findings include increased anxiety-like behaviour, changes in memory, attentional control and decision making, reduced cortical thickness and spine density, and finally increased thalamic area. Exercise and sport training in adolescence was found to positively impact cognition in young adulthood.

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## LIST OF ABBREVIATIONS

AID	Agranular insular dorsal cortex
BDNF	Brain-derived neurotropic factor
Cg3	Cingulate cortex
CR	Cognitive reserve
EF	Executive function
Fr1	Frontal area 1
IGF-1	Insulin-like growth factor-1
IL	Infralimbic
LO	Lateral orbital
LTP	Long-term potentiation
mPFC	medial prefrontal cortex
MO	Medial orbital
MWM	Morris water maze
OFC	Orbital frontal cortex
P	Post-natal day
Par2	Parietal cortex area 2
PFC	Prefrontal cortex
PL	Prelimbic
PVN	Paraventricular nucleus
rIGT	Rodent iowa gambling task
VEGF	Vascular endothelial-derived growth factor

## **Chapter 1**

### **General Introduction**

Our brain is an incredible and complex organ that shapes our personal experiences, which in turn, shape our brain. Extensive research has gone into elucidating the mysterious workings of our brains. One main area of focus in the field of neuroscience is behaviour; specifically, how behaviour is produced and how it is modified. To understand behaviour, we must first understand how the brain develops over a lifetime. Kolb and Gibb (2011) highlight that a single experience could be acting on a very different brain depending on the stage of development that the individual or organism is in when the experience occurs. Therefore, research is aimed at understanding how experience can modify the organization of the brain. Especially since experiences can influence our brain throughout our life, including negative events such as stress and drugs or positive events such as sensory and motor experience, and enriched environments.

This thesis contributes to our understanding of how a positive experience, such as physical activity (more specifically exercise and sport), impacts brain development and subsequent morphology and behaviour, using a rodent model. Physical activity can be defined as bodily movements that are produced by skeletal musculature that result in energy expenditure above what is normally exerted in a resting state. Physical activity includes occupational activities, sports, and daily tasks. This thesis will be taking physical activity one step further and discussing it in terms of exercise. Exercise is a subset of physical activity that is planned or structured in such a way that the intent is

increasing physical health. Furthermore, this work explores a relatively underexamined stage of life, adolescence. The majority of research in this field is conducted on the possibility of using exercise as an intervention for cognitive decline in aging populations. The following sections will briefly outline the importance of exercise as a component of maintaining holistic health, because of its positive influence on physical and mental health throughout our life span. Traditionally, exercise has been most strongly associated with physical health, such as body mass index or cardiac health. However, exercise has a strong influence on mental health as well. Specifically, exercise has a big influence during critical periods during the early years and adolescence (the focus of my thesis), but there is evidence that it has profound impacts well into the later years of life. This introduction will discuss the impact of exercise at different life stages such as early life, adolescence, and late adulthood. As well as, whether the type of exercise performed changes the potential benefits.

## **1.1 Exercise and Health**

In general, it is agreed that for an individual to be healthy, they have to be in good standing regarding physical and mental health. These two things, however, are not easily teased apart and they ultimately have a significant influence on each other. For the purpose of this next section, physical health will be regarded as the optimal functioning of the body and mental/brain health will be the optimal functioning of behaviour.

### **1.1.1. Physical Health**

There are many modifiable lifestyle choices that affect physical health. A few examples of some of these choices are diet, drug use, and exercise. The first two can have a positive or negative impact while exercise predominantly increases physical health. Exercise and health have a linear relationship, such that an increase in exercise can lead to improvements in health (Warburton, Nicol, & Bredin, 2006). Furthermore, exercise is effective in both primary and secondary prevention of death from several chronic diseases. According to Statistics Canada in 2017 the two leading causes of death are still cancer and heart disease. Numerous studies over the past decades have highlighted the importance of using exercise to increase physical health and reduce cardiovascular mortality. Studies have shown that increased physical activity reduces the risk for cardiovascular-related mortality (Hu et al., 2004; Erikssen et al., 1998) and these protective effects can be seen with even moderate increases in physical activity, such as walking an hour a week (Oguma & Shinoda, 2004). In addition to preventing a diagnosis of cardiovascular disease, exercise also benefits those with already established disease (secondary prevention) (Taylor et al., 2004).

The other leading cause of death in Canada is cancer and exercise has been shown to have a protective effect on some site-specific cancers. After reviewing observational studies, Thune and Furberg (2001) found that colon and breast cancer risk is lowered (by 20-40%) in physically active populations compared to inactive ones. Furthermore, exercise is also important for maintaining and improving quality of life for those who already have a cancer diagnosis or have gone through cancer treatment. Cancer treatments are generally aggressive and can result in bone-mineral density loss and

muscular atrophy. An effective way to circumvent or minimize these effects is to maintain a resistance training exercise program (Galvão & Newton, 2005).

### **1.1.2. Mental/Brain Health**

Brain health starts as soon as ones' brain starts to develop. Therefore, it is important, even in early to mid-childhood,, to start thinking about building executive functions (EF). EF is a diverse set of neurocognitive processes that allow us to regulate our thoughts, emotions, and actions. Periods of rapid functional and structural brain development coincide with rapid increases in EF. These periods of rapid EF development include approximately ages 2-6 and 9-11 (Moriguchi & Hiraki, 2009).

Adolescence is a critical time in development to maintain a healthy brain. There are immense changes, hormonally and physically, which create windows of plasticity where the brain is sensitive to the environment. In particular, significant development of the prefrontal cortex occurs during adolescence. This area is responsible for EF. Just as this period is a peak time for developing mental capacities that are positive, such as EF, there is also the potential for negative impacts which may manifest as mental disorders. Several mental disorders are more likely to emerge during adolescence, schizophrenia and substance abuse diagnoses, for example, peak right in the middle of the teenage years (Lee et al., 2014).

Mental health is not only important early in development, but it continues to be important into old age. Cognitive function generally declines with age and are associated with increases in neurodegenerative diseases. However cognitive reserve (CR) can help combat this decline due to age, disease, or a combination of the two. CR is the brains

ability to find alternative solutions to everyday problems by being able to use different networks, brain structures and processes even in the presence of impairments. CR is the ability to remain flexible and individuals who build it up over the course of their lifespan tend to prolong the years before neurodegeneration is diagnosed. Our brains are plastic and highly vulnerable to experiences; this vulnerability can be used for good, as in increasing CR. Humans have natural critical periods where the brain is especially plastic, but periods of plasticity can be engineered by giving the brain certain kinds of experience. One such experience, the focus of this thesis, is exercise. Exercise is an experience that enhances plasticity and therefore, our ability to increase CR by working on EF (Hillman, Erickson, & Kramer, 2008).

## **1.2. Exercise During Different Life Stages**

Our brain is continually developing, in one way or another, and the same experience can have a different impact on brain development depending on when the experience occurs. This section will be broken into four subsections that align with developmental epochs, where substantial brain changes are taking place. The first life stage to be discussed is the prenatal period, a stage where the brain is first developing and highly sensitive to the environment. The next stage is early life, which encompasses infancy to pre-puberty. This is an important stage because proper childhood development is important for normal functioning in adulthood. This stage is followed by adolescence, a period of high plasticity where the brain is changing rapidly and is highly susceptible to the environment. The brain is never really done developing, because of its plastic nature, it continues to change into adulthood. The last stage to be discussed is old age that is

typically characterized by lower levels of plasticity and a higher prevalence of neurodegeneration.

### **1.2.1. Prenatal**

Prenatal exercise is beneficial for both mother and offspring. Pregnancy is a time fraught with different stressors and hormonal changes that have the potential to influence the mental health of, not only the mother but the future development of the offspring. Thus, it becomes imperative to protect the mental health of mothers as best as is possible. During the last two trimesters of pregnancy, maintaining above average levels of physical activity improves mood stability (Poudevigne & O'Connor, 2005). Even engaging in low levels of physical activity, regardless of trimester, has been shown to be beneficial in lowering depression, anxiety, and stress in the pregnant mothers (Da Costa, Rippen, Dritsa, & Ring, 2003). The benefits of exercise don't stop as soon as pregnancy is over but continue to be influential in the postpartum period. In one study, not only did postpartum exercise intervention improve fitness level, which has a myriad of benefits on its own, but significantly improved depression symptomology (Armstrong & Edwards, 2003).

Moyer, Reoyo, and May (2016), summarized the influence of prenatal exercise on offspring health. It was found that at as early as 1 year old, IQ scores were significantly higher in offspring whose mothers exercised during pregnancy. This trend continues throughout development; at 5 years old Weschler scores (IQ measures) are also significantly higher and in pre-puberty (8-12 years old) as well as late adolescence/early

adulthood (17-20 years old) academic performance is better, when compared to mothers who did not exercise prenatally.

### **1.2.2. Early Life**

Early childhood is an important time to start developing EF. Individual differences in EF set a foundation for later developmental outcomes. A review by Diamond and Lee (2011) compared different interventions, including different types of exercise and their efficacy in aiding EF development. They found that the most beneficial type of exercise was of an aerobic nature, with exercise focusing on bimanual coordination, having positive effects on EF, while resistance training had none. They also noted that exercise alone was not as efficacious in developing EF as exercise paired with character development, such as martial arts or exercise and mindfulness. It was mentioned that little study has been done on sports training, but extrapolating from current research on exercise in general, it could be beneficial. In a second review, it was found that improvements in mental functioning due to exercise were seen on tasks that involved some sort of EF (Tomporowski, Davis, Miller, & Naglieri, 2008). It was concluded that exercise can be a simple and effective way to enhance EF, which is central to later cognitive and social development. Aside from increasing EF, early physical activity such as play, improves motor skill development (Graf et al., 2004). This is important because there is a bi-directional relationship between motor skills and EF; developing one skill enhances the other (Gonzalez et al., 2014). At any rate, exercise in early childhood is important for healthy brain and motor development with the two being intimately intertwined.

### **1.2.3. Adolescence**

Just as it is important for children to be engaging in physical activity or exercise, it is important for participation to continue into adolescence. As mentioned earlier, several disorders start to emerge during adolescence due to abnormal or altered behaviour. These disorders include depression, schizophrenia, drug abuse, and impulse control disorders. Hueston and Nolan (2017) suggest that altered hippocampal neurogenesis is at the root of these disorders. In the case of depression, reduced neurogenesis has been proposed to explain the cognitive deficits (Sahay & Hen, 2007). Sahay & Hen (2007) also highlighted that one of the important mechanisms behind the effectiveness of antidepressants and other interventions like exercise is their ability to stimulate hippocampal neurogenesis. Since adolescence is a time of high stress due to changes in physical development, social interactions, and cognitive growth, developing and continuing healthy habits such as regular exercise may be important in attenuating the negative impact of these different stressors because they can ultimately result in a reduction of hippocampal neurogenesis (van Praag, 2008).

Not only does exercise help attenuate the effects of negative experiences to preserve cognition, it helps enhance cognitive functioning. One study done on adolescent rats found that both voluntary and involuntary exercise improved learning and memory in the Morris Water Maze (Uysal et al., 2015). Looking at a human study, regularly exercising teens showed better performance on neuropsychological assessments of frontal and medial temporal lobe function. These improvements were in memory, inhibitory control, and cognitive flexibility, all of which are components of EF (Lee et al., 2014).

#### **1.2.4. Late Adulthood**

Development doesn't come to a halt as we enter adulthood. Brain development is a lifelong process and continually changes throughout our lifetime. Our brain is highly plastic and therefore, susceptible to different experiences. Exercise is a type of experience dependent plasticity, because it is not required for proper development but, nonetheless it changes our brain. In the case of exercise, this change is typically for the better and exercise although there is no critical period associated with exercise the evidence is overwhelming; it changes the brain at all stages of life. The majority of research into exercise and brain health has been done on aged populations, with a focus on ways to decrease cognitive decline and the incidence of neurodegenerative diseases. As we age, so does the brain and this can have negative consequences. Over time our cortex and hippocampus start to atrophy and memory functions declines (Golomb et al., 1996). However, it seems that exercise may be able to attenuate some of the consequences of aging.

The concept of CR comes back into play, the idea being that the higher the cognitive reserve, the less cognitive decline or a dampening of symptomology related to neurodegenerative disease. Two aged individuals with the same amount of brain atrophy have the potential to display very different behavioural outcomes. The individual with higher CR could be leading a fairly normal life while the other, with a less than ideal reserve, could have full blown dementia (Stern, 2002). Why is this? Just because the structure of the brain is degenerating, functional networks are not always compromised. CR is based on the idea that the brain can optimize performance by recruiting different brain networks or employing alternate cognitive strategies to solve the same problem.

This means that individuals with higher CR can maintain high functional outcomes even with brain deterioration or damage because their brain is able to efficiently use alternate networks to solve every day problems. Exercise seems to be an effective behavioural intervention for increasing CR and delaying the effects of neurodegeneration. Exercise has a disproportionately larger effect on executive control processes and their supporting brain regions (eg. prefrontal, hippocampus, basal ganglia). This is important because these are some of the first areas to show substantial age-related decline such as neuronal loss and degradation of neuronal pathways (Hillman et al., 2008).

### **1.3. Does Type of Exercise Matter?**

It is becoming increasingly clear that exercise has a profound effect on brain development throughout the life span, but some questions still remain unanswered. Does playing a sport make a difference in comparison to a general training regime? Playing sports require a lot more moving parts than just running, for example. Playing team sports requires knowledge of game tactics, what your opponents are trying to do, and interaction with teammates. Even solo sports such as gymnastics requires heavy interaction with apparatuses and complex sequencing of movements. It is conceivable that with all the extra cognitive load that comes with sports, that it would have a different or possibly enhanced effect on the brain in comparison to just exercising for the goal of physical fitness. This is a relatively understudied area because presently there is no suitable animal model for sports participation, so studies currently involve human participants which makes it hard to control for all the diverse life experiences between each individual that

could be confounding variables. Nonetheless, several studies have looked at the effect that participating in sports has on cognition.

In the first study examined, Chaddock et al. (2011) looked at multitasking ability using a virtual street crossing task and processing speed using a reaction time task. During the street crossing task, the goal was for participants to make it across the street while avoiding traffic. There were three different conditions, no distraction and two different types of distractions (cellphone and i-pod). While the reaction time task evaluated response speed of participants when fixed on a central point while responding to asterisks appearing in the periphery. Athletes (versus non-athletes) had a higher success rate during the virtual street crossing task reflected by significantly fewer collisions with vehicles in all conditions of the task. Athletes also showed faster processing speed on the reaction time task. Taken together, the authors concluded that there is a beneficial relationship between sport, which is a multitasking activity and speed of information processing.

A second review provides a more in-depth look at the type of sport and moderating factors such as sex and expertise (Voss et al., 2009). Overall, it was found that there was a significant effect of sport, regardless of type, on cognitive measures. The largest difference was found in the interceptive group, which was defined as sports requiring coordination between the athletes' body, parts of the body, or a held implement and an object in the environment (eg. tennis). The next largest difference was in the strategic group, which was defined as sports requiring the processing of a large amount of information regarding self, teammates, and opponents. On all tasks, athletes showed enhanced cognitive performance, but their increased performance on varied attentional

paradigms and faster processing speed was the most significant. Regarding sex differences, males showed the largest effects on the cognitive measures. This review also looked at level of expertise, comparing college versus professional athletes, with professional level athletes showing the largest effect on the cognitive measures. Taken together, the above study and review give evidence that participating in sports can give you an edge in the cognitive domain, and even more so if you are at a professional level.

#### **1.4. Objective of Thesis**

The purpose of this thesis was to investigate the effect that exercise, and more specifically, sport involvement in adolescence has on cognition in young adulthood. To accomplish this goal, a rat model was used to analyze behavioural changes after being subjected to exercise or sport intervention. A behavioral test battery was used to establish if there were any changes to cognition and subsequent anatomical analysis was done to determine whether any of the changes in behavior was reflected in a change in the brain's architecture.

##### **1.4.1. Using a Rodent Model**

This research uses a rodent model, Long-Evans rats specifically, to address the limitations of human studies. Using an animal model controls for confounding variables. With human studies, every individual has a different history and lifestyle, making it difficult to disentangle the object of study, such as exercise, with other components that may have positive impacts on the measurements being made. Animal research addresses this issue by providing a controlled environment and life experience for all the subjects.

Another advantage for using an animal model is the ability to look at the brain after the experiment. This allows us to find potential mechanisms for changes in behavior and see what brain processes and structures are changed by environmental manipulations.

**1.4.1.1. Measuring Cognition.** The cognition of the rats in this experiment was evaluated using a behavioral test battery following completion of their training. This battery utilized tests that evaluate different components of EF and factors that may influence it. The first test of the battery was the activity box to assess locomotor activity and anxiety. The former is important because of the bi-directional relationship with motor skill and EF (Gonzalez, et al. 2014) and latter because of its potential impact on test performance. The elevated plus maze (EPM) is another test of anxiety that has shown to be consistent and reliable in animal models (Walf & Frye, 2007). Three different paradigms of the Morris Water Maze (MWM) were used to evaluate various components of EF. The first spatial paradigm is a test of learning and spatial memory (Morris, 1984). The second paradigm, a reversal of the first, is used to evaluate reversal learning, which is the ability to extinguish previously learnt information and update it to learn a new set of information (Vorhees and Williams, 2006). The final paradigm is a test of working memory that requires animals to learn a new location each day while inhibiting previous days locations (Anisman & McIntyre, 2002). The final test used was the Rodent Iowa Gambling Task (rIGT), which is designed to evaluate long-term decision making and efficiency of behavior.

**1.4.1.2. Anatomical Analysis.** Several different anatomical analyses were used to determine if sport training had any effect on brain architecture. Cortical thickness was used to evaluate the brain at several different points in five different planes. Two prefrontal planes, five points of measurement in each, were used because of the PFCs substantial involvement in EF. Medial, central and lateral measurements were also taken at five more posterior planes to identify any other regions that may have been affected and because the prefrontal cortex has vast connections with many different brain regions. The second analysis done was thalamic area because of its involvement in memory, attention, and other executive functions (Van der Werf et al., 2003). Finally, spine density was determined because a modification of synaptic structure is often found after environmental manipulations. Specifically, two areas were analyzed, Cg3 (mPFC in humans) and AID (OFC in humans), both of which are located in the PFC.

## **1.5. Theory**

This research is guided by the fundamental theory of neuroplasticity which states that *the brain has the ability to change throughout life in response to our environment and experience, thus changing subsequent behaviour.*

## **1.6. Hypothesis**

I hypothesize that sport training in adolescence will affect the brain and behaviour of rodents in young adulthood.

### **1.6.1. Predictions**

1. I predict that both exercise and sport will have a positive impact on cognition, reflected through behavioural testing.

2. I predict that playing a sport will have a greater impact on cognition than exercise alone, and playing multiple sports will have the greatest impact on cognition, reflected through behavioural testing.

3. I predict that both exercise and sport will change brain architecture.

### **1.7. Organization of Thesis**

Chapter 2 describes the methods used in this rodent model, including the adolescent training paradigms and the subsequent behavioural test battery, used to assess cognition. It also describes the statistical analyses used throughout the thesis. Chapter 3 discusses the effect that adolescent sport training had on the behaviour of the rats in young adulthood. Chapter 4 examines the effects that the adolescent sport training had on brain morphology. Finally, Chapter 5 provides a general discussion of the major findings of the study and their implications for the field of cognitive research.

## Chapter 2

### General Methods

#### 2.1. Animals

All experimental procedures performed were approved by the University of Lethbridge Animal Care and Use committee and are in accordance with the Canadian Council of Animal Care. Eleven Long-Evans rat pairs were mated, resulting in six successful pregnancies. In total, among the six litters there were 38 females and 32 males. Animals were maintained on a 12-hr light/dark schedule (light on 7:30 to 19:30) in a temperature controlled (21°C) room. Water and food were available *ad libitum*, with the exception of a food restricted 2-week period during one of the behavioural tasks. All subjects remained with their mothers until weaning on postnatal day (P) 21. Animals were then randomly assigned to groups (Table 2.1) and balanced as much as possible with regards to litter and sex. The animals were housed in their same-sex groups: Control, Exercise Control, Single-sport #1, Single-sport #2, Single-sport #3, and Multi-sport. Following completion of the third behavioural testing, groups were split based on sex and exercise group, to allow for staggering of the remaining tests to accommodate for lengthy testing times.. For 1 week immediately prior to training, each animal received daily handling and was introduced to the food reward (Frootloops®).

Table 2.1. Experimental Group Composition: Overview of animals according to group and sex.

<b>Group</b>	<b>Female</b>	<b>Male</b>
<i>Control</i>	5	5
<i>Exercise Control</i>	7	5
<i>Single-sport #1(Rope Pulling)</i>	6	6
<i>Single-sport #2(Rope Climbing)</i>	7	5
<i>Single-sport #3(Uneven Ladder Rung Walking)</i>	7	5
<i>Multi-sport (All three of the above activities)</i>	6	6

## 2.2. Training

Training commenced on approximately P30 and lasted 3 weeks. Each animal received a single 10-minute training session, according to their experimental condition, per day for 6 days in a row, with the 7th as a rest day. In this rodent model, complex motor tasks were surrogates for ‘sports’. These complex motor tasks included rope pulling, rope climbing, and uneven ladder rung walking. The ‘multi-sport’ group was trained on all three complex motor tasks. To accomplish this, they trained on each complex motor task in 2-day blocks for the 6 training days each week for a total of 10 minutes. The order of each trained skill by the multi-sport group was counter-balanced for sex and task. The three ‘single-sport’ group were each trained on a single task. The exercise control group ran on running wheels for the duration of training and were given a food reward immediately following the session. A final control group had no form of exercise but was taken to the testing area for the duration of training and received the food rewards. Frootloops were used as food rewards to motivate the animals to complete

the complex motor tasks. Each rat received the equivalent of two Footloops by the end of each training session.

The rope-pulling task was adapted from Whishaw and Miklyaev (1996). Rats were placed in a Plexiglas cage with wire mesh floor and one wall of 3mm wide metal bars spaced 10mm apart. Rats were placed singly in the cages, side by side and encouraged to pull in a piece of 1mm thick cotton string with a Frootloop tied to the end. Initially, the Frootloop was placed on a short piece of rope and the end of the string was placed near the bars, as training progressed the string was lengthened. On the third week, the end of the string was placed an inch from the bars so the rats had to reach for the string in order to be able to pull it in.

The rope climbing task was adapted from Klintsova *et al.* (1998). During the task the rats were placed in a black Plexiglas® box measuring 30x30x60cm (at younger ages ~P30 to P40) or the same box with a height of 60cm (at older ages ~P40-P55). A polypropylene rope was suspended from a Plexiglas bar running across the length of the box. Rats (2-4) were placed in the box and were encouraged by the experimenter to climb up the rope. Upon reaching the top, the rats were removed to a holding area given a short rest and piece of Frootloop before being placed back into the box for additional climbs until the 10 minutes were up. The rope was modified in several different ways throughout the 3-week training to allow for skill progression. At the start, the rope had knots spaced close together and was placed in the corner of the box against the walls to allow the rat to use it for assistance. For the second week, the knots were spaced out and the height was extended, and on the third week the rope was removed from the corner and placed away from the wall.

The uneven ladder rung walking task was adapted from Metz & Whishaw (2002). During this task the ladder, measuring 60cm in length and 15cm in width, was set up between two transport tubs (46cm X 25cm X 20cm). Rats (2-4) were placed on one end of the ladder with their cage mates and encouraged to traverse to the other transport tub. Upon successful traversing in the middle of the ladder (not the sidewalls), rats were given Frootloops and encouraged to go back to the opposite side. A mat (sealed and easy to decontaminate) was placed under the ladder to prevent injury in the event an animal fell off. Each week additional rungs were taken out to accommodate the animal's growth and to increase difficulty level as the training progressed.

### **2.3. Statistical Analysis**

All statistical analysis was done with RStudio for Mac. Two-way ANOVAs with group and sex as factors were used for behavioral analysis. Although this study did not was not specifically designed to study sex difference, because sex-differences often occur in behavioral and anatomical analyses, sex was included as a factor. If there was no significant main effect of sex or any significant interactions, the data were collapsed and all animals were analyzed just by group. Behavioural tests across multiple days were analyzed the same way except as a repeated measures ANOVA. Three-way ANOVAs with condition, sex, and hemisphere were used to analyze all anatomical measures. Tukey HSD tests, to make multiple comparisons of the means, were utilized for all post hoc analysis. Graphs and tables were constructed using ggplot in RStudio for Mac and Excel (Microsoft) for Mac, respectively.

## **Chapter 3**

### **Effects of Adolescent Sport Training on Young Adulthood Behavior**

#### **3.1. Methods**

##### **3.1.1. Activity Box**

Animals were tested at ~P56. The Versamax Animal Activity Monitor system (Accuscan Instruments, Inc.) was used to record the animal's activity. Individual rats were placed in clear Plexiglas® boxes (42cm x 42cm x 30cm) which were placed within the monitoring system. The rat's activity was recorded for 10, 1 min samples, for 10 total minutes of activity recording. The amount of horizontal activity, total time spent active, and time spent in either the center or perimeter of the box was recorded by the software. All boxes were cleaned with Virkon® between animals. One data point per animal per measure was obtained by summing across the 10, 1 min samples.

##### **3.1.2. Elevated Plus Maze**

Animals were tested on the EPM at ~P53 (see Fig. 3.2). The testing apparatus was constructed completely out of black opaque Plexiglas®. The apparatus measures 1m tall with four arms, each 40cm long and 10 cm wide. Two of the arms had no walls, the other two had 40cm high walls. The distal 20cm of each open arm was marked with a white tick to make it clear when the rats crossed the point into the “titanic zone” (end of the open arms). To start testing, each rat was placed in the maze such that the head and

shoulders were in the center square and the body on an open arm. Animals were left undisturbed for 5 minutes of recording, and the maze was cleaned with Virkon between each animal. Videos were scored for the length of time spent in each area of the maze (center square, closed arm, open arm, titanic zone), the number of entries into each area, and latency to enter a closed arm after first being placed on the maze. A rat has considered to enter an area once their shoulders had completely passed into that new area (i.e. once where the fur changed from black to white crossed into a different area of the maze).

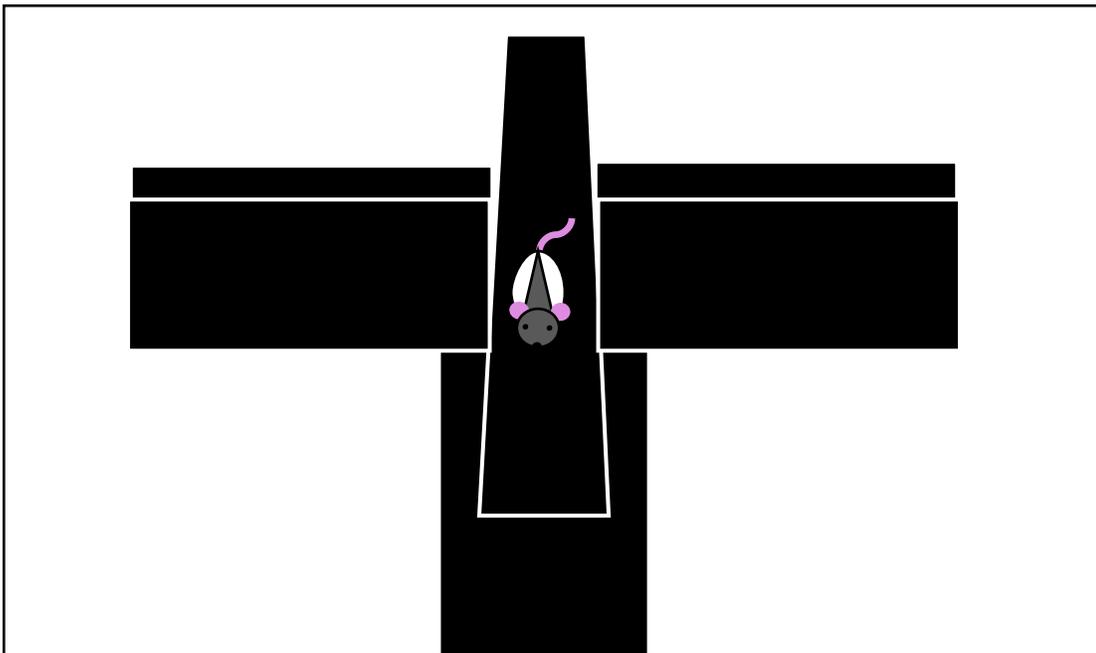


Figure 3.1: Elevated Plus Maze. Animals were tested on ~P56. The apparatus was constructed out of black Plexiglas® and had two “open” arms and two “closed” arms. Each arm was 40cm long and 10cm wide; the apparatus was 1m above the floor.

### 3.1.3. Morris Water Maze

Animals were tested on three different versions of the MWM: 1) A standard spatial place task version as described by Morris (1984) 2.) A reversal of the standard place task and 3.) A matching to place paradigm as described by Anisman and McIntyre

(2002). In all paradigms, a circular pool (1.5m diameter, 45 cm high) was located in a room with visible distal cues located on the walls (see Fig. 3.2). The pool was filled with  $23 \pm 0.5^{\circ}\text{C}$  water made opaque with  $\sim 250$  ml non-toxic liquid Tempera paint. A clear, square platform (12 cm x 12 cm) was placed in the pool  $\sim 2$  cm beneath the surface of the water. To start each trial, the rats were placed in the water facing the wall at one of the four cardinal points and allowed a maximum of 60 s to search for the platform (with the exception of probe day, which was 30 s with no platform). After each trial the rat was removed from the pool, dried off, and placed in the holding cage for  $\sim 2$ -5 minutes before starting a new trial (depending on how many rats [between 6-8] in each training group). The swim distance, swim speed, and latency to find the hidden platform was recorded for each trial. On probe days the amount of time spent in each quadrant was recorded.

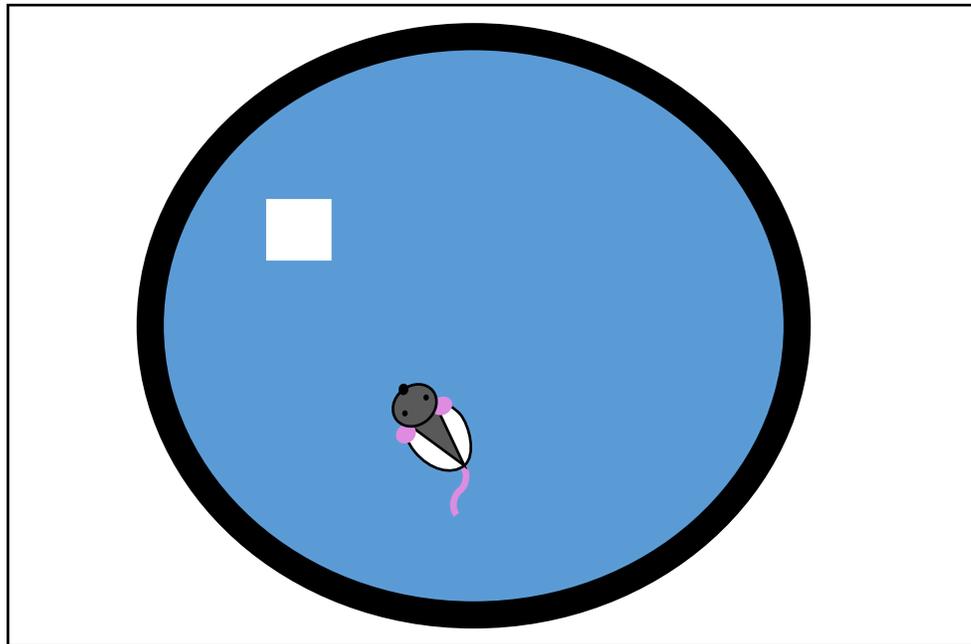


Figure 3.2: Morris Water Maze. Animals were tested on three separate paradigms to test long-term memory, reversal learning, and working memory.

**3.1.3.1. Spatial Paradigm.** Animals were tested at ~P60. In the spatial paradigm, each animal was given four trials per day in which they were started (pseudo-randomly) from four different cardinal locations (north, east, south, west) and were tasked with finding a hidden platform using distal room cues. They completed training for 4 consecutive days (no platform movement) and on the fifth day they were given a probe trial to test acquired learning of the hidden platform location. On the probe day the platform was removed and their behaviour was observed for 30 secs.

**3.1.3.2. Reversal Paradigm.** The reversal paradigm was used the day immediately following completion of the spatial paradigm to test reversal learning. In the reversal paradigm, each animal was given another 4 trials per day for 4 consecutive days with a probe trial on the 5<sup>th</sup> day. The only difference between this paradigm and the spatial paradigm was that the hidden platform was moved to the quadrant directly opposite the original position.

**3.1.3.3. Matching to Place Paradigm.** The final version of the MWM was tested on ~P85 and ~P101 because of the need for separation into two groups as mentioned in section 2.1. This matching-to-place paradigm is a test of working memory. Each rat received 8 trials per day, 2 trials at each cardinal location, for 6 consecutive days. Each day, the hidden platform moved to a new location, such that each location was used no more than twice and the same location was not used within a time frame of three days. There was no probe trial for this paradigm.

### **3.1.4. Rodent Iowa Gambling Task**

Animals were tested on this task at ~P71 and ~P89 because of the need for separation into two groups as mentioned in section 2.1. This rodent version of the Iowa Gambling Task was conducted as described by Van den Bos et al. (2006). This test utilized a gambling box constructed out of black opaque Plexiglas® (see Fig.3.3). Rats were placed in a start box which opened up into a choice area containing four arms. The start box was 60cm long, 20cm wide, and 30cm high; the choice area was 20cm long, 60cm wide and 30cm high; the arms were each 30cm high, 80cm long, and 15cm wide.

The rats were given one habituation day with a 10min exploration session where they were free to explore the entirety of the gambling box. Following habituation, each animal received 10 trials per day, for 12 days. Trials began by placing the rat in the start box and lifting the slide door allowing them to enter the choice area. Once the animals entered an arm (made a choice), a door was lowered to prevent them from leaving the arm. The animals had an inter-trial rest of 15sec in the start box and had a maximum of two minutes to explore the arms/eat the food reward. The gambling boxes were cleaned with Virkon® between each animal.

The gambling task itself utilizes cued arms to specific food rewards, two of the arms were not rewarded to control for aspecific effects such as general exploration, while the other two contained food rewards. In the “long-term advantageous” arm there was always one sucrose food pellet and in 2 out of the 10 trials that pellet was quinine treated, which is bitter and therefore used as a negative reward. In the “long-term disadvantageous” arm there was always three sucrose food pellets. In 9 out of 10 trials those 3 pellets were quinine treated. The sugar/quinine treated pellets were placed at the

end of the arm in a white plastic weigh boat, 1cm in diameter. Cues were placed on the sidewall at the entry to each arm to help the rats distinguish between the arms. The cues were red or white circles and crosses (10 cm x 10 cm), shapes rather than colors placed in adjacent arms to make each arm even more distinguishable from the neighboring arm(s).

The 10 trials per day were prearranged and randomized so that the rats had no rules to follow to determine when the arms were to be rewarded, and to what extent. Rewarded and empty arms, as well as the “good/bad” arms were counterbalanced for subjects to avoid bias due to preference of arms. Each rat was introduced to the sugar pellet rewards in their home cage for one week prior to testing. Animals began food restriction two days prior to testing to encourage reward seeking behaviour. Each rat was weighed daily and their bodyweight was maintained such that their weight did not drop below an acceptable level for maintaining health and proper development, according to the standard growth curve for Long-Evans rats from Charles River Laboratories. The original percentile for each rats’ weight was determined by averaging each animals weight for three days prior to food restriction and then using the growth curve to find their starting (original) percentile and the minimal acceptable percentile (15 percentiles below original). Animals were fed within 1 hr. after completing the training. The “quinine pellets” were created by soaking the sucrose pellets in 180mM quinine (Sigma Aldrich) for several minutes, air dried on paper towel then stored in containers shielded from light until used.

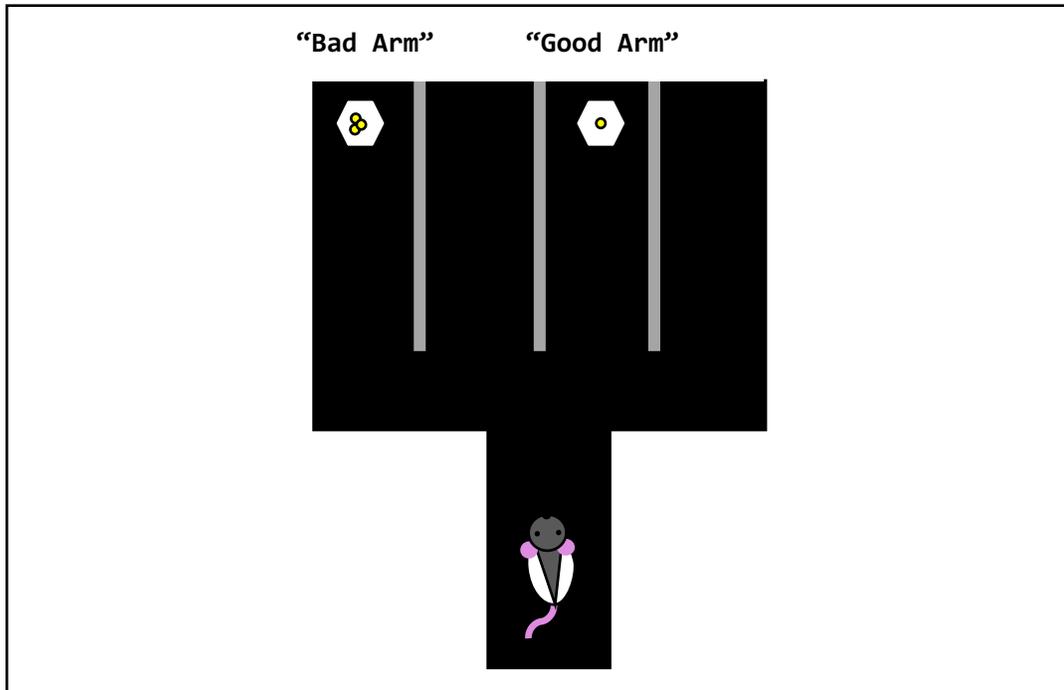


Figure 3.3: Rodent Iowa Gambling Task. Animals were placed in the start box and allowed free choice to enter one of the four arms, each marked with different spatial cues. Two arms were empty, one was a long term advantageous “good” arm containing one food reward, one was long term disadvantageous “bad” arm containing three food rewards.

## 3.2. Results

### 3.2.1. Activity Box

Group had no effect on horizontal activity or total distance moved in the Activity Box ( $F(5,58)=1.167, p=0.336$  and  $F(5,58)=1.438, p=0.224$ , respectively). However, there was a sex effect; females were significantly more active than males (Fig. 3.5a) and travelled a longer distance (Fig. 3.5b) ( $F(1,58)=10.050, p=0.002$  and  $F(1,58)=7.858, p=.007$ , respectively). Group did have an effect on the time spent exploring the perimeter of the Activity Box ( $F(5,58)=3.034, p=0.017$ )(Fig. 3.5c). Post hoc analysis found that the single-sport group #3 spent significantly more time in the

perimeter than the exercise control group ( $p = 0.028$ ). Table 3.1 summarizes all main effects and interactions, significant or not.

Table 3.1: Activity Box results summary. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

Activity Box	Sex Effect		Group Effect		Sex*Group Interaction
	Change	$p$	Change	$p$	$p$
Overall Activity	Female ↑	2.43e-03***	---	0.336	0.552
Total Distance	Female ↑	0.007**	---	0.224	0.832
Perimeter Time	---	0.088	Sport 3 ↑ vs Exercise Control	0.017*	0.798

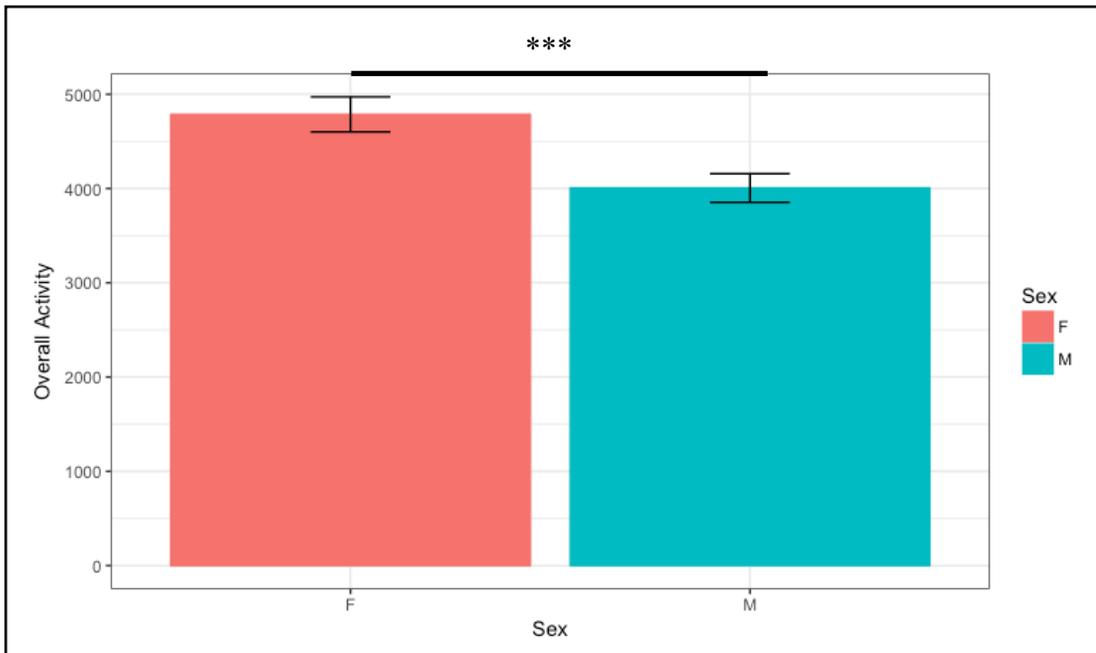


Figure 3.4a: Activity box. Overall horizontal activity; females were significantly more active than males. Error bars = SE. \*\*\* =  $p < 0.001$ .

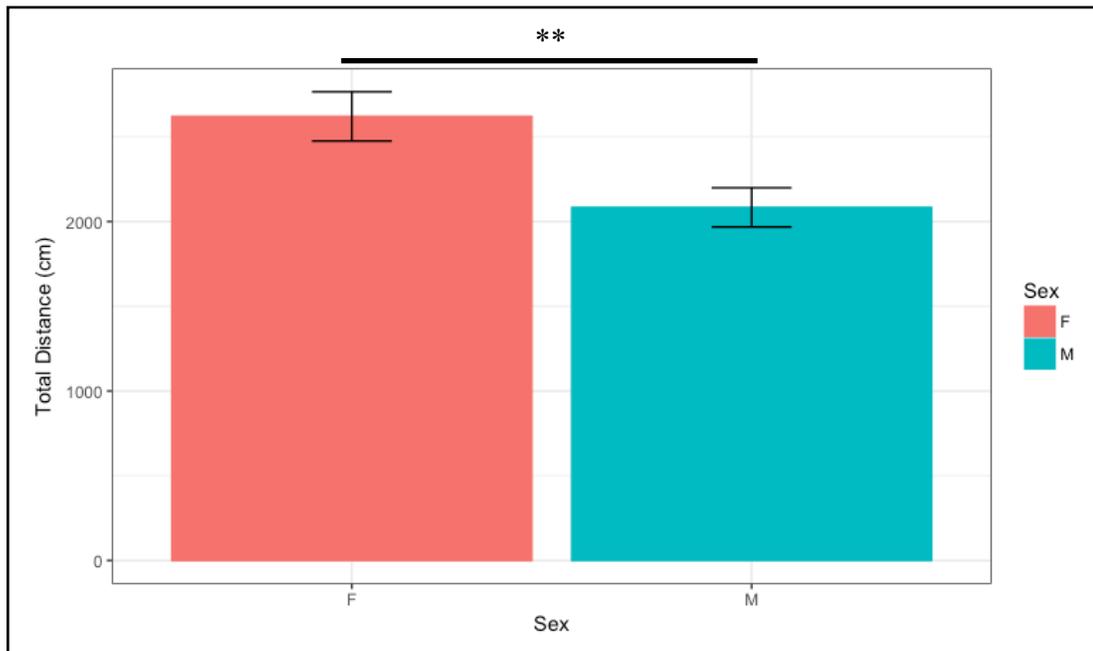


Figure 3.4b: Activity box. Total distance travelled; females travelled a significantly larger distance than females. Error bars = SE. \*\* =  $p < 0.01$ .

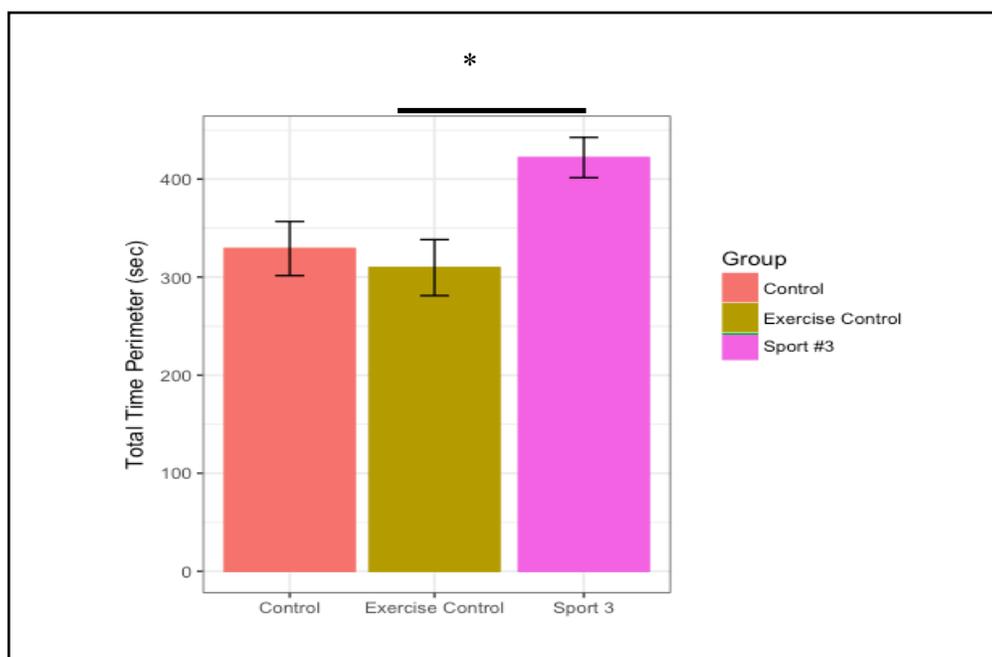


Figure 3.4c: Activity box. Perimeter time; single-sport #3 spent significantly more time in the perimeter than exercise controls. Error bars = SE. \* =  $p < 0.05$ .

### 3.2.2. Elevated Plus Maze

There were no sex differences in the EPM, therefore sex was collapsed for group comparisons. Effects of group were seen in several different observations. There was a significant group effect for open arm time ( $F(5,56)=3.246, p=.012$ ) (Fig. 3.6a), closed arm time ( $F(5,56)=2.95, p=.019$ ) (Fig.3.6b), and titanic zone time ( $F(5,56)=5.305, p=.001$ ) (Fig. 3.6c).

Post hoc analysis revealed that Single-sport #2 group spent significantly more time in the open arms than Single-sport #3 ( $p= 0.006$ ). Conversely, Single-sport #3 spent significantly more time in the closed arms than Single-sport #1 and Single-sport #2 ( $p= 0.028$  and  $p= 0.024$ , respectively). Furthermore, the Exercise Control, Single-Sport #1, and Single-Sport #2 group spent more time in the titanic zone than the Single-sport #3 group ( $p= 0.006, p= 0.001, \text{ and } p= 0.003$ ), respectively. Table 3.2 gives a complete summary of all main effects and interactions, significant or not.

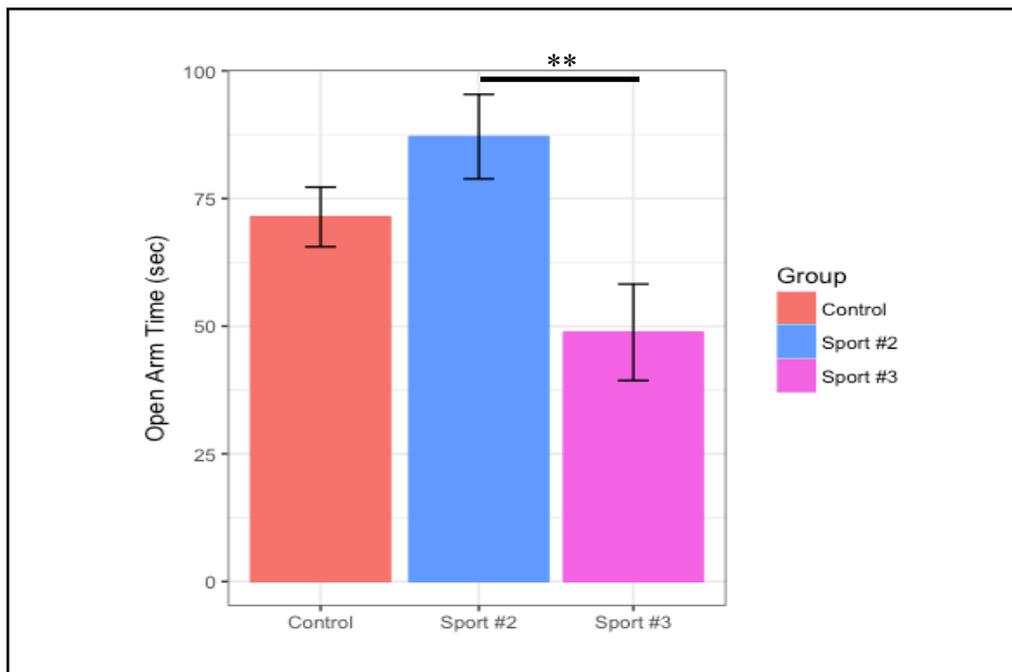
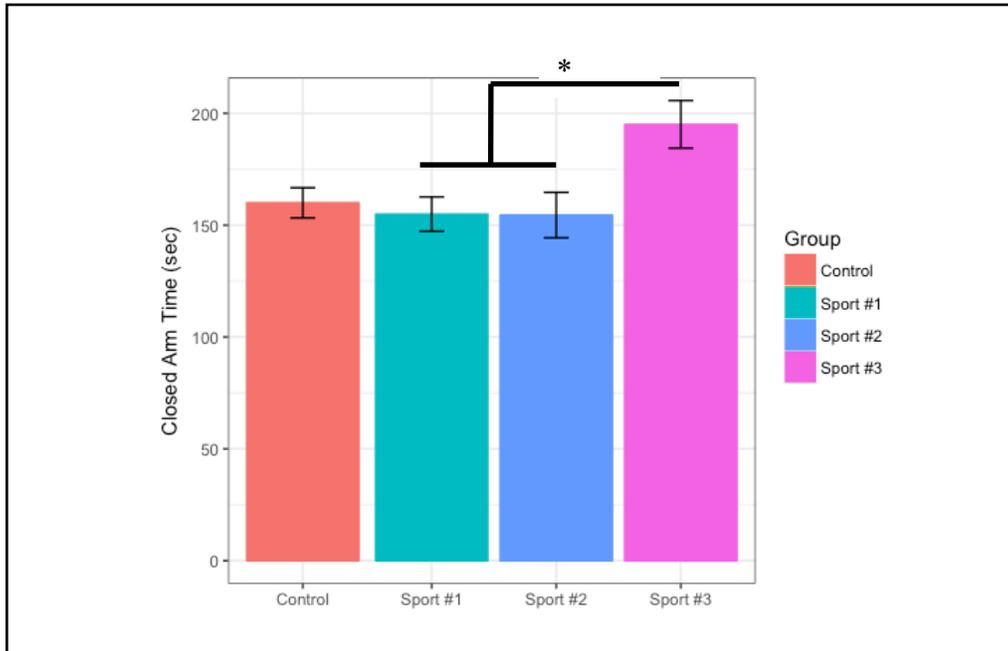


Figure 3.5a: Elevated plus maze. Open arm time; single-sport #2 spent significantly more time than single-sport #3. Error bars = SE. \*\* =  $p < 0.01$ .



3.5b: Elevated plus maze. Closed arm time; single-sport #3 spent significantly more time than single-sport groups #1 and #2. Error bars = SE. \* =  $p < 0.05$ .

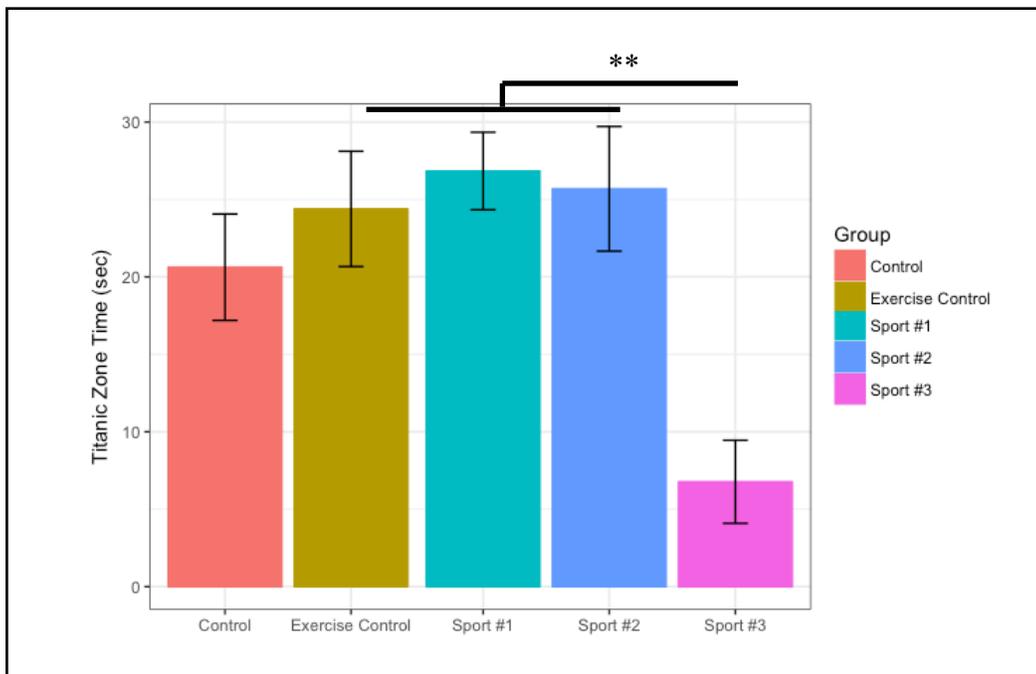


Figure 3.5c: Elevated plus maze. Titanic zone time; exercise control, single-sport #1 and #2 groups spent significantly more time than the single-sport #3 group. Error bars = SE.

\*\* =  $p < 0.01$ .

Table 3.2: Elevated Plus Maze results summary. \* =  $p < 0.05$ , \*\*\* =  $p < 0.001$ .

EPM	Sex		Group		Interaction
	Change	<i>p</i>	Change	<i>p</i>	<i>p</i>
<b>Open Arm Time</b>	----	0.491	Sport 2 ↑ vs Sport 3	0.012*	0.684
<b>Closed Arm Time</b>	----	0.491	Sport 3 ↑ vs Sport 1/2	0.020*	0.144
<b>Titanic Zone Time</b>	----	0.145	Exercise Control, Sport 1/2 ↑ vs Sport 3	0.000***	0.972

### 3.2.3. Morris Water Maze

**3.2.3.1. Spatial Paradigm.** During the four days of training measures of average latency (Fig. 3.7a), average path length (Fig 3.7b), and velocity (Fig. 3.7c) were taken. Females were omitted from velocity and path length because at the time of testing they were too small in size to be picked up by the overhead motion tracker. There was no effect of group on latency, path length, nor velocity ( $F(5,58)= 0.794, p= 0.558$ ,  $F(5,26)=1.081, p= 0.394$ , and  $F(5,26)=1.765, p=0.155$ , respectively). There was a no effect of sex on latency ( $F(1,58)=3.781, p=0.057$ ). On probe day, the amount of time spent in the quadrant where the hidden platform was recorded (Fig. 3.7d). Neither sex nor group had an effect on time spent in the quadrant ( $F(1,58)=2.064, p=0.156$  and  $F(5,58)=.265, p=.930$ , respectively). Table 3.3 provides a summary of main effects and interactions, significant or not.

Table 3.3: Morris Water Maze spatial paradigm summary. There were no significant main effects or interactions.

MWM Spatial	Sex Effect		Group Effect		Interaction
	Change	<i>p</i>	Change	<i>p</i>	<i>p</i>
Target Quadrant	----	0.156	----	0.930	0.677
Average Latency	----	0.057	----	0.559	0.513
Average Path Length	N/A	N/A	----	0.394	N/A
Velocity	N/A	N/A	----	0.155	N/A

**3.2.3.2. Reversal Paradigm.** During the four days of training, measures of average latency (Fig. 3.8a), average path length (Fig. 3.8b), and velocity (Fig. 3.8c) were taken. Females were omitted from velocity and path length because at the time of testing they were too small in size to be picked up by the overhead motion tracker. There was not a significant effect of group on latency, path length, or velocity ( $F(5,58)=0.430$ ,  $p=0.826$ ,  $F(5,26)=0.285$ ,  $p=0.917$ , and  $F(5,26)=0.986$ ,  $p=0.445$ , respectively). There was a significant effect of sex on latency; males took a shorter a time duration to reach the platform ( $F(1,58)=10.546$ ,  $p=0.002$ ). There was also a significant effect of sex on time spent in the target quadrant (Fig. 3.8d); males spent more time in the quadrant where the platform was placed for the 4 days prior ( $F(1,58)=6.232$ ,  $p=0.015$ ). However, there was no significant effect of group on time spent in the target quadrant during the probe trial ( $F(5,58)=0.871$ ,  $p=0.506$ ). Table 3.4 provides a summary of all main effects and interactions, significant or not.

Table 3.4: Morris Water Maze reversal paradigm summary. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

MWM Reversal	Sex Effect		Group Effect		Interaction
	Change	$p$	Change	$p$	$p$
Target Quadrant	Males ↑	0.015*	----	0.506	0.924
Average Latency	Males ↓	0.002**	----	0.826	0.750
Average Path Length	N/A	N/A	----	0.917	N/A
Velocity	N/A	N/A	----	0.445	N/A

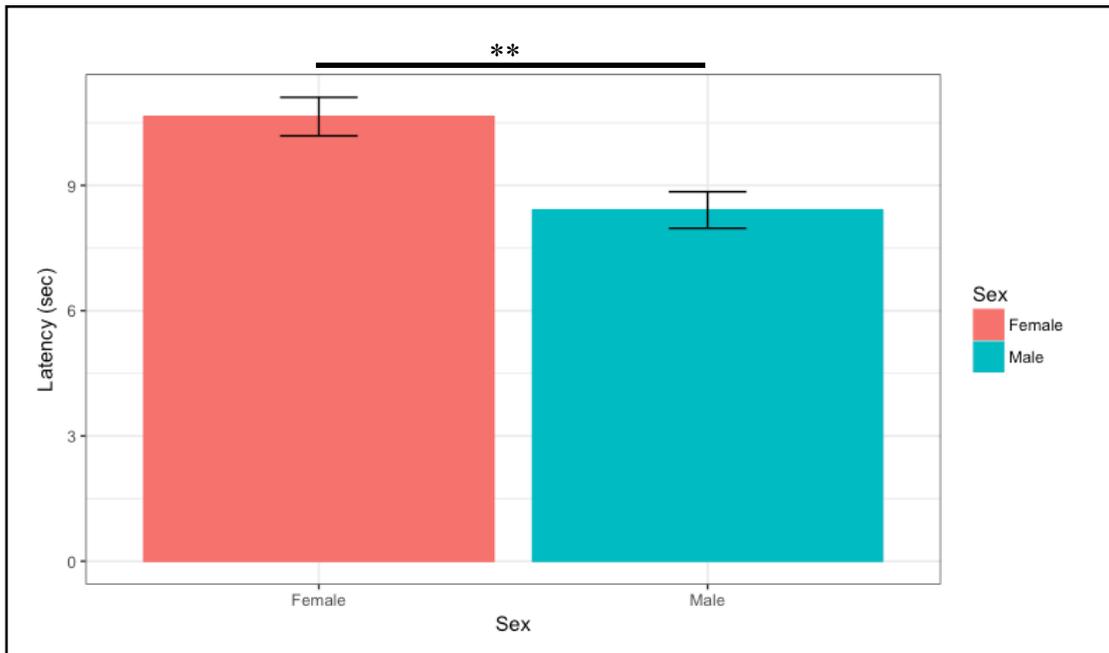


Figure 3.6a: Morris water maze reversal paradigm. Latency; males had a significantly lower average latency to find the hidden platform compared to females. Error bars = SE. \*\* =  $p < 0.01$ .

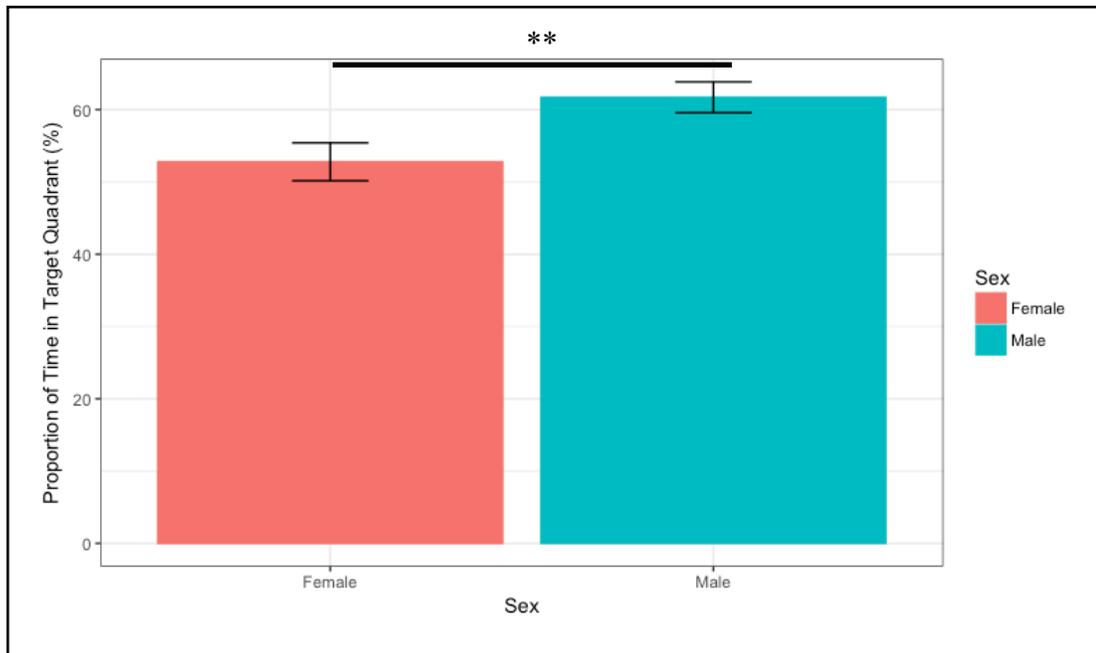


Figure 3.6b: Morris water maze reversal paradigm. Target quadrant; males spent significantly larger proportion of time in the target quadrant than females. Error bars = SE. \*\* =  $p < 0.01$ .

**3.2.3.3. Matching to Place Paradigm.** At the time of testing females were large enough to be picked up by the overhead motion tracker, thus they are included in all measures. During this paradigm there was a significant effect of sex on both average latency (Fig. 3.9a) and path length (Fig. 3.9b), males having shorter latency and shorter path lengths ( $F(1,57)=8.740, p=0.004$  and  $F(1,57)=6.255, p=0.015$ , respectively). There was no effect of sex on velocity ( $F(1,57)=0.000, p=0.996$ ). There was no effect of group on latency or path length ( $F(5,57)=1.792, p=0.129$  and  $F(5,57)=0.645, p=0.667$ , respectively). However, there was a significant effect of group on velocity ( $F(5,57)=2.655, p=0.032$ ) (Fig. 3.9c). Post hoc testing revealed the Exercise Control, Single-sport #1, Single-sport #2, Single-sport #3, and Multi-sport groups had significantly slower velocities than the Controls ( $p < 0.000$  for all groups). The Exercise Control, Single-sport #2, Single-sport #3, and Multi-sport groups had a significantly

slower velocity than the Single-sport #1 group ( $p=0.014$ ,  $p=0.012$ ,  $p<0.000$ , and  $p<0.000$ , respectively). The Single-sport #3 group had a significantly slower velocity than the exercise control and Single-sport #2 groups ( $p=0.005$  and  $p=0.004$ , respectively). Table 3.5 provides a summary of a main effects and interactions, significant or not.

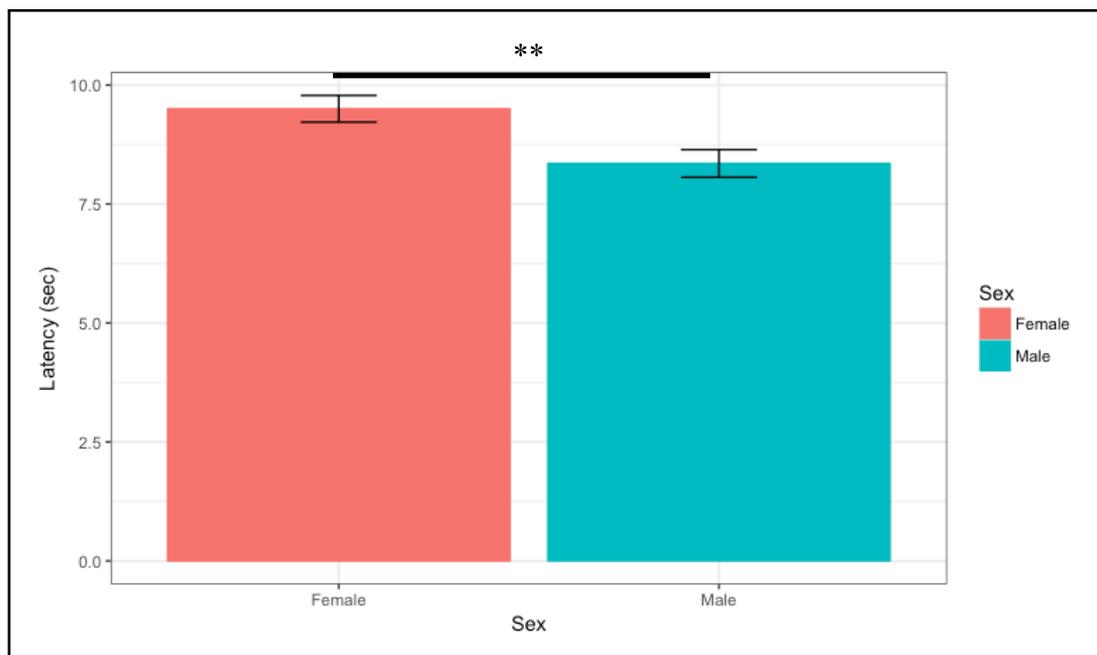


Figure 3.7a: Morris water maze matching to place paradigm. Latency; males had a significantly faster average latency than females. Error bars = SE. \*\* =  $p < 0.01$ .

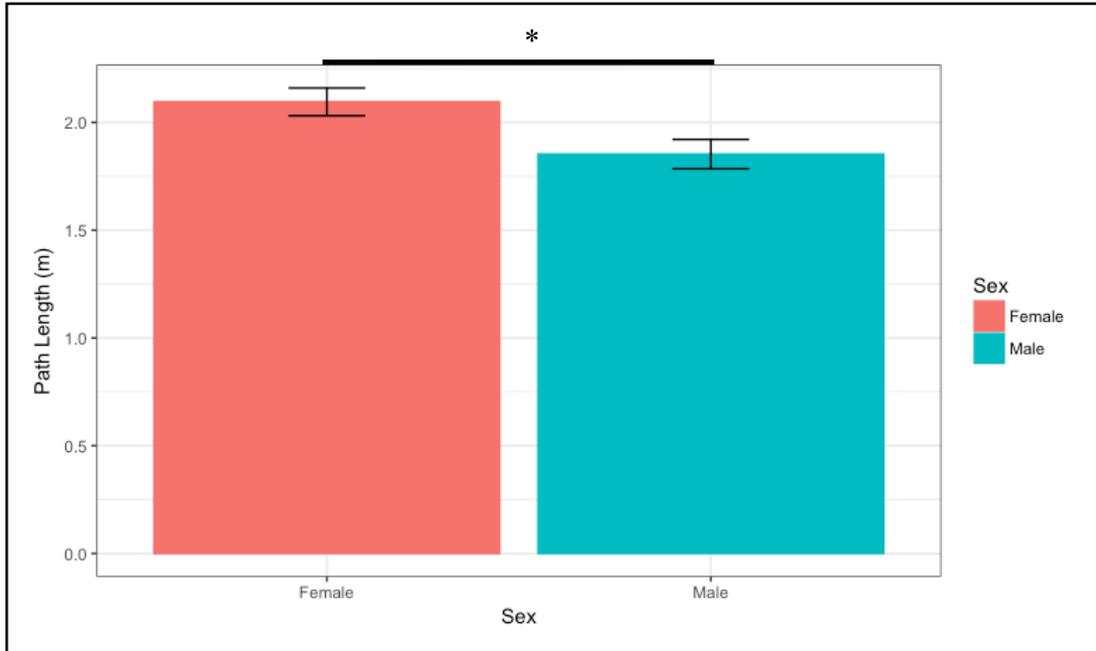


Figure 3.7b: Morris water maze matching to place paradigm. Path length; males had a significantly shorter average path length than females. Error bars = SE. \* =  $p < 0.05$

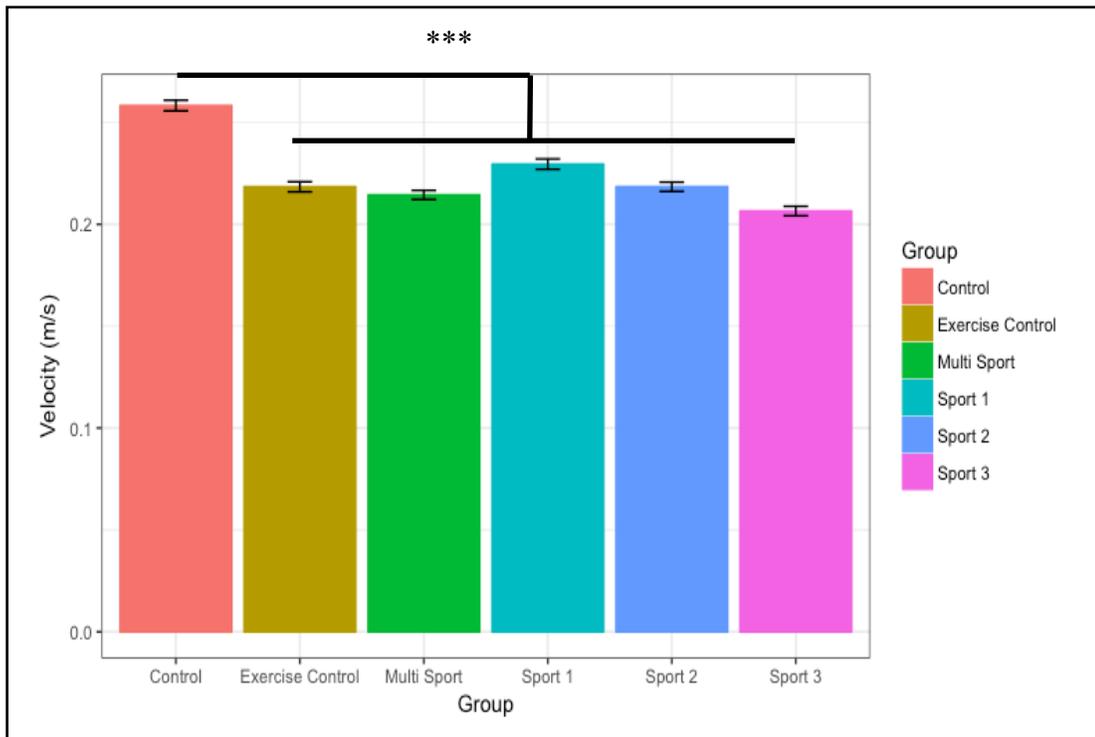


Figure 3.7c: Morris water maze matching to place paradigm. Velocity; group had a significant effect on average velocity. \*\*\* =  $p < 0.001$ .

Table 3.5: Morris Water Maze matching to place paradigm summary. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

MWM Matching to Place	Sex Effect		Group Effect		Interaction
	Change	<i>p</i>	Change	<i>p</i>	
<b>Latency</b>	Males ↓	0.005**	----	0.129	0.370
<b>Path Length</b>	Males ↓	0.015*	----	0.667	0.498
<b>Velocity</b>	----	0.996	Exercise control, Sport 1/2/3, and Multi-sport ↓ vs Control; Exercise control, Sport 1/2/3 and Multi-sport ↓ vs Sport 1; Sport 3 ↓ vs Exercise control and Sport 2	0.032*	0.709

### 3.1.4. Rodent Iowa Gambling Task

There was no effect of sex on any of the measures, therefore sex was collapsed for group analysis. There was a significant effect of group on the proportion of bad arm entries (Fig. 3.10a), the ratio between good and bad arm entries (Fig. 3.10c) but not on the proportion of good arm entries (Fig. 3.10b) ( $F(5,719)=6.842, p<0.000$ ,  $F(5,719)=2.405, p=0.036$ , and  $F(5,719)=1.486, p=0.1920$ , respectively). Post hoc testing found the Exercise Control group had a significantly lower proportion of entries into the bad arms in comparison to Control, Single-sport #1 and Single-sport #2 ( $p=0.002$ ,  $p=0.001$ , and  $p<0.000$ , respectively). Table 3.6 provides a summary of all main effects and interactions, significant or not.

Table 3.6: Rodent Iowa Gambling Task summary. \* =  $p < 0.05$ , \*\*\* =  $p < 0.001$ .

rIGT	Sex Effect		Group Effect		Interaction
	Change	<i>p</i>	Change	<i>p</i>	<i>P</i>
<b>Good Arm Entries</b>	----	0.567	----	0.192	0.164
<b>Bad Arm Entries</b>	----	0.798	Exercise control ↓ vs Control, Sport 1 and Sport 2	0.000***	0.467
<b>Good to Bad Ratio</b>	----	0.512	Sport 3 ↑ vs Sport 2	0.036*	0.105

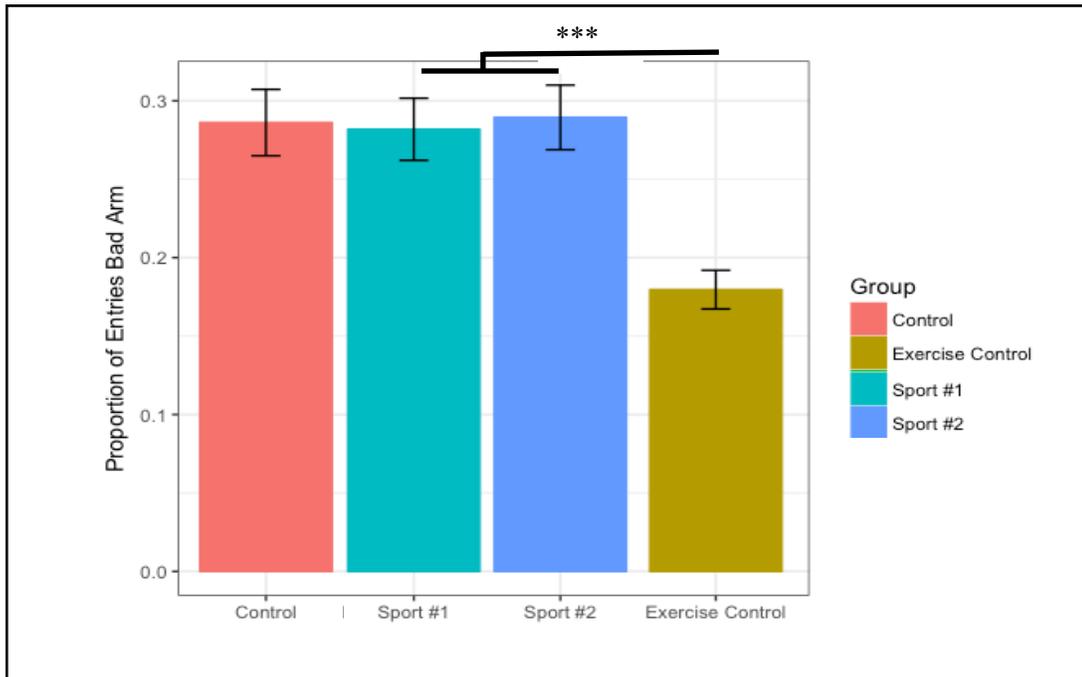


Figure 3.8a: Rodent Iowa gambling task. Bad arm entries; the exercise control group had a significantly lower proportion of entries than the control, and single-sport #1 and #2 groups. Error bars = SE. \*\*\* =  $p < 0.001$ .

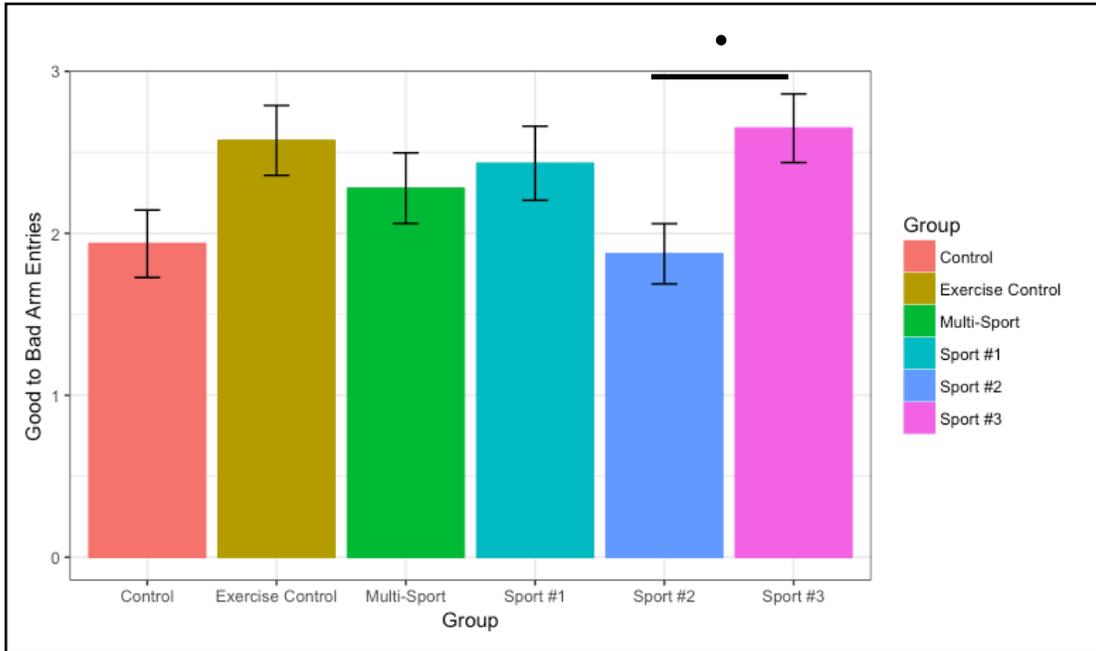


Figure 3.8b: Rodent Iowa gambling task. Good to bad arm entries ratio; there was a significant effect of group. Error bars = SE. • =  $p < 0.09$ .

### 3.3. Discussion

#### 3.3.1. Activity Levels

Although activity levels does not directly correspond to levels of cognition, it does allow us to say with more certainty that sport training in adolescence is what affected cognition later, rather than a general increase in exercise. There was no significant difference between groups for activity levels, but as seen in the results, there were several significant differences between groups in other behavioral measures.

Although we were not directly looking a sex differences, there was a significant effect of sex on activity levels. Females were significantly more active than the males, shown as higher overall activity and larger total distances travelled in the activity box.

Sex differences in voluntary physical activity is common. Rosenfeld (2017) discusses how sex differences in physical activity is common for many rodents, rats and mice included, with females showing higher physical activity levels.

### **3.3.2. Anxiety Level Depends on Type of Training**

In the elevated plus maze, increased anxiety is associated with more time spent in the closed arms, while more time spent in the “titanic zone” implies low-level anxiety (Walf & Frye, 2007). Anxiety can also be assessed in the activity box. Rats prefer to remain close to objects and walls in order to be less vulnerable to predators. This adaptive function is known as thigmotaxis, the more positive thigmotaxis the more anxious the animal (Harris, D’Eath, & Healy, 2009). Single-sport #3 emerged as having significant differences in several different anxiety measures in comparison to several groups. They spent less time in the open arms and titanic zone, while spending more time in the closed arms of the EPM. This is also supported by the activity box results, they spent more time and travelled more distance around the perimeter of the box in comparison to the center. This increased anxiety isn’t necessarily a bad thing. It has been known for a long time that there is an optimal arousal level for optimal performance, this is the Yerkes-Dodson Law (Cohen, 2011). Anxiety is essentially the body preparing to respond quickly to a threatening situation by increasing arousal levels and therefore alertness (Hoehn-Saric & McLeod, 2000). Therefore, more arousal can lead to an enhancement in later performance. As we will see shortly, this increased anxiety levels that Single-sport #3 group shows, does not hinder cognitive performance but may, actually, increase it as shown in the rIGT.

### 3.3.3. Sport Training May Enhance Components of Memory

Overall, there was no significant effect of group in either the spatial or reversal MWM paradigms. Interestingly, in the first paradigm (spatial version), there were no sex differences. This could have occurred for two different reasons; 1) there was actually no difference between males and females, or 2) there were improvements in the spatial memory of females in one or more of the exercise/sport groups that were big enough to null the traditional sex effect. The latter is more plausible as there is a large body of literature that supports males having better spatial memory than females (eg. Perrot-Sinal, Kostenuik, Ossenkopp & Kavaliers, 1996; Saucier et al., 2008). However, there was a significant effect of sex on the reversal paradigm, with males having shorter latencies to find the hidden platform. This is unsurprising as this paradigm is more challenging because of the requirement for the rats to suppress the previously learned location and learn another immediately after the first paradigm was completed.

Neither exercise nor sport training had a huge impact on the matching-to-place paradigm, which assesses short-term memory by switching platform locations each day. The, in turn, rats have to rely on memories formed between trials rather than days (Anisman & McIntyre, 2002). One interesting finding is that all the exercise and sport groups had significantly slower velocities than the control group. This may explain why there was not a significant effect of group on latency or path length as the numbers came close but not enough to reach a significant level. Overall, this may indicate that these groups had lower anxiety about the platform changing locations each of the 6 days of training and were better able to cope with the changing demands of the task. This does not conclusively support the notion that exercise improves short-term memory, but it

does indicate potential. However, the lower anxiety displayed by these rats during the task, points to an improvement in another area of executive functioning, attentional control. Miyake et al. (2000) identified three basic control functions of executive function: inhibition, shifting, and updating. Two of the three, it has been suggested, are important for attentional control (Eysenck, Derakshan, Santos & Calvo, 2007). Inhibition uses attentional control by preventing attention from being used to on irrelevant stimuli, while shifting uses attentional control by allocating or shifting attention to remain focused on task relevant stimuli. In the case of this study, the exercise and sport group rats were able to inhibit old representations of platform location and shift attention to learn the new locations. They were able to do this more effectively, as shown by decreased swim velocity, than the control rats because they were less anxious. This is important because anxiety has significantly adverse effects on performance that relies heavily on attentional control (Eysenck, Derakshan, Santos & Calvo, 2007).

#### **3.3.4. Exercise Affects Decision Making**

There was a significant effect of group on the proportion of bad arm entries in the rIGT. Specifically, the exercise control group had significantly fewer entries into the bad arms. However, they did not have significantly more good arm entries or a higher good to bad ratio. This could indicate less risky behavior as they didn't differ in good arm entries but consistently chose to enter the non-baited (empty) arms rather than take a loss in the bad arms over the 12 days testing. A significant effect of group was also seen in the good to bad arm ratio. The single sport #3 group was trending towards, but not quite hitting, a significantly higher ratio of good to bad arm entries. A study in humans by Giorgetta *et al.*

(2012) demonstrated, using a clinically anxious population and the IGT, that anxious and non-anxious individuals respond differently when faced with risky choices. Specifically, they avoided risky choices and were more risk avoidant after a gain. This corroborates the results of this study, the more anxious rats (single sport #3) were more likely to take less risk, so they chose an arm with less reward to gain a positive outcome and were less likely to switch arms and therefore had less entries into arms with a higher risk of bad outcome.

### **3.4. Conclusion**

Sport training, and exercise in general, during adolescence affected later behaviour in young adulthood but not in a uniform way. Sport training that relied more heavily on hindlimb coordination increased resting anxiety level. However, this may have enhanced later decision making on the rIGT. Furthermore, exercise and sport training may improve spatial memory in females. Exercise and sport training improved executive functioning in the form of increased attentional control. The next chapter focuses on structural brain changes that accompanied the behavioural changes.

## Chapter 4

### Effect of Adolescent Sport Training on Young Adulthood Brain

#### 4.1. Methods

##### 4.1.1. Perfusion and Staining

All animals were euthanized immediately after completing their last day of testing. Each animal was administered an intraperitoneal injection of pentobarbital (300 mg/kg), intracardially perfused with 0.9% saline for Golgi-Cox staining and decapitated for brain removal. Brains were promptly removed, weighed, and placed in Golgi-Cox solution.

The brains were kept in light-shielded bottles with Golgi-Cox solution for 14 days. After which, the Golgi-Cox was drained and replaced with a 30% sucrose solution. The brains remained in the sucrose for a minimum of two weeks, sucrose being replaced every two weeks, until sectioning. Brains were sectioned at 200 $\mu$ m on a vibratome and mounted on 2% gelatin coated microscope slides. Slides were kept moist for a minimum of 3 days before staining. Brains were stained according to procedure described by Gibb and Kolb (1998). Slides were coverslipped with Permount immediately following staining and kept out of direct light to dry several weeks before analysis.

#### 4.1.2. Anatomical Measures

**4.1.2.1. Cortical Thickness.** Neocortical thickness was measured by projecting the Golgi-Cox stained slides on a Zeiss 2 POL projector set at 17.5X magnification. Measurements were taken at 7 different planes. Five measures (MO/IL, LO, AID, Fr1, PL) were taken at two prefrontal planes, shown in Figure 4.1, corresponding to Bregma 3.70mm and 3.20mm. Three measures (Medial, Central, Lateral) were taken at the remaining 5 planes, shown in Figure 4.2, corresponding to Bregma 2.20mm, -0.30mm, -2.12mm, -4.52mm, and -6.30mm. Each measurement was taken using a ruler measuring from the edge of the white matter to the outer edge of the cortex.

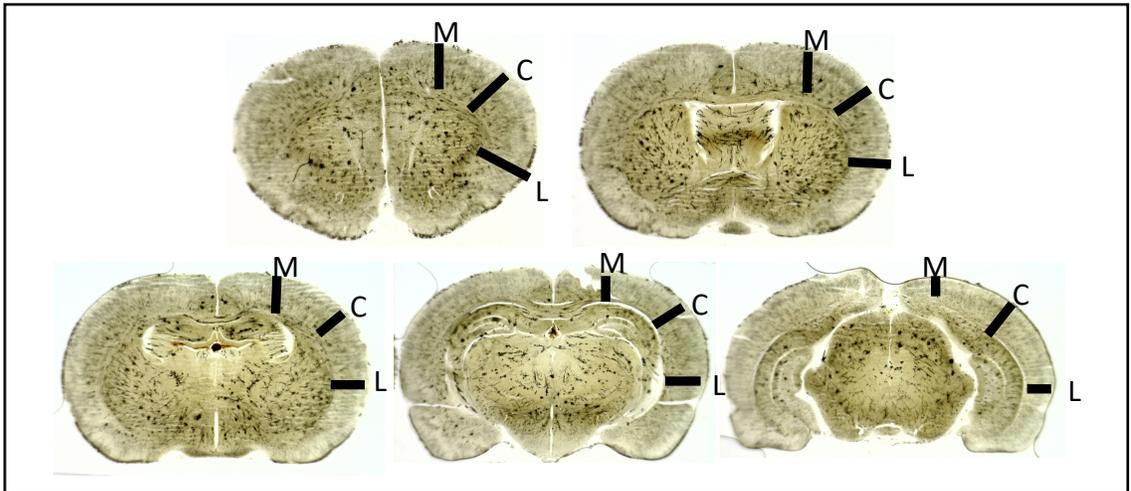


Figure 4.1: Prefrontal Cortical thickness; thickness was measured in the left and right hemispheres at the 5 locations indicated at each plane.

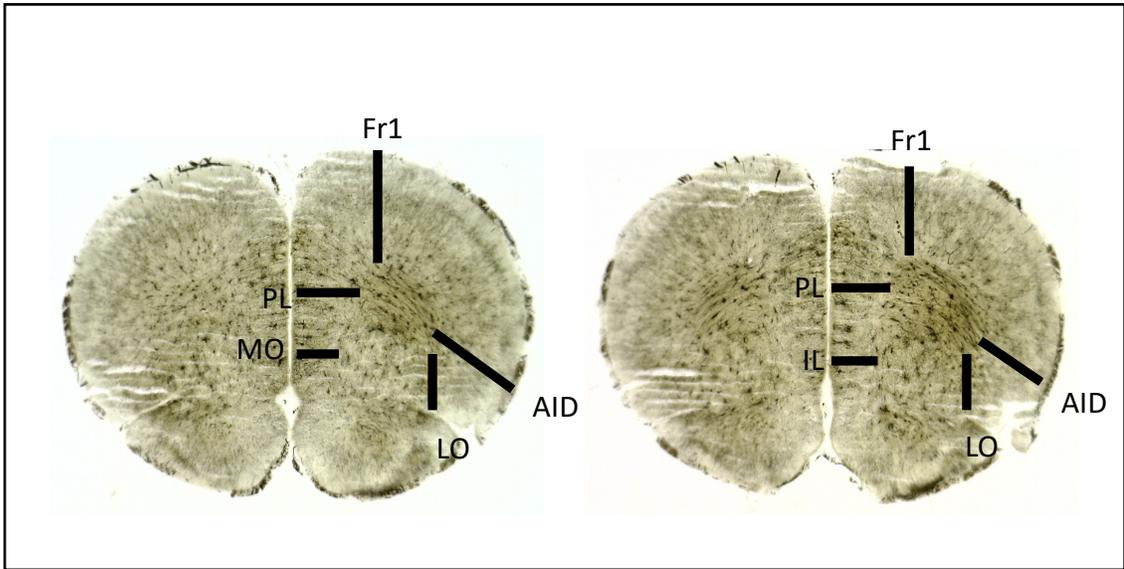


Figure 4.2: Cortical thickness; thickness was measured in the left and right hemispheres at the 3 locations indicated at each of the 5 planes.

**4.1.2.2. Thalamic Area.** Thalamic area was measured using images of the Golgi-Cox stained slides taken on the DinoCam Lite computer program and analyzed using Fiji. Measurements were taken at an anterior and posterior plane (Fig. 4.3), corresponding to Bregma -2.12mm and -4.52mm, respectively.

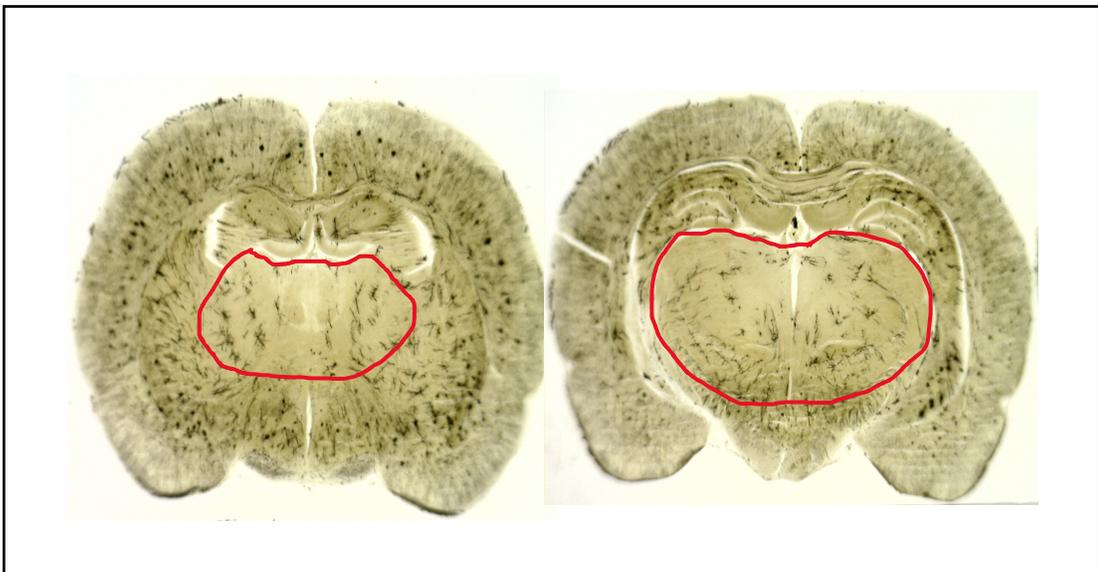


Figure 4.3: Thalamic area; area was measure at two thalamic planes, anterior (left) and posterior (right).

**4.1.2.3. Spine Density.** The dendritic architecture of pyramidal cells from layer III of area AID and layer V of Cg3 were examined from the Golgi Cox stained brains. Dendrites were traced at 1000x magnification using a compound microscope. For each region of interest, 5 terminal branches from each hemisphere (10 neurons total per area per brain) were analyzed for spine density. For each pyramidal neuron, spines were counted on one terminal tip (3<sup>rd</sup> order or higher). An average number of spines per 10 $\mu$ m in each hemisphere was calculated for each area. It was ensured that each dendritic segment traced met the criteria of not being obscured by neighboring dendrites or blood vessels, and that it was well impregnated with stain.

## 4.2. Results

### 4.2.1. Brain and Body Weight

Sex had a significant effect on both brain (Fig 4.4) and body weight (Fig. 4.5), males being heavier ( $F(1,58)=21.190, p<.000$  and  $F(1,58)=229.330, p<.000$ , respectively). There was no effect of group on either brain or body weight ( $F(5,58)=.079, p=.995$  and  $F(5,58)=.888, p=.495$ , respectively). No significant interactions between Sex and Group were observed for brain or body weight ( $F(5,58)=.374, p=.864$  and  $F(5,58)=.083, p=.995$ , respectively).

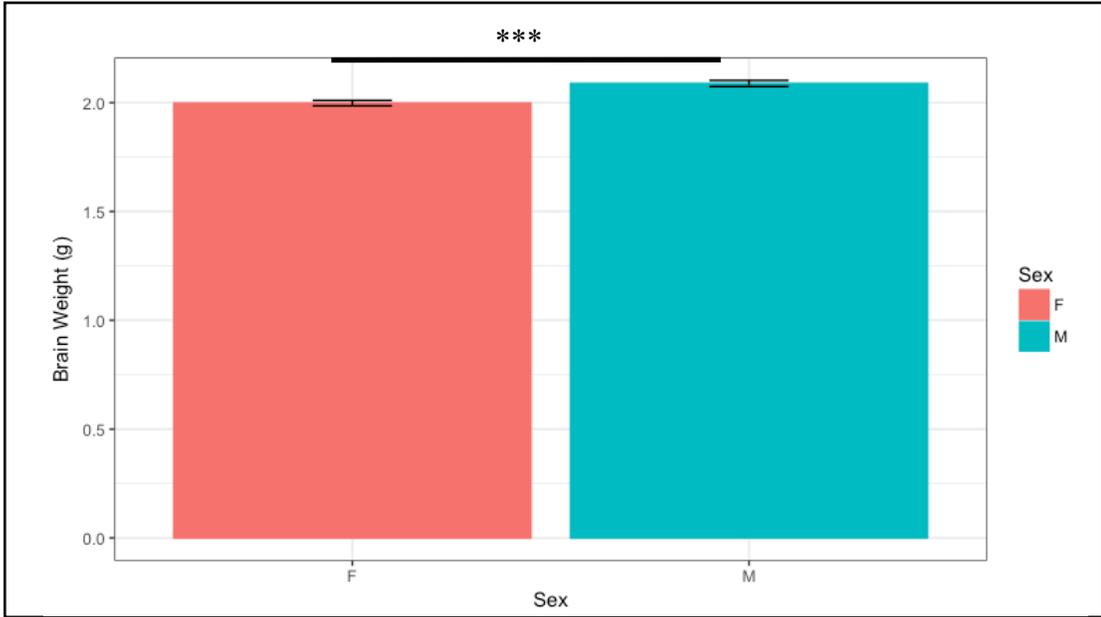


Figure 4.4: Brain weight; males had significantly heavier brains than females but no significant effect of group. Error bars = SE. \*\*\* =  $p < 0.001$ .

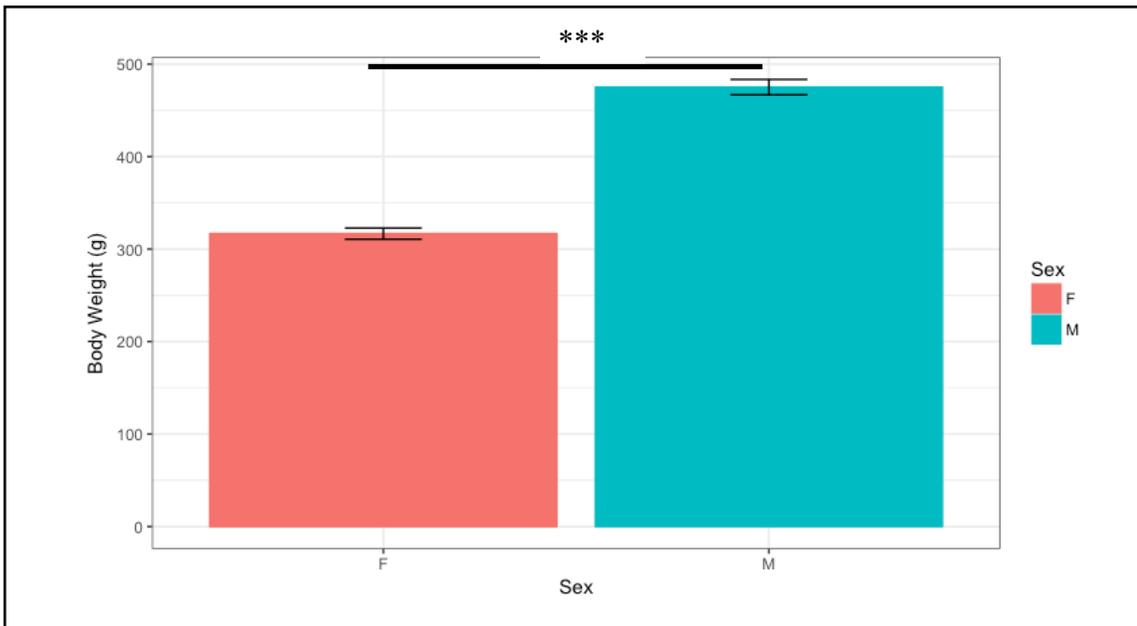


Figure 4.5: Body weight; males were significantly heavier than females. Error bars = SE. \*\*\* =  $p < 0.001$ .

#### 4.2.2. Cortical Thickness

First, the results of pre-frontal areas will be discussed. Sex effects were seen in all area except PL, which was not significant ( $F(1,116)=.127, p=.722$ ). In both the LO (Fig. 4.6a) and AID areas, the cortical thickness was significantly larger in males ( $(F(1,116)=44.455, p<.000)$  and  $(F(1,116)=11.329, p=0.001)$ , respectively). Conversely, in the Fr1 and IL (Fig. 4.6b) areas, the cortical thickness was significantly larger in the females ( $F(1,116)=4.667, p=.033$  and  $F(1,116)=9.858, p=.002$ , respectively). There was a significant effect of group in two different areas, the LO and IL ( $F(5,116)=2.347, p=.045$  and  $F(5,116)=6.855, p<.000$ , respectively). The post hoc analysis revealed that in the LO of Single-sport #1 animals had a significantly thicker cortex than Single-sport #3 animals ( $p=0.036$ ). In the IL, the Multi-sport and Single-sport #1 animals had significantly thinner cortex than the Controls ( $p=.002$  and  $p=.014$ , respectively). Also, the Multi-sport, Single-sport #1 and Single-sport #3 animals had significantly thinner cortex than the Exercise control group ( $p<.000, p=0.002$ , and  $p=0.010$ , respectively). There were several interactions; LO, AID, and IL ( $F(5,116)=2.936, p=0.016, F(5,116)=3.558, p=.005$ , and  $F(5,116)=3.557, p=.005$ , respectively). In area LO, non-control males had thicker cortex. In area AID, exercise control and multi-sport males had thicker cortex. In area IL, control and exercise control females had thicker cortex.

With regards to the results of the post-frontal areas of the brain. It was found that there was a significant effect of sex where males had a significantly thicker cortex in the central and lateral measures ( $F(1,116)=20.915, p<.000$  and  $(F(1,116)=9.007, p=.003$ , respectively). There were effects of group in the medial and central measures ( $F(5,116)=2.765, p=.021$  and  $F(5,116)=3.521, p=.005$ , respectively). In the medial

measure (Fig. 4.7c), the controls had significantly thicker cortex than Single-sport #2 animals ( $p=.047$ ). In the central measure (Fig. 4.7d), the Multi-sport group had significantly thicker cortex than Single-sport #1 group ( $p=.003$ ), according to post hoc analysis. There was no significant interactions. Figure 4.1 gives a summary of all the main effects and interactions, significant or not.

Table 4.1: Cortical thickness results summary. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

	Sex		Group		Interaction
	Change	$p$	Change	$p$	$p$
<b>LO</b>	Male ↑	0.000***	Sport 1 ↑ vs Sport 3	0.045*	0.016*
<b>AID</b>	Male ↑	0.001**	----	0.912	0.005**
<b>Fr1</b>	Female ↑	0.033*	----	0.130	0.097
<b>PL</b>	----	0.722	----	0.061	0.212
<b>IL</b>	Female ↑	0.002**	Multi and Sport 1 ↓ vs Control; Multi, Sport 1/3 ↓ vs Exercise Control	0.000***	0.005**
<b>Medial</b>	----	0.081	Control ↑ vs Sport 2	0.021*	0.358
<b>Central</b>	Male ↑	0.000***	Multi ↑ vs Sport 1	0.005**	0.130
<b>Lateral</b>	Male ↑	0.003**	----	0.834	0.094

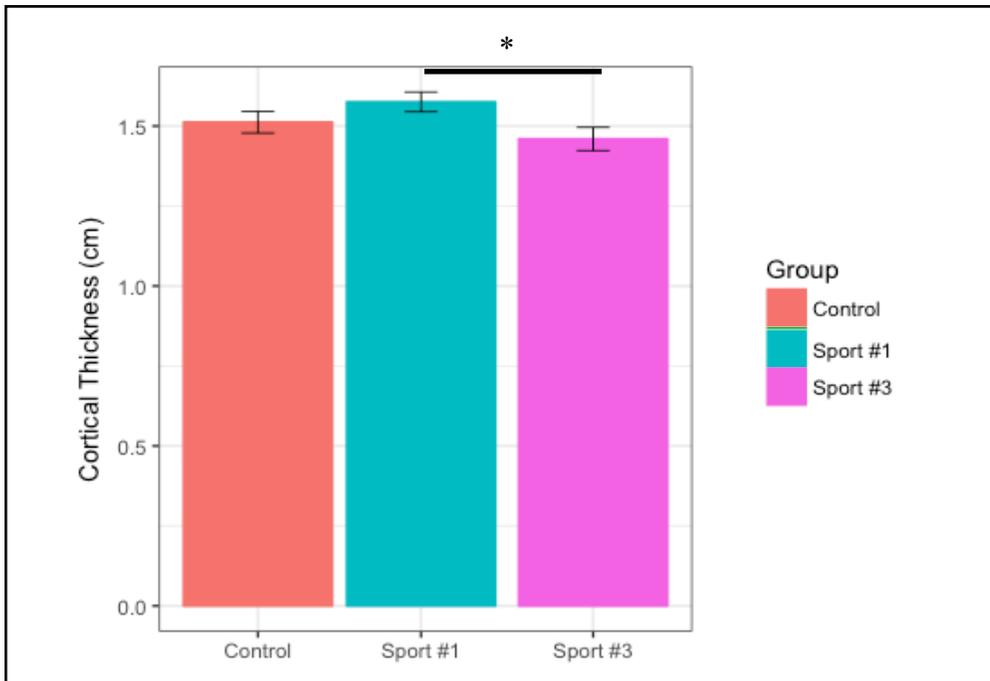


Figure 4.6a: Prefrontal cortical thickness. LO; single-sport #1 had significantly thicker cortex than single-sport #3. Error bars = SE. \* =  $p < 0.05$ .

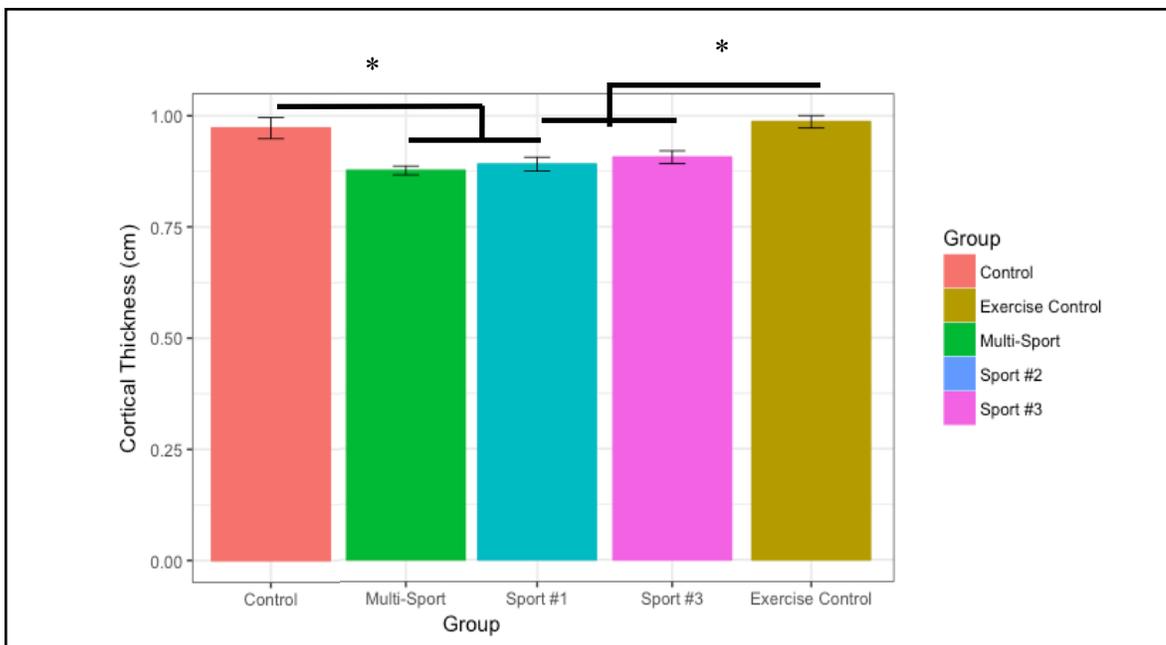


Figure 4.6b: Prefrontal cortical thickness. IL; there was significant effect of group. Error bars = SE. \* =  $p < 0.05$ .

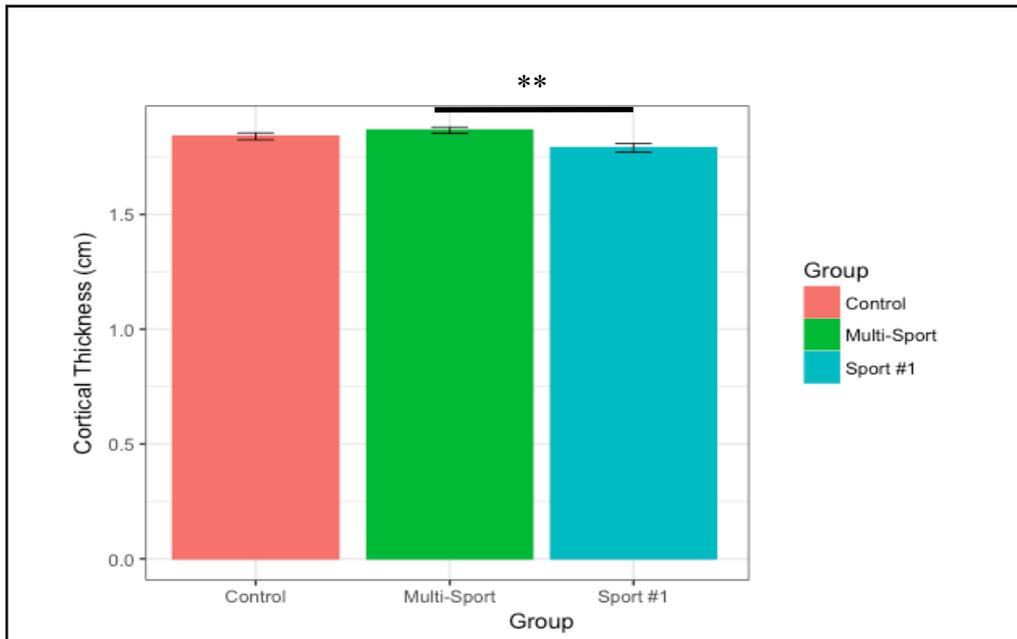


Figure 4.7a: Cortical thickness. Central; multi-sport group had significantly thicker cortex than the single-sport #1 group. Error bars = SE. \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

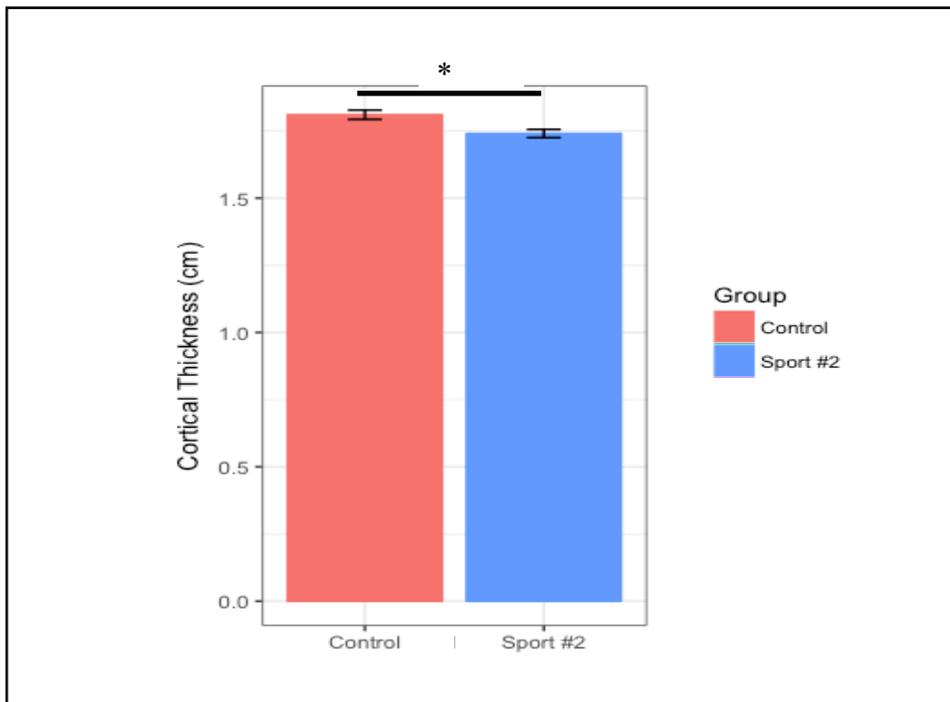


Figure 4.7b: Cortical thickness. Medial; the control group had significantly thicker cortex than the single-sport #2 group. Error bars = SE. \* =  $p < 0.05$ .

### 4.2.3. Thalamic Area

In the anterior plane, there was no significant differences found with regards to sex or group on thalamic area ( $F(1,58)=0.000, p=.990$  and  $F(5,58)=1.471, p=.213$ , respectively). However, there was an interaction between Sex and Group in the anterior thalamic area ( $F(5,58)=2.596, p=.035$ ), control females had a larger area. In the posterior plane (Fig. 4.8) there was a significant effect of sex on area, males being larger ( $F(1,58)=5.342, p=.024$ ). There was also a significant effect of group on posterior thalamic area ( $F(5,58)=2.491, p=.041$ ). Post hoc analysis found that the single-sport #3 group had a significantly larger posterior thalamic area than the Exercise Controls ( $p=.043$ ). There was no significant interaction between Sex and Group ( $F(5,58)=1.027, p=.410$ ). Table 4.2 gives a summary of all main effects and interactions, significant or not.

Table 4.2: Thalamic area summary. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

	Sex Effects		Group Effects		Interaction
	Change	<i>p</i>	Change	<i>p</i>	<i>p</i>
<b>Anterior</b>	---	0.990	---	0.213	0.035*
<b>Posterior</b>	Male ↑	0.024*	Sport 3 ↑ vs Exercise Control	0.041*	0.410

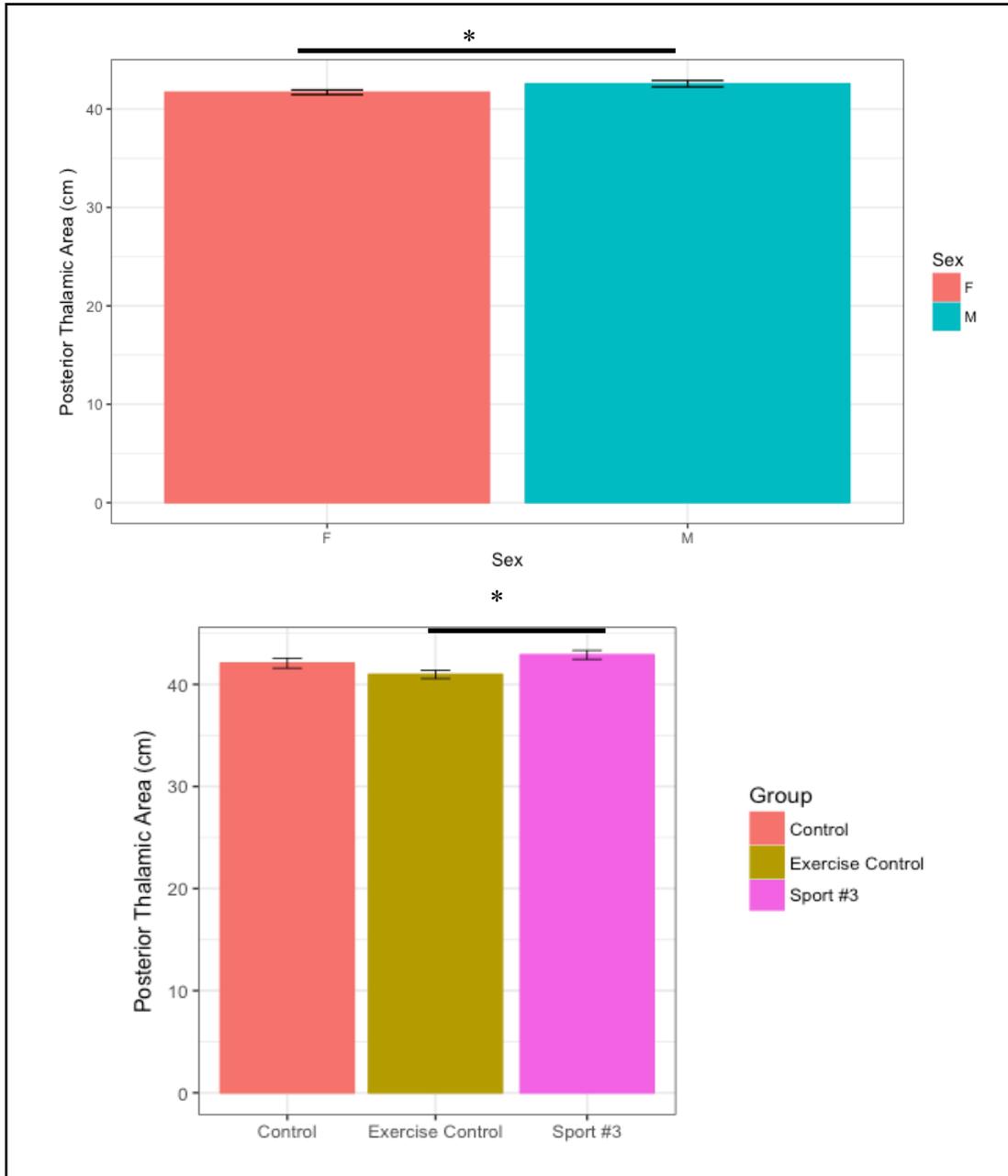


Figure 4.8: Posterior Thalamic Area; males had significantly larger thalamic area (top) and the single-sport #3 group had a significantly larger thalamic area than the exercise control group (bottom). Error bars = SE. \* =  $p < 0.05$ .

#### 4.1.4. Spine Density

There was no significant effect of side, therefore, all the data was collapsed, each animal having two measurements (left and right) in each area. In area AID there was a significant effect of sex (Fig. 4.9a), males having greater spine density, but no significant effect of group ( $F(1,112)=25.105, p<.000$ , and  $F(5,112)=1.350, p=.249$ , respectively). There were no significant interactions for area AID. In area Cg3 there was a significant effect of sex, males having greater spine density, and a significant effect of group ( $F(1,112)=63.451, p<.000$ , and  $F(5,112)=8.597, p<.000$ , respectively) (Fig. 4.9b). Post hoc analysis found regarding the effect of group, the Multi- sport group had significantly higher spine density than Single-sport #1 and #2 animals ( $p= .006$  and  $p= .003$ , respectively). It was also found, Single-sport #3 animals had significantly higher spine density than the Exercise control and Single-sport #1 groups ( $p=.001$  and  $p<.000$ , respectively). There was also significant interaction in area Cg3, males in sport groups had increased spine density ( $F(5,112)=3.778, p=.003$ ). Table 4.3 gives a summary of all main effects and interactions, significant or not.

Table 4.3: Spine density summary. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

	Sex Effect		Group Effect		Interaction
	Change	$p$	Change	$p$	$p$
<b>AID</b>	Male ↑	0.000***	---	0.249	0.054
<b>Cg3</b>	Male ↑	0.000***	Multi-sport ↑ vs Sport 1 and Sport 2; Sport 3 ↑ vs Exercise Control and Sport 1	0.000***	0.003**

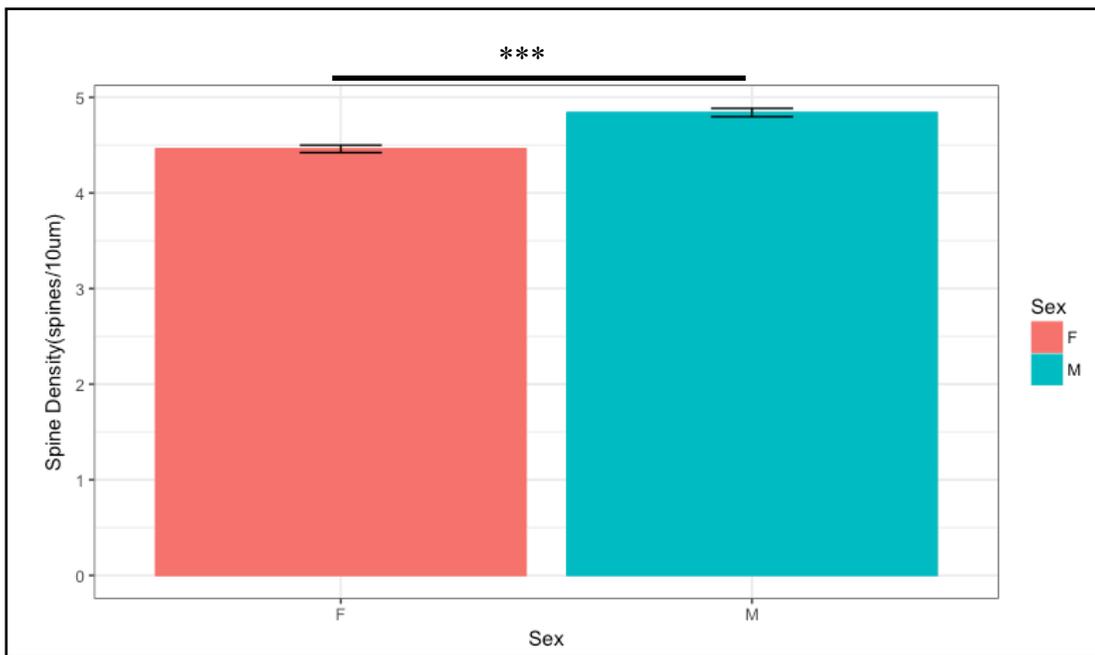


Figure 4.9a: Spine density. AID; males had significantly higher spine density than females. Error bars = SE. \*\*\* =  $p < 0.001$ .

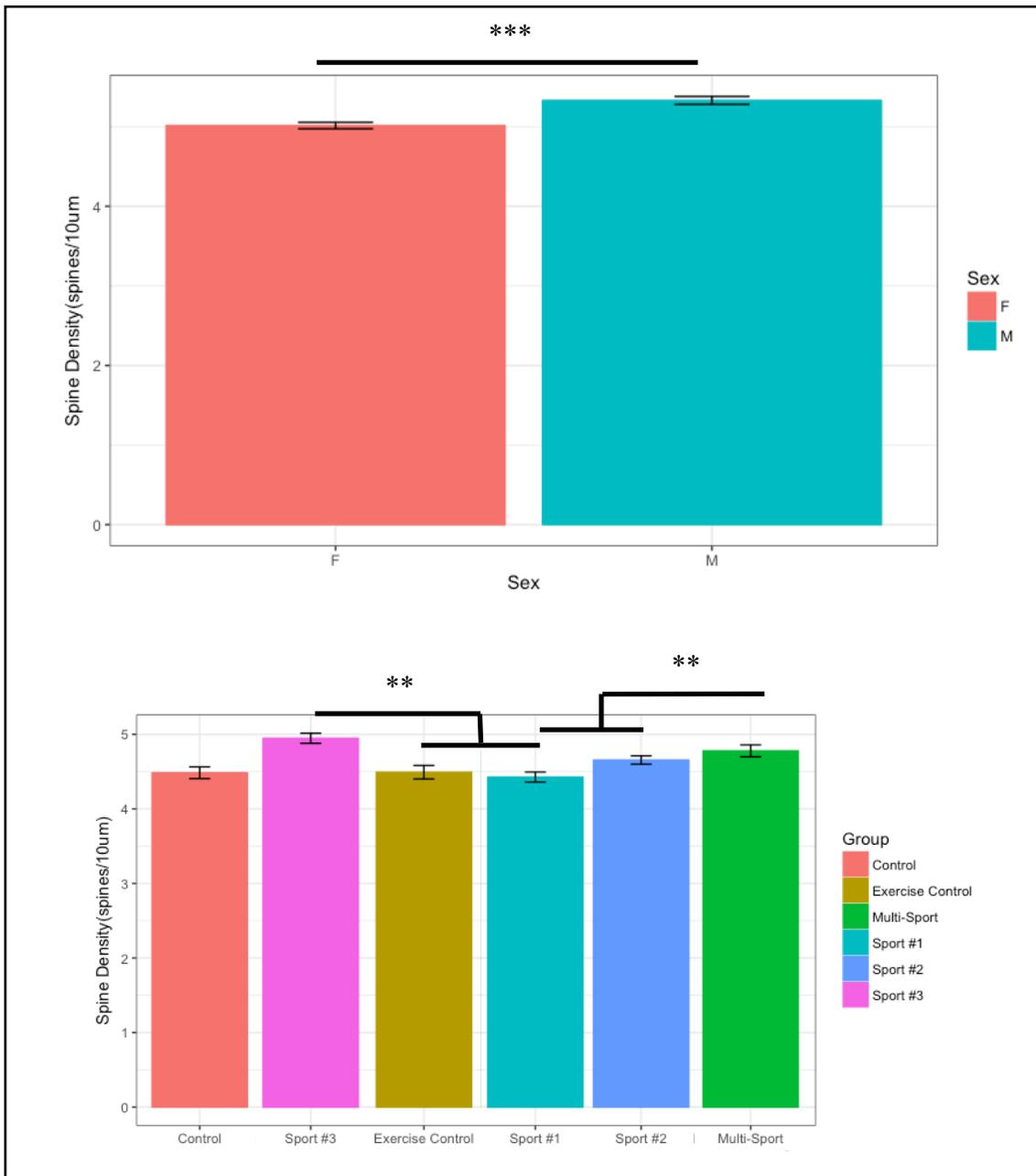


Figure 4.9b: Spine density. Cg3; males had a significantly higher spine density than females (top). There was a significant effect of group (bottom). Error bars = SE. \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ .

## **4.3. Discussion**

### **4.3.1. Sport Selectively Produces Changes in Cortical Thickness**

Changes in cortical thickness is a normal part of brain development. Adolescence is a period of extensive cortical thinning, especially in the frontal regions (Squeglia et al., 2013). It has been shown that intelligence and change in cortical thickness is closely related. The more superior the intelligence, the more rapid cortical thinning (Shaw et al., 2006). It is also important to note that cortical thinning, especially in the prefrontal cortex, leads to improvements in executive functioning (Tamnes et al., 2009). One of the most significant results found in the cortical thickness measures was a reduction in the IL plane of the cortex in some of the sport groups compared to controls and exercise controls. This could, in part, help explain some the improvements in attentional control and decision making. All of these behaviors can be seen as maintenance of behavioral flexibility, which is one of the critical functions of the IL (Barker, Taylor, & Chandler, 2014).

### **4.3.2. Exercise Changes Spine Density in Area Cg3**

As with cortical thickness, spine density is also reduced during brain maturation. During early brain development there is a general overgrowth of synapses, the subsequent pruning later in development help reconfigure the brain into its adult form (Tau & Peterson, 2010). Area Cg3, also referred to as the prelimbic cortex, is important for a variety of cognitive processes including attentional functions, working memory, and response-selection (Granon & Poucet, 2000). Accelerated pruning could conceivably

mature the cognitive processes associated with the area, which could corroborate the improvements seen on the MWM and rIGT behavioral tests, as mentioned in the section above. However, the differences seen between groups doesn't match up completely, so it is unlikely changes in spine density is the sole reason for the improvement.

There was a significant effect of group on spine density in Cg3. The exercise controls, single sport #1 and #2 had decreased spine density. These same groups also displayed less anxiety in the behavioral tests. Exercise controls spent significantly less time in the perimeter of the activity box, more time in the titanic zone and open arms and less time in the closed arms of the EPM. Single sport #1 spent significantly less time in the closed arms and more time in the titanic zone of the EPM. Single sport #2 spent significantly more time in the open arms and titanic zone and less time in the closed arms of the EPM. In a review by Leuner and Shors (2013), it was discussed how changes in dendritic and synaptic structure in areas involved in anxiety, such as prefrontal cortex, can produce changes in anxiety behaviors. This may be one connection linking the changes to brain morphology to some of the behaviours seen in the current study.

#### **4.3.3. Changes in Thalamic Volume Reflect Differences in Anxiety**

Area measurements of the thalamus in two planes were used to evaluate overall size differences. The only significant results were in the posterior plane. The posterior plane contains the paraventricular nucleus (PVN). Li et al., (2010) reports multiple studies indicating the PVNs role in regulating anxiety behaviors through connections with the forebrain. In this study, both the activity box and EPM results show Single-sport 3 as being significantly more anxious than several groups, and of particular interest, the

Exercise Control group. The posterior thalamus measurements showed that the Single-sport #3 group had significantly larger area in comparison to the Exercise Controls. This structural change in the posterior thalamus may be in part responsible for this difference in anxiety.

#### **4.4. Conclusion**

Anatomical changes occurred in response to both exercise in general and sport training. Cortical thinning was seen in response to sport training, and the most significant results were seen in the prefrontal cortex. There was a decrease in spine density in groups who displayed less anxiety behaviors, and these changes were only seen in are Cg3. Changes in the thalamus were restricted to the posterior plane and only seen in a select group. This change in the thalamus, as mentioned above, may reflect the difference in anxiety behavior seen between the two groups. It is not clear whether these changes in anatomical structure are in part responsible for the increased executive functioning in the groups that showed a differences in behavior.

## **Chapter 5**

### **General Discussion**

#### **5.1. Using Rats as a Model for Sport**

To our knowledge, this is the first time a rat model has been used to study sport training and its effects on cognition. Although the complex motor tasks were not an exact replica of a sport, they were a good stand in for the general characteristics a sport includes, such as complex motor training. No two sports are exactly identical and all utilize different body parts and combinations of body parts. The rope pulling task focused on forelimb (upper body) coordination, the uneven ladder rung focused on hindlimb (lower body) coordination, and the rope climbing was a combination of the two. This allowed us to compare whether all types sports are the same, finding in the end that not all sports are equal. Finally, sports include rules and regulations that everyone must follow. The simplistic design used in this thesis had one rule, complete the complex motor task correctly and a food reward will be given. In the end, this is a good framework for future sport models using rats.

This present research is similar to that of Kleim et al. (1998). They trained a group of rats on an obstacle course requiring substantial motor coordination (acrobatic condition), while a second group had access to running wheels (voluntary exercise condition). The obstacle course contained items such as beams to traverse and ladders to

climb. Their acrobatic group is similar to our multi-sport group as they were also required to learn several complex motor tasks. Although our single-sport groups only learned a single motor task, those tasks were similar to single components of the obstacle course (eg. rope climbing vs rope ladder climbing). The voluntary exercise group, similar to our exercise control group, also used running wheels. There were a few main differences comparing these studies. Firstly, Kleim and colleagues trained their rats on the obstacle course for 30 consecutive days rather than 21 days with every 7<sup>th</sup> day off. However, they only had 5 trials per/day, which was 5 mins total time or less, compared to 10 minutes of continuous training utilized for this thesis. Secondly, their acrobatic condition was exposed to all obstacles each day rather than one at a time, like the multi-sport group in the current paradigm. The voluntary exercise group had free access to wheels while the exercise controls in the current study only had 10 minutes of access during training days. Kleim and colleagues found a significant increase in cortical thickness and increase synaptic density, in the cerebellum, in the acrobatic condition compared to their exercise controls. The multi-sport group utilized in this thesis had significantly higher spine density in area Cg3 compared to some of the Single-sports. The multi-sport group had significantly thinner cortex in area IL, however; they had significantly thicker cortex in the central measure of the more posterior planes. These similarities help validate our prediction that complex motor training changes brain architecture, although different measures were taken in different areas by Kleim and colleagues. However, Kleim and colleagues didn't directly test for behavioral changes after the training like we did, rather they assessed functional changes to the brain. They saw substantial functional changes to the cerebellum in their acrobatic condition which could presumably, if they would have

tested for, produced behavioral differences as well, potentially corroborating the current finding that sport training changes some subsequent behaviours.

Environmental enrichment (EE) in the context of laboratory animals is the modification of their environment to provide stimulation via physical and social surrounding. In a review by Kramer et al. (2004), they discussed how EE changed the brain function and structure in dramatic ways. They argue that EE is an experience-induced way to increase cognitive reserve capacity. This is what this study set out to accomplish. We wanted to determine if sport training could enhance cognitive functioning. The sport training used in this thesis is, arguably, a form of EE because animals were exposed to an environment that required substantial interaction. The main difference between classical EE and our study was the length of exposure. In most cases, exposure to EE is extend for several hours and often periods of days or months (eg. Johnson, 2013; Young et al., 1999; Birch & Kelly, 2019). Our exposure was limited to 10-minute periods/day for a span of 3 weeks. Although we saw changes in behaviour that suggest enhanced executive functioning, and therefore increased cognition, it wasn't as dramatic or widespread across behavioural tests as other studies. This could be due to the difference in exposure time as mentioned above. Leal-Galicia, Castañeda-Bueno, Quiroz-Baez, and Arias (2008) found that animals exposed to life-long EE starting in their youth, had enhanced short-term memory and reduced anxiety in novel environments. We were beginning to see these outcomes even in young adulthood. Our results were not as prominent, but this could be due to length of exposure. They exposed animals to EE for several hours a day for 15 days and then moved to several hours per week for 18 months.

Taken all together, this study lines up with current and past literature findings and even fills in some of the blanks. We have seen that complex motor training changes brain architecture. We also have seen that EE changes behavior. This study combines the two, as changes were seen in both brain and behavior. Although the changes weren't as prominent as the other studies discussed above, it shows that there could be great potential with protocol refinement.

## **5.2. Limitations and Future Direction**

This research provides a base for developing rodent models of sport training. However, the inconsistency and lack of significant results in some areas calls for a refinement in protocol. It is conceivable that the amount of training time the animals were subjected to was not long enough. Typical high performing athletes train multiple times a day, often for extended lengths of times spanning hours over the course of years. Since this was an animal model designed from scratch, it was difficult to know how long of a training sessions the rats could handle. A prior pilot study suggested that 10 minutes was reaching maximum attention span and amount of food reward to be given. Since Frootloops were used as a food reward, the amount given was consciously monitored because of its relatively high caloric level. Finding an alternate food reward with lower calories but keeping motivational value high would allow for longer training sessions without the potential negative consequences of the food reward itself. Longer training sessions should produce more prominent results based on the current and previous studies on both complex motor training and enrichment.

Aside from mirroring typical athlete training patterns, changes in behavior and brain architecture could have been masked for other reasons. It is well known that even training the rats during the behavioral testing changes the brain as well. These changes could have easily masked any subtle changes from the exercise/sport training sessions as the behavioral test battery lasted weeks longer than the training itself. The only way to address the issue adequately would be to include another group who received training but none of the testing, allowing for anatomical comparisons between groups who received the experience of the test battery training and those who did not. We also know that social play peaks in the adolescent period, with cage-mates playing for around an hour a day (Pellis & Pellis, 2007), much longer than the training sessions they received for the experimental protocol. Play is important for normal brain development, specifically refinement of the mPFC (Bell, Pellis, & Kolb, 2010; Juvenile peer play experience and the development of the orbitofrontal and medial prefrontal cortices) and thus any changes that may have resulted from sport training may have been masked by play behavior. By socially isolating the rats during the training period the current study may have found that sport training has a similar effect on EFs that play does, thus confirming that complex sport can enhance and train the brain of rats (Baarendse et al., 2013; Pellis & Pellis, 2017; Vanderschuren, 2010)

Another limitation was the number of animals used in the study. The lower end of the groups included 5 animals while the most was 7. This was not an ideal group size to obtain accurate results. Too few subjects can lead to significant results to appear where there shouldn't be any or it may have prevented trending results from becoming significant (Charan & Kantharia, 2013). In any case, the amount of research, time, and

space ultimately led to group sizes being small in order to run the entirety of the experiment at one time and in a timely manner. It would be advisable to break up the experiment into smaller groups to facilitate longer training time and larger number of animals per group.

Further anatomical analysis need to be done. In this study the only anatomical measures that were reported were thalamic area, cortical thickness, spine density, and brain weight. Within these measures, spine density only included the basilar dendrites of areas Cg3 and AID. In future, it would be beneficial to explore the apical dendrites of those areas, as well dendritic length and complexity, and include other areas commonly associated with executive functioning (such as the hippocampus and parietal cortex (Turner & Spreng, 2012)) to get a more holistic view of sport training's effect on synaptic structure. Other measures on the same Golgi-Cox stained tissue could include dendritic length and complexity. Future studies in this area should also include Cresyl Violet staining of some brains in order to quantify neuron and glial density.

### **5.3. Conclusion**

The present research demonstrated that exercise and sport during the adolescent period has the potential to positively affect behaviour and cognition in young adulthood. Major findings included increase anxiety-like behavior, changes in spatial memory, increased attentional control, and altered decision making in rats exposed to exercise or sport training. Overall, my general hypothesis was supported. My first prediction was supported; both exercise and sport had a positive impact on cognition. My second prediction, however; was not. It was unclear whether sport training or multi-sport training

had a greater impact on cognition than exercise alone. My third prediction was supported; both exercise and sport training produced architectural changes in the brain. Further studies should include analysis of neurogenesis and/or neurotropic factor concentrations to attempt to provide a mechanism behind these behavioral changes.

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