SEX DIFFERENCES IN THE EFFECTS OF AEROBIC EXERCISE ON NEUROGENESIS AND COGNITIVE FUNCTION IN ADULT C57BL/6 MICE

by

ANA-STEFANIA GHEORGHE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Neuroscience)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

December 2017

Abstract

Aerobic exercise has positive effects on the brain and is associated with improved cognition. One mechanism through which exercise can promote brain health is by increasing adult hippocampal neurogenesis, a phenomenon that produces new function brain cells after the developmental period and throughout life. Meta-analyses of exercise interventions have shown a sex-difference in the efficacy of exercise on cognition in older adults. This current study sought to determine whether there are sex-differences in the efficacy of exercise for promoting neurogenesis and improving cognition in healthy, adult mice. Adult male and female mice were given access to a running wheel (or a disassembled wheel) for 28 days and on day 22 were given 6 days in the Morris water maze, for spatial learning, memory and reversal training. Mice were then sacrificed and examined for neurogenesis in the dentate gyrus using the endogenous protein doublecortin (DCX), a marker for immature neurons. Mice that ran for 28 days showed increased hippocampal neurogenesis, regardless of sex. All mice, irrespective of exercise intervention or sex, showed learned the reference memory version of the Morris water maze. There was a positive correlation between time spent in the platform zone and doublecortin-expressing neurons in the dorsal dentate gyrus in males, but not females. Future studies should continue to address sex-differences in the effects of exercise on neurogenesis and cognition by using more specific and challenging cognitive tests for young, healthy rodents, or using animals with impaired or naturally decreased levels of neurogenesis.

Lay Summary

Aerobic exercise has positive effects on brain health. Exercise drastically increases the production of new brain cells (neurogenesis) in a region of the brain called the hippocampus in adulthood and into older age. However, exercise interventions in older humans have shown sex-differences in the effect of exercise on cognition, with women showing a greater benefit of exercise on cognition than men. The research conducted in this thesis sought to determine whether there are sex-differences in the effects of exercise on neurogenesis and cognition in healthy adult mice. Mice that exercised for 28 days showed increased neurogenesis compared to sedentary controls, but there were no sex differences in total number of new cells. All mice performed equally well on the learning and memory tests, but there was no effect of exercise or difference between sexes. Future research should employ more challenging tasks for testing cognition in young, healthy animals.

Preface

This thesis is an unpublished, original work written by Ana-Stefania Gheorghe, with the following exceptions: a paragraph regarding the differences between forced and voluntary exercise in the introduction and two paragraphs in the discussion regarding the interactions between hormones and exercise. These sections were also written by Ana-Stefania Gheorghe, but were submitted as part of a book chapter and is still under review.

Table of Contents

List of Tables

List of Figures

Acknowledgements

Thank you to my supervisor Liisa Galea and committee members Teresa Liu-Ambrose and Alasdair Barr. Thank you to Cindy Barha for ongoing guidance regarding experimental design, statistics, and analysis. Thank you also to all the lab members: Stephanie, Paula, Jessica, Kim, Remi, Emily, Laura, Shunya, Sam, Rand, Wansu, and Aarthi.

Introduction

Regular physical exercise is a modifiable component of daily living that can have profound benefits on several domains of health (Warburton et al., 2006). Research has consistently shown that higher physical activity is associated with reduced mortality from cardiovascular disease, decreased risk of diabetes II, and a decreased likelihood of developing specific forms of cancer (Warburton et al., 2006). Additionally, in randomized control trials, exercise interventions elicit positive benefits in individuals already diagnosed with cardiovascular disease, diabetes, and metabolic syndrome, indicating that physical activity is an excellent approach for secondary prevention (Warburton et al., 2006). The benefits of physical activity also extend to brain health. There has been a plethora of research showing a positive relationship between physical activity and cognitive function. The positive effects of exercise on cognition have been observed throughout all stages of life from childhood (Sibley and Etnier, 2003) through adolescence (Esteban-Cornejo et al., 2015), and into adulthood and older age (Hillman et al., 2008).

The mechanism through which aerobic exercise affects cognitive function is likely multi-faceted and remains an area of active research. Exercise has been shown to increase blood flow (Holschneider et al., 2007), affect neurotransmitter release (Meeusen and De Meirleir, 1995), increase growth and trophic factors (Cotman et al., 2007; Neeper et al., 1996), increase angiogenesis (Swain et al., 2003; Kerr et al., 2010), and increase adult hippocampal neurogenesis (van Praag et al., 1999 a,b). Physical exercise can improve some forms of learning and memory, which usually relies on the integrity and proper functioning of the hippocampus. Adult neurogenesis is the production of new neurons in adulthood, and this phenomenon is restricted to two areas of the brain, one of which is the hippocampus. Thus, for this research study, I will be focusing on the effects of voluntary exercise on neurogenesis and hippocampal-dependent learning and memory.

The hippocampus is a small sea-horse shaped structure in the medial temporal lobe of the brain that plays an important role in some forms of learning, memory, and spatial function (Fanselow and Dong, 2010) and is also implicated in emotional responses and mood (Fanselow and Dong, 2010). The hippocampus can be divided into two regions: the dorsal and the ventral hippocampus, both of which undergo neurogenesis. However, several lines of evidence including lesion studies (Moser et al., 1995), anatomical analyses (Vivar et al., 2016), and gene expression results (Thompson et al., 2008) have indicated that the dorsal hippocampus is crucial for spatial learning and memory, while the ventral hippocampus may be more involved in

limbic area associated functions including mood regulation. Recent research has found that exercise increases neurogenesis to a greater degree in the dorsal region than in the ventral region (Vivar et al., 2016), further validating the link between exercise, increased neurogenesis, and improved cognition. In adult hippocampal neurogenesis, new neurons originate from neural stem/progenitor cells in the subgranular zone of the dentate gyrus (DG). These progenitor cells proliferate and form two daughter cells, at least one of which will become a mature granule neuron. These new neurons become integrated into the functional hippocampal circuitry (Toni and Schinder, 2016). However, whether new neurons form appropriate connections or contribute in a positive way to the function of the DG depends on a number of factors including the environment into which the new neuron was produced and matured (Jakubs et al., 2006). For example, seizures cause increased hippocampal proliferation, but these new cells differ from those that are born under non-pathological conditions. New cells created after seizures show decreased spontaneous excitatory potentials and increased spontaneous inhibitory potentials compared to new cells born under normal physiological conditions (Jakubs et al., 2006). Additionally, seizure-induced new cells can ectopically migrate into the hilus of the hippocampus, a region that does not typically show neurogenesis in normal physiological conditions. These findings suggest that new adult generated cells differ based on the environment under which they are produced.

New cells can be visualized using endogenous or exogenous markers. Endogenous proteins such as Ki67, a nuclear protein that is expressed exclusively during cell proliferation (with the exception of G_0), or doublecortin (DCX), a microtubule-associated protein that is expressed in immature neurons, are often used depending on the question of interest. Exogenous markers such as thymidine analogs are used to label cells that have divided during a short time window. Bromodeoxyuridine (BrdU), a synthetic nucleoside, is incorporated into dividing cells during a 2-hour long period after injection, and can be used to quantify cell proliferation and/or survival of new cells depending on the timeline between BrdU incorporation and sacrifice. However, it is important that when using BrdU or other synthetic nucleosides, to be aware of several caveats mainly around the dose, toxicity, and timing of BrdU exposure relative to any manipulations and sacrifice as these can affect outcome (see Taupin, 2007). In both rodents and primates, neurogenesis persists throughout life (reviewed by Cameron and Mckay, 2001; Spalding et al., 2013) but it is important to be aware that the timeline of maturation of new neurons in the hippocampus differs between species including a longer timeline of maturation in mice compared to rats (Snyder et al., 2009).

Aerobic exercise is a strong positive regulator of neurogenesis (van Praag et al., 1999 a,b; van Praag et al., 2005; Lou et al. 2008; Redila and Christie, 2006; Eadie et al., 2005). Van Praag et al. (1999) first showed that running drastically increases hippocampal neurogenesis in young adult female mice via increases in both cell proliferation and subsequent survival of new neurons, an effect that was also confirmed in males (van Praag et al., 2005). Increased neurogenesis in the dentate gyrus after exercise has also been observed in juvenile male rats (Lou et al., 2008), adult male and female Sprague-Dawley rats (Redila and Christie, 2006; Eadie et al., 2005), and C57BL/6 mice (Creer et al., 2010; Fischer et al., 2014). Furthermore, the beneficial effects of exercise on neurogenesis is not limited to young or adult animals. Van Praag et al. (2005) showed that male mice who began a 4-week voluntary exercise program at 19 months of age, an age that is equivalent to ~ 70 years in humans, exhibited increased neurogenesis via both an increase in proliferation and differentiation of new cells into neurons compared to age-matched sedentary controls. Similarly, Speisman et al. (2013) found that aged male rats who voluntarily exercised for 18 weeks showed increased neurogenesis compared to sedentary controls. In both studies, increased neurogenesis was accompanied by improved water-maze performance, suggesting that exercise can improve cognition even in older age.

In humans as well, research has shown that aerobic exercise interventions started in older age benefit cognitive health (Colcombe and Kramer, 2003; Erickson and Kramer, 2009; Barha et al., 2017a). Interestingly, emerging trends show that females and males may not benefit to the same extent from these interventions, suggesting that exercise-efficacy for improving cognition may be dependent on sex, particularly in older age. This sex-difference was first observed by Colcombe and Kramer (2003), whose 18-study meta-analysis showed that sex was a moderating variable for the effect of exercise on cognition in older adults. Specifically, there was a greater effect size for the ability of exercise to improve cognition in studies with a high percentage (>50%) of female participants, compared to those with a high percentage (>50%) of male participants. Confirming these findings, Baker et al. (2010) also found that elderly females showed a greater benefit on certain executive function tasks than elderly males when subject to an aerobic exercise intervention. In this study, Baker et al. (2010) conducted a 6-month aerobic exercise intervention on previously inactive older adults with mild amnesic cognitive impairment. Compared to the sedentary stretching control group, the exercise group showed improvements in executive function after 6 months of exercise. Separate analyses by sex showed that there was a larger effect size for women compared to men in certain executive function tasks including the category fluency task and symbol digit task. There were also sex differences in the

effect of exercise on performance on the Stroop test; while exercise improved performance for women, there was no effect of the exercise intervention for men.

More recently, Barha et al. (2017a) published a meta-analysis on sex differences in exercise efficacy for improving cognition in older humans. This meta-analysis included 39 randomized control trials (RCTs) that met the following criteria: participants were at least 45 years old, did not have any neurodegenerative disorders or clinical disorders, the exercise intervention was at least two months in duration and was undertaken at least once a week, and cognitive performance was measured for several different cognitive domains, including certain ones that were specifically reliant on integral hippocampus functioning such as episodic memory and visuospatial function. These RCTs were coded as either high female (>71%) or low female (≤ 71%) to investigate the role of sex as a moderating variable in the effect of exercise on cognition. Barha et al. (2017a) found that aerobic exercise improved executive function compared to controls, and that high female studies showed a greater effect size than low female studies. Similarly, exercise improved visuospatial performance compared to controls and in this case, the effect was only significant in high female studies. Thus, the literature on exercise intervention in older humans consistently shows a trend of exercise favouring improvements of cognition in women more than men.

Given that rodents are often used as animal models for looking at exercise efficacy on cognition, it was expected that these sex-differences would also be observed in older rodents. Barha et al. (2017b) also published a meta-analysis on sex differences in exercise efficacy for improving cognition in older rodents. This meta-analysis included 17 rodent studies that met the following criteria: the subjects were cognitively healthy, at least 8 months of age, not models of any kind of neurological disease, the exercise intervention was at least 3 days in duration, a proper control group was implemented, and performance on a cognitive task (of any domain) was used as an outcome measure. Cognitive performance analysis was organized based on the specific type of task and the cognitive domain that was targeted. Specifically, this meta-analysis looked at the effect sizes in the following groups: spatial learning in the Morris water maze (MWM), spatial memory in the MWM, other hippocampal-dependent tasks, conditioned avoidance memory, and non-spatial memory tasks. Barha et al. (2017b) found that while voluntary aerobic exercise improved both spatial learning and memory in the MWM, and performance in other hippocampal-dependent tasks, there no sex-differences were observed in efficacy of exercise on cognition. However, sex differences favouring female older rodents for hippocampal dependent learning and memory became apparent when looking specifically at forced exercise

interventions. Rodent exercise can typically be classified as either voluntary or forced. Voluntary running usually occurs in a running wheel, while forced running usually occurs on a motorized rodent treadmill (Ang et al., 2006, Liu et al., 2009), or motorized running wheel (Leasure and Jones, 2008). Voluntary running typically allows animals to run *ad libitum*, thus permitting them to control both the amount and intensity of exercise. However, this makes it challenging for researchers to control for total distance, time, and intensity of running across animals, which can lead to high variability in the exercise group. This variability can be circumvented by using a forced exercise paradigm. With the motorized treadmill or motorized running wheel, researchers can control the intensity and duration of exercise (Leasure and Jones, 2008; Ang et al., 2006; Liu et al., 2009). However, in some forced exercise paradigms, mild electric shocks can be administered if the animal steps off the treadmill prematurely to motivate continued running (Ang et al., 2006; Hayes et al., 2008). This added stress will undoubtedly influence the outcome measures and must be considered when comparing results between voluntary and forced exercise paradigms. Thus, that Barha et al. (2016) found sex-differences favouring females on the effects of exercise on hippocampal-dependent learning only when the exercise was forced suggests an interaction between stress, sex, and exercise on cognition. However due to the interference of forced exercise with stress and the plethora of work indicating sex differences in the influence of stress, I chose to examine voluntary exercise in this study.

While there has been research looking at sex-differences in aerobic exercise efficacy for improving cognition in older mammals, it is currently unknown whether these sex-differences exist in young adult, healthy animals. Looking at these sex-differences in young animals is crucial as levels of endogenous sex hormones during the reproductive years affect neurogenesis (Mahmoud et al., 2016) and cognition (Hausmann et al., 2000, Horst et al., 2012) both on their own, and in interactions with exercise (Jin et al., 2008; Okamoto et al., 2012). To date there have been no studies on potential sex differences in exercise-promoting effects on neurogenesis to improve cognition. Thus, this research project sought to fill in an current gap in the literature. Using young adult female and male C57BL6/J mice, I hypothesized the following:

1. That 28 days of voluntary exercise would increase neurogenesis in a sex-specific manner.

2. That there would be a sex-difference in the efficacy of exercise for improving spatial learning, memory, and reversal learning, correlating with increased neurogenesis.

3. That exercise-induced neurogenesis increase in the dorsal hippocampus would correlate with Morris water maze probe trial performance in a sex-specific manner.

Methods

An overview of the experimental timeline is shown in Figure 1, with more detailed explanations following.

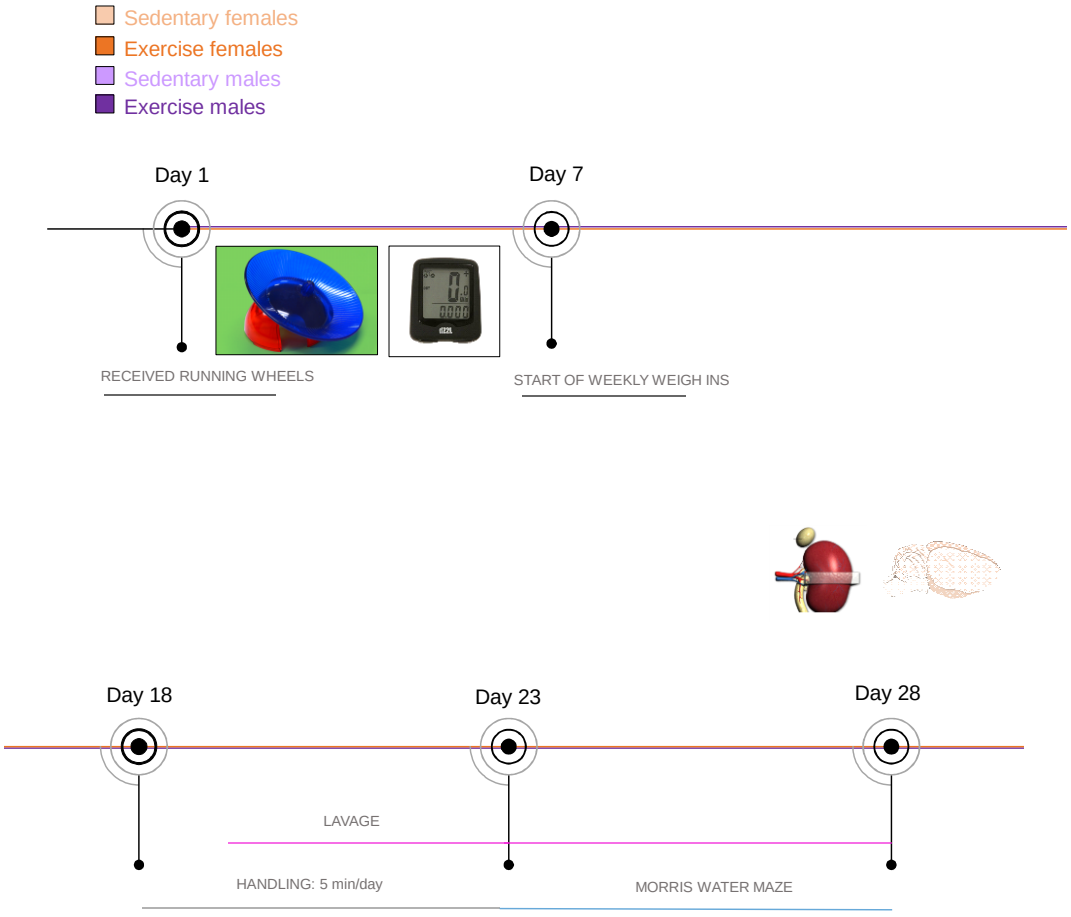

Figure 1. Experimental timeline. Exercise animals received their running wheels on day 1 of the experiment. Animals were weighed weekly beginning on day 7 (days 14, 21, 28 not shown). Animals were handled for 5 minutes a day from day 18 to day 23, with females getting lavaged daily starting on day 20. Morris water maze testing was done on days 23-28. Animals were euthanized on day 28, with adrenals and brains collected for analysis.

Animals

Female (n=14) and male (n = 18) C57BL/6 (J) mice were obtained from Jackson Laboratories at approximately 2.5 months of age. This strain of mice was used because it is a conventional wild-type laboratory strain that is used in many studies looking at the effects of exercise on neurogenesis and/or cognition (van Praag et al. 1999a,b, van Praag et al., 2005). Upon arrival, mice were group-housed (2-3 per cage) with others of the same sex. Males and females were housed in separate colony rooms. After one week of acclimatization to the Center of Disease Modeling (CDM) animal facility at the University of British Columbia, mice were put into their own cages and single-housed for the remainder of the experiment. Animals were designated to one of four groups: sedentary females (n = 7), exercise females (n = 7), sedentary males (n = 9), and exercise males (9). All animals were weighed beginning on day 7 of the experiment using a scale that rounded to the nearest gram. Starting on day 21 or day 28 (depending on the group), a different scale was used that measured to the closest 0.1 gram. Cages were changed on either day 8 or 15, depending on the cohort. All procedures were performed in accordance with ethical guidelines set by the Canadian Council on Animal Care, and approved by the Animal Care Committee at the University of British Columbia.

Exercise

Exercise animals received a Fast-Trac running wheel (BioServ, New Jersey, USA) atop of a plastic igloo dome (BioServ, New Jersey) in their home cage. Two magnets were attached to the underside of the wheel and a bicycle tracker (Filzer DZ2L Cycling Computer, Mountain Equipment Co-Op) was attached to the underside of the cage to measure the distance traveled by the mice. The tracker was calibrated to track for this specific wheel with a circumference of 479 mm. Exercise mice received the running wheel on day 1 of the experiment and remained in the cage throughout the experiment. Sedentary mice received the plastic dome, but no running wheel. Exercise animals had *ad libitum* access to running wheel for 28 days while in their home cage. Food pellet crumbs (equivalent to approximately 1/3 of one pellet) were sprinkled on the wheel for the first 3 days to encourage mice to explore the wheel. Trackers were checked, and distance was recorded twice daily at 7 am (lights on) and 7 pm (lights off).

Morris Water Maze

All animals were handled for 5 minutes per day for 5 days prior to the start of the Morris water maze testing (days 18-22 of the experiment). The water was kept at 25-26°C and was made opaque with white, non-toxic tempera paint (FunStuff brand). The platform (3-inch platform

diameter) was painted white using the same white, non-toxic tempera paint. In trials that included the platform, it was submerged 1 cm below the top of water. Each trial lasted until the mouse found the platform, up to a maximum of 60 seconds. If the mouse did not find the platform, it was guided to the platform. Each mouse was given 10 seconds atop the platform to orient itself to the spatial cues around the room. ANY-Maze software (Stoelting, CO, USA) was used to track and analyze the testing. Males were tested in the morning and females were tested in the afternoon. The pool was partially drained and refilled between sexes to minimize any olfactory cues from the opposite sex. The Morris water maze test consisted of four phases: acquisition, probe trial (to test for spatial memory of location of the hidden platform), reversal learning, and a visual platform test (to test for visual acuity).

Acquisition

Each mouse received 6 trials a day for the first 5 days (days 23-27 of the experiment) of testing. The platform was located in the NE quadrant. There was an inter-trial interval of 60-90 seconds. The mice were released from one of four pseudorandom locations on each trial. The location of the release points varied from day to day.

Probe Trial

On day 6 of testing (day 28 of the experiment), mice were tested in the probe trial 24 hours after the last acquisition trial. The platform was removed, and the animal was released into the pool from a random location. ANY-maze was used to determine distance swum in the target quadrant and the target area where the platform was previously located. The trial lasted 60 seconds.

Reversal Learning

On the same day following the probe trial, the platform was placed in the SW quadrant (the opposite quadrant to the acquisition trials) and animals were again released from pseudorandom locations to find the platform. Eight trials were conducted with an inter-trial interval of 60-90 seconds.

Visual Control Test

Following the reversal learning trials, the platform was moved to the centre of the maze and a high-contrast flag was attached to the middle of the platform. This test was included to ensure that none of the animals suffered from any visual deficits that would impact their spatial learning and memory. Animals were released from pseudorandom locations. This stage consisted of two

trials up to a maximum of 60 seconds. The distance to platform from the two trials were averaged together to get one visual test score.

Vaginal Lavage

Female mice were vaginally lavaged once a day between 1 and 3 pm starting on day 20 of the experiment. The mouse was manually restrained ("scruffed") and a plastic pipette tip containing 10 µL of ambient temperature water was inserted into her vagina. The vagina was flushed 2-3 times and the cell/water solution was collected back inside the pipette tip after the final flush. The solution was placed onto a glass slide (Fisher Scientific), left to dry, and then stained with cresyl violet for easier cell identification. Cells were identified using a Nikon Alphaphot-2 microscope at 40 x magnification. Males were handled and scruffed for the same amount of time.

Immunohistochemistry

Animals were euthanized by live decapitation. The brains were immediately removed and halved. The right half was put into cold 4% paraformaldehyde for fixation and was left at 4°C for 24 hours. The halved brains were then transferred to a 30% sucrose solution at 4°C until sectioned. The brains were sectioned coronally at 40 µm thickness. Sections were placed in Eppendorf tubes containing ethylene glycol and glycerol in phosphate buffer, and were stored at -80°C until used for immunohistology. The left half of each brain was immediately placed on ice for use in protein assays, but ultimately not used.

All rinses and antibody incubations in this protocol were done on a shaker. Sections were rinsed in 0.1 M phosphate-buffered saline (PBS, pH 7.4) 2 x 10 minutes at RT and then rinsed in PBS overnight at 4 °C. On the following day, sections were rinsed 3 x 10 minutes in PBS, treated with 0.3 % hydrogen peroxide for 30 minutes, and then incubated in DCX goat polyclonal primary antibody (1:1000) (SantaCruz Biotechnology, Santa Cruz, CA, USA) with 0.04% Triton-X and 3 % normal rabbit serum for 3 hours at room temperature (RT), followed by 21 hours at 4 °C. Sections were then rinsed 5 x 10 minutes in PBS and incubated in rabbit anti-goat secondary antibody (1:1000) (Vector Laboratories, Burlington, ON, Canada) for 1.5 hours at RT, followed by 21 hours at 4 °C. Sections were washed again 5 x 10 minutes in PBS and incubated in avidin-biotin solution (ABC Elite Kit; Burlington, ON, Canada) for 4 hours at RT. After 2 x 2-minute washes in 0.175 M (pH 7.3) sodium acetate at RT, sections were developed using diaminobenzidine with nickel (DAB Peroxidase Substrate Kit, Vector) and mounted on slides. After a 72-hour drying period, sections were dehydrated using increasing concentrations

of ethanol (50% for 2 minutes, 70 % for 2 minutes, 95 % for 2 minutes, 100 % for 10 minutes, xylene for 10 minutes) and then coverslipped with Permount (Fisher Scientific).

Slides were coded so that the experimenter was blind to the treatment groups when examining the DCX+ cells. DCX+ cells were counted in every 10^{th} section of the hippocampus, using the 40x objective on a Nikon Eclipse E600 microscope. The dorsal and ventral regions of the hippocampus were analyzed separately. Digitizing imaging software was used to capture photos of the sections at 4x magnification. The granular cell layer was traced and measured on these photos using ImageJ (NIH Bethesda, MD, USA) for density calculations (cells/mm^2). From each animal, 25 cells were chosen from both the dorsal and ventral regions, for a total of 50 cells. These cells were chosen randomly across 4 sections (2 dorsal, 2 ventral) and were categorized as type 1, 2, or 3 DCX+ cells based on morphology, as described in Plumpe et al. (2007).

Adrenal Mass

Adrenals were removed bilaterally immediately after euthanasia, placed in Eppendorf tubes on ice, and weighed within 3 hours of removal. Adrenal mass was divided by euthanasia (day 28) body mass to get an adrenal:body mass ratio for each mouse.

Blood Collection

Immediately following decapitation, 2 Eppendorf tubes of blood were collected from each animal. This blood was to be used for hormone analysis and/or brain-derived neurotrophic analysis, but both analyses were ultimately not performed.

Fecal Sample and Cecum Collection

On the final day following lavage, animals were scruffed for a maximum of an additional 3 minutes to collect fecal samples. After euthanasia, additional fecal samples were dissected from the large intestine of each animal. The cecum was also dissected out from each animal and stored in glass jars at -80°C for later microbiome analysis which was ultimately not performed.

Statistics

All statistical analyses were conducted using Statistica software. Distance run was analyzed using a repeated measures ANOVA with days as the within-subjects factor and sex as the between-subjects factor. Performance on the Morris water maze acquisition and reversal learning was analyzed using a repeated measures ANOVA with days (acquisition) or trials (reversal) as the within-subjects factor, and sex and treatment as the between subjects factors.

Performance on the probe trial was analyzed using a factorial ANOVA with sex and treatment as the between-subjects factors. DCX+ cell density was analyzed with a repeated measures ANOVA, with region (dorsal, ventral) and type (1, 2, 3) as the within-subjects factors, and sex and treatment as the between-subjects factors. Fisher's least-significant difference (LSD) post-hoc was used where applicable.

Results

Female mice ran greater distances than male mice on certain days.

Females ran a greater total distance across all days than males ($p < 0.05$) (Figure 2A). Over the 28-day period, there was a significant effect of sex ($F(1, 14) = 8.16$, $p = 0.01$) and a significant day by sex interaction ($F(27, 378) = 1.70$, $p = 0.02$) in distance run (Figure 2A). Post hoc testing showed that females ran greater distances than males on day 10 ($p=0.04$), day 11 ($p= 0.01$), day 12 ($p=0.03$), day 13 ($p < 0.01$), day 15 ($p=0.02$), day 17 ($p < 0.01$), day 18 ($p = 0.04$), day 21 ($p=0.02$), day 22 ($p=0.03$), day 23 ($p = 0.01$), day 26 ($p=0.02$). Females showed a significant drop in running amount between days 23 and 24, coinciding with the start of Morris water maze testing ($p < 0.01$) (Figure 2B), while males did not ($p = 0.21$).

Males weighed more than females, but there was no significant effect of exercise on body mass

Body mass was measured weekly starting on day 7 of the exercise intervention. There was a significant effect of sex, with males weighing more than females ($F(1, 28) = 77.990$, $p < 0.01$; Figure 3A). There was also a significant effect of time ($F(3,84) = 40.195$, $p < 0.01$), with the LSD post-hoc test showing that body mass increased significantly each week. (Figure 3B). There was no significant sex by time interaction ($F(3,84) = 1.798$, $p = 0.15$), sex by exercise interaction ($F(1,28) = 0.886$, $p = 0.36$), or sex by time by exercise interaction ($F(3,84) = 0.68$) for body weight (Figure 3C).

Females had a larger adrenal/body mass ratio than males.

There was a significant effect of sex on the adrenal weight to body weight ratio ($F(1, 28) = 17.008$, $p < 0.01$), with females having a greater ratio than males (Figure 4). There was no significant main effect of exercise ($p = 0.98$) and no sex by exercise interaction ($p = 0.77$).

Morris Water Maze

Spatial learning: All animals learned to find the hidden platform

The layout of the spatial cues for the entire Morris water maze testing period is shown in Figure 5. All animals learned to navigate to the hidden platform over the course of 5 days; there was a significant effect of time for all the animals combined ($F(4,104) = 96.396$, $p < 0.01$). Post-hoc tests showed that there was a significant decrease in distance to platform between day 1 and day 2, and between day 2 and day 3 ($p<0.01$). There was no main effect of exercise($F(1,26) = 0.610$, $p = 0.44$) or sex ($F(1,26) = 1.344$, $p = 0.26$) . There was no significant exercise by time

interaction ($p = 0.81$) (Figure 6A), but there was a significant sex by time interaction ($F(4,104) = 2.490$, $p = 0.05$), with post-hoc tests showing males swimming significantly less distance to reach the platform than females on day 1 ($p<0.01$) (Figure 6B). There were no significant interactions of exercise by sex ($p = 0.17$) or sex by exercise by time interaction ($p = 0.51$; Figure 6C and 6D). Significant differences between the sedentary and exercise groups occurred in only 3 trials, as shown in Table 1.

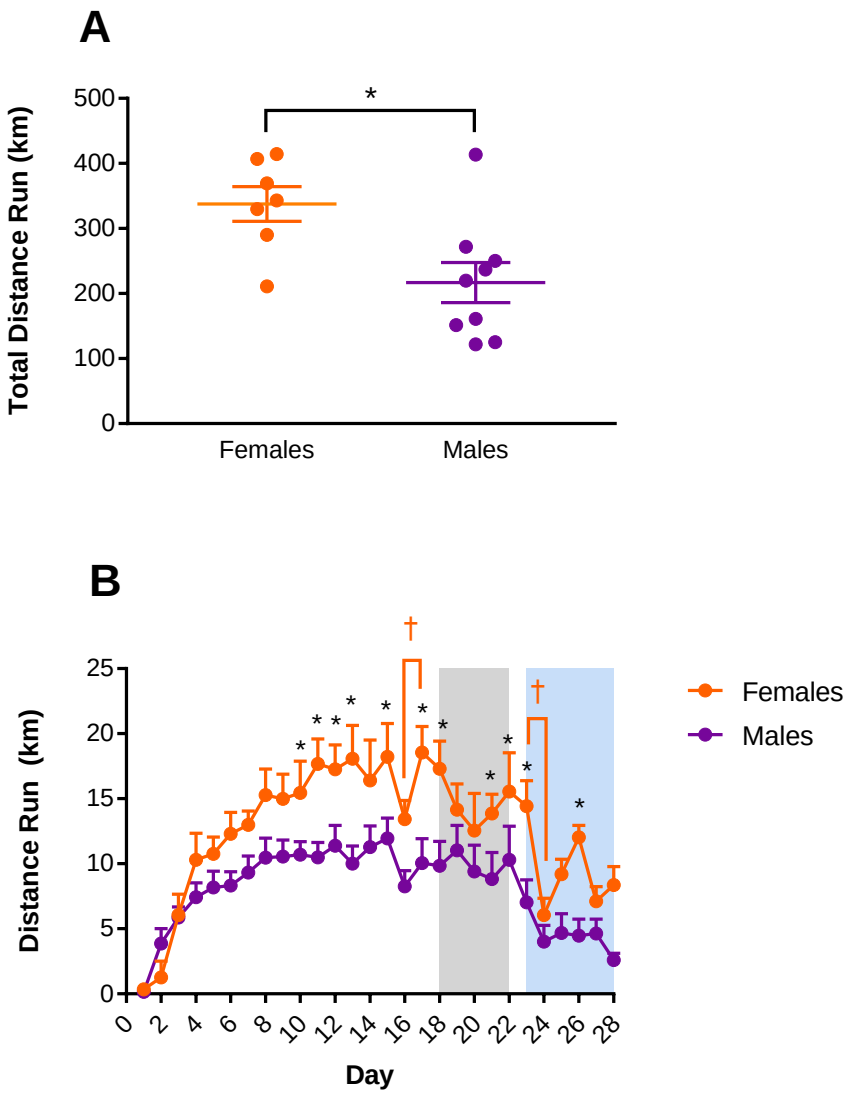

Figure 2. Voluntary running distances. **A** Average total distance run by exercising females (n=7) and exercising males (n=9) over the 28-day period; asterisk denotes significance ($p<0.05$). **B** Average daily distance run by exercising females and exercising males for 28 days. Grey shading shows days when animals were handled prior to behavioural testing. Blue shading shows days of Morris water maze testing. Asterisk denotes a significant difference ($p<0.05$), between females and males on same day. Dagger denotes a significant difference ($p<0.05$) in distance run between consecutive days in animals of the same-sex; the colour of the dagger indicates in which sex it was significant (orange = females, purple = males).

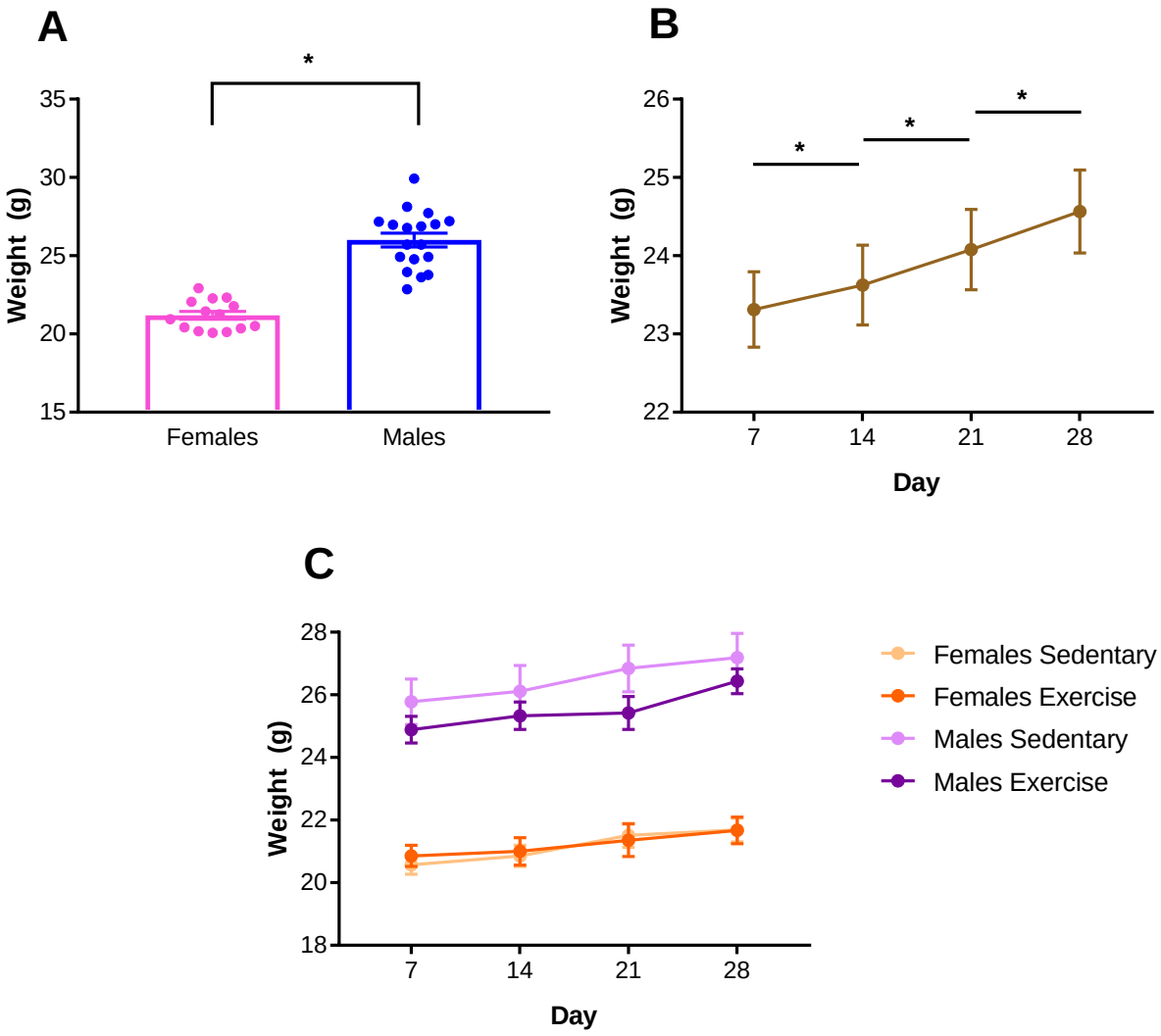

Figure 3. Body mass. **A** There was a significant effect of sex on body mass, with females weighing less than males. **B** There was a significant effect of time on body mass, with significant increases from week to week. **C** There were no significant sex by time, sex by treatment, or sex by time by treatment interactions on body mass. Asterisk denotes significance ($p < 0.05$).

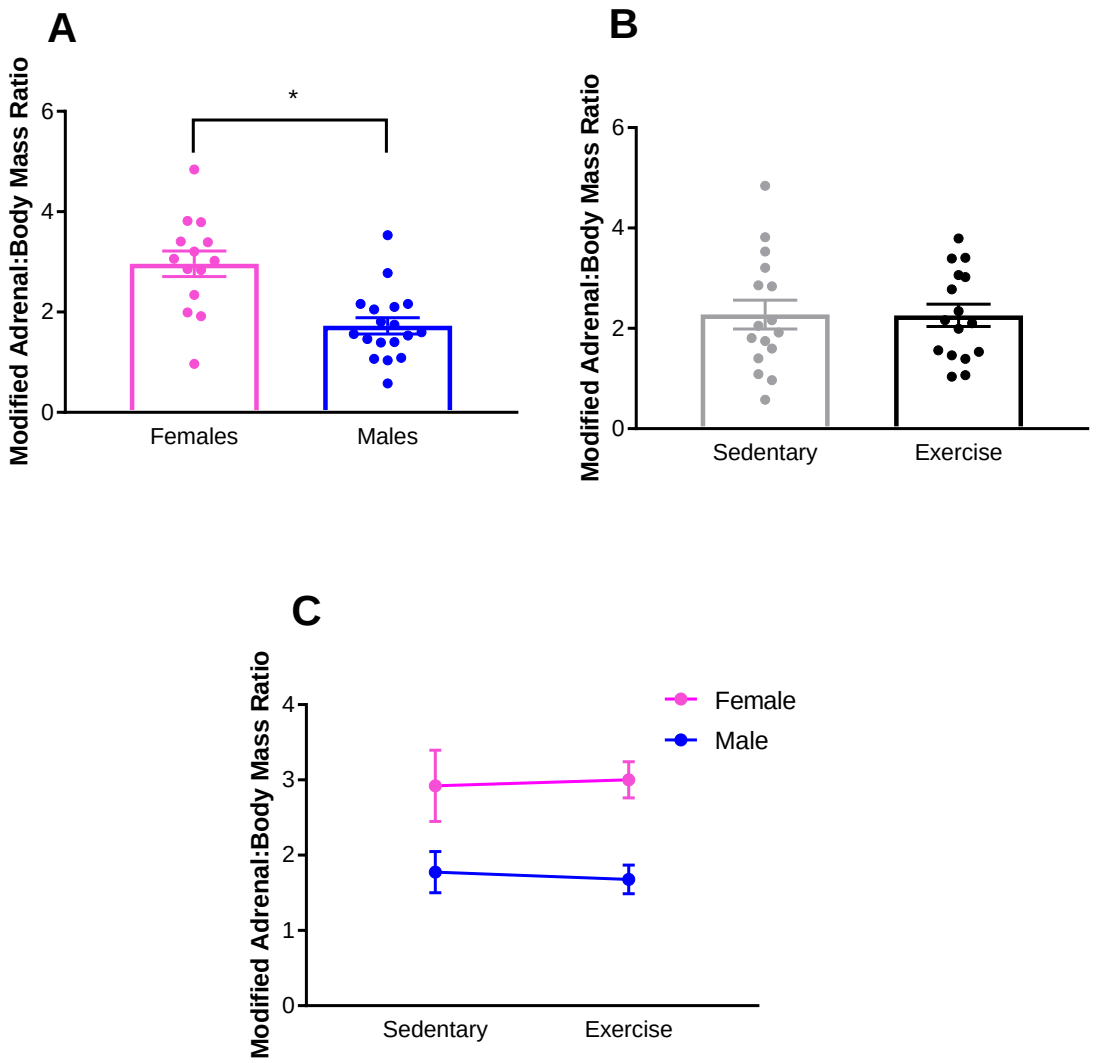

Figure 4. Modified adrenal mass to euthanasia day body mass ratios; raw values were $x10^{-4.}$ **A** Females had a significantly larger ratio than males. **B and C** There was no effect of treatment, nor a sex by treatment interaction on the adrenal to body mass ratios. Asterisk denotes significance ($p<0.05$).

Figure 5. Layout of spatial cues in the Morris water maze room. On the room door, there was another similar sized black rectangle cue with a large white circle in the middle (not shown). The computer and tester were situated in a corner near the door (not shown) throughout all the tests.

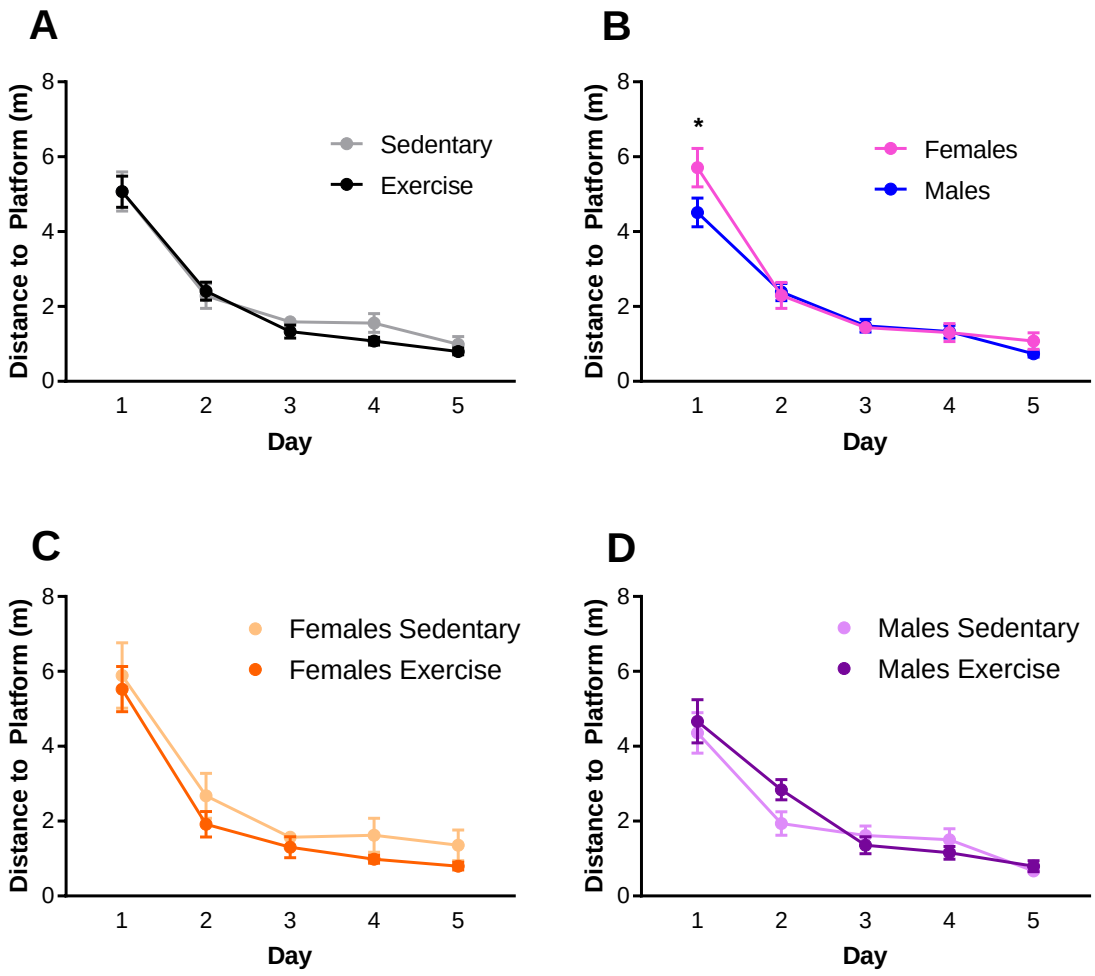

Figure 6. Distance to platform in the Morris water maze during the spatial acquisition phase. Performance during the 6 trials each day were averaged to one representative value for the day. **A** No significant effect of exercise and no significant exercise by day interaction on performance, collapsed across sexes. **B** There was a significant sex by time interaction, collapsed across treatment groups. Significant difference only occured on day 1 of testing, with males swimming less distance to the platform. **D and E** No significant exercise by time interaction for either females or males. Asterisk denotes significance ($p<0.05$).

Table 1. Group means for distance to platform (m) during the spatial acquisition task in each trial. Standard error of the mean given in parentheses. Red indicates a significant difference of $p<0.05$ compared to the sedentary same-sex counterpart group.

		Females		Males	
	Trial	**Sedentary**	**Exercise**	**Sedentary**	**Exercise**
Day 1	1	8.45 (1.71)	8.97 (0.95)	8.47 (1.11)	5.99 (1.06)
	2	4.57 (1.46)	6.22 (1.77)	5.32 (1.36)	5.85 (1.27)
	3	4.93 (1.89)	4.61 (0.88)	3.98 (1.14)	3.96 (0.70)
	4	6.15 (1.76)	6.87 (0.93)	4.64 (1.28)	5.26 (1.22)
	5	5.80 (1.91)	4.63 (1.16)	1.77 (0.59)	3.13 (0.73)
	6	5.46 (1.14)	1.85 (0.65)	1.95 (0.30)	3.80 (0.96)
Day 2	1	3.25 (1.51)	3.26 (1.06)	2.30 (0.97)	3.41 (0.71)
	2	5.11 (1.35)	3.10 (1.50)	4.20 (1.43)	3.97 (1.21)
	3	3.36 (1.23)	2.80 (0.66)	1.66 (0.46)	2.25 (0.64)
	4	0.91 (0.53)	0.47 (0.15)	0.97 (0.38)	5.23 (1.55)
	5	2.62 (0.70)	1.48 (0.25)	2.16 (0.50)	1.51 (0.32)
	6	1.27 (0.67)	0.40 (0.06)	0.33 (0.05)	0.66 (0.15)
Day 3	1	0.98 (0.39)	2.16 (1.21)	1.24 (0.60)	1.30 (0.31)
	2	1.72 (0.58)	1.19 (0.28)	1.23 (0.35)	1.61 (0.54)
	3	1.58 (0.34)	1.44 (0.39)	2.61 (0.59)	2.43 (1.07)
	4	2.87 (0.80)	1.16 (0.36)	2.32 (0.56)	1.29 (0.24)
	5	0.83 (0.31)	1.10 (0.33)	0.90 (0.31)	0.57 (0.15)
	6	1.42 (0.70)	0.76 (0.15)	1.38 (0.33)	0.95 (0.23)
Day 4	1	1.03 (0.15)	0.92 (0.19)	2.12 (0.61)	1.05 (0.25)
	2	1.18 (0.19)	1.12 (0.37)	2.55 (0.87)	1.76 (0.57)
	3	1.81 (0.73)	1.00 (0.19)	1.02 (0.19)	0.95 (0.18)
	4	0.53 (0.19)	0.86 (0.42)	0.60 (0.28)	0.39 (0.13)
	5	2.51 (0.77)	1.12 (0.27)	1.58 (0.53)	1.29 (0.32)
	6	2.68 (1.29)	0.88 (0.19)	1.15 (0.25)	1.56 (0.44)
Day 5	1	2.35 (1.26)	0.84 (0.09)	0.66 (0.09)	1.34 (0.49)
	2	1.91 (0.71)	0.66 (0.06)	1.05 (0.24)	0.84 (0.11)
	3	2.08 (1.15)	0.89 (0.30)	0.87 (0.40)	0.94 (0.20)
	4	0.24 (0.07)	0.49 (0.14)	0.32 (0.09)	0.64 (0.39)
	5	0.84 (0.13)	1.08 (0.23)	0.76 (0.10)	0.78 (0.25)
	6	0.72 (0.32)	0.84 (0.18)	0.36 (0.09)	0.25 (0.05)

Spatial memory: All mice spent more time in the quadrant that previously held the hidden platform

There was a significant effect of quadrant during the probe trial ($F(3,78) = 58.553$, $p<0.01$) (see Figure 7A for schematic of quadrants). Animals from all groups traveled a significantly greater

distance in the NE (target) quadrant than any of the other quadrants ($p<0.01$ compared to NW, SW, and SE; Figure 7B). There were no significant interactions of sex by quadrant ($F(3,78) = 0.491$, $p = 0.69$), exercise by quadrant ($F(3,78) = 0.276$, $p = 0.84$), or sex by exercise by quadrant ($F(3,78) = 0.910$, $p = 0.44$) (data not shown).

Looking only at the platform zone ('the target zone', see Figure 7C for schematic) in the first 30 seconds of the probe trial, there was also no significant sex by exercise interaction ($F(1, 26)$ $p = 0.33$) (Figure 7D). There was also no significant difference in distance traveled in the target zone between sexes ($F(1, 26) = 2.249$, $p = 0.15$) (Figure 7E) or exercise groups ($F(1, 26) = 0.044$, $p = 0.84$) (Figure 7F).

All mice learned the reversal task and performed the visual platform task

All animals learned to find the platform in the new location over the course of 8 trials; there was a significant effect of time (trials) ($F(7,182) = 11.423$, $p < 0.01$), with a significance drop in distance to platform only seen between trial 1 and trial 2 ($p<0.01$). There were no other main or interaction effects on reversal learning (all $p>0.05$) (Figure 8A-D). However, there was a trend for an exercise by sex by time interaction ($F(7,182) = 1.795$, $p = 0.09$), but this was driven by a significant difference between the sedentary males and females only on the first reversal learning trial ($p < 0.01$) (Figures 8E and 8F). All animals swam similar distances to reach the visual platform ($p>0.05$) (Figure 9).

<u>Female mice did not differ in the number of estrous cycles with exercise</u>

Baseline estrous cycling was not determined prior to the exercise intervention, and thus a within-animal comparison could not be made to determine how exercise affected the estrous cycle. There was no effect of exercise on the number of estrous cycles observed during the last 9 days of the exercise intervention ($X^2(3) = 1.333$, $p = 0.72$) (Figure 10).

<u>Exercise increased the number of DCX+ cells in the hippocampus</u>

Exercise increased the number of DCX+ cells in the hippocampus (main effect of exercise: $F(1,28) = 32.843$, $p < 0.01$:Figures 11A and 11B), seen in both females and males (Figure 11E). There was a significant effect of region on the number of DCX+ cells, with the dorsal region having significantly greater number of DCX+ cells than the ventral region ($F(1,28) = 23.623$, $p<0.01$) (Figure 11D). However, there were no other significant main or interaction effects ($p =$

0.80 for exercise by sex interaction, p = 0.35 for region by sex interaction, p = 0.89 for exercise by region interaction, p = 0.12 for exercise by sex by region interaction) (Figure 11E).

Exercise increased the proportion of Type 3 DCX+ cells in the hippocampus

There was a significant exercise by cell type interaction ($p < 0.01$), with exercise mice having a lower percentage of Type 1 cells ($p < 0.01$), and a higher percentage of Type 3 cells ($p < 0.01$) than the sedentary animals, but no significant difference in Type 2 cells ($p = 0.23$) (Figure 12)). After converting the percentages to approximate density, there was still a significant exercise by cell type interaction ($p=0.01$), but the significant difference in Type 1 cells was no longer observed ($p = 0.62$) (Figure 12). Instead, there was a significant difference in the density of Type 2 cells between exercise and sedentary animals ($p = 0.02$), with exercise animals having a greater density than sedentary animals. Exercise animals also had a significantly greater density of Type 3 cells than the sedentary animals ($p < 0.01$) (Figure 12).

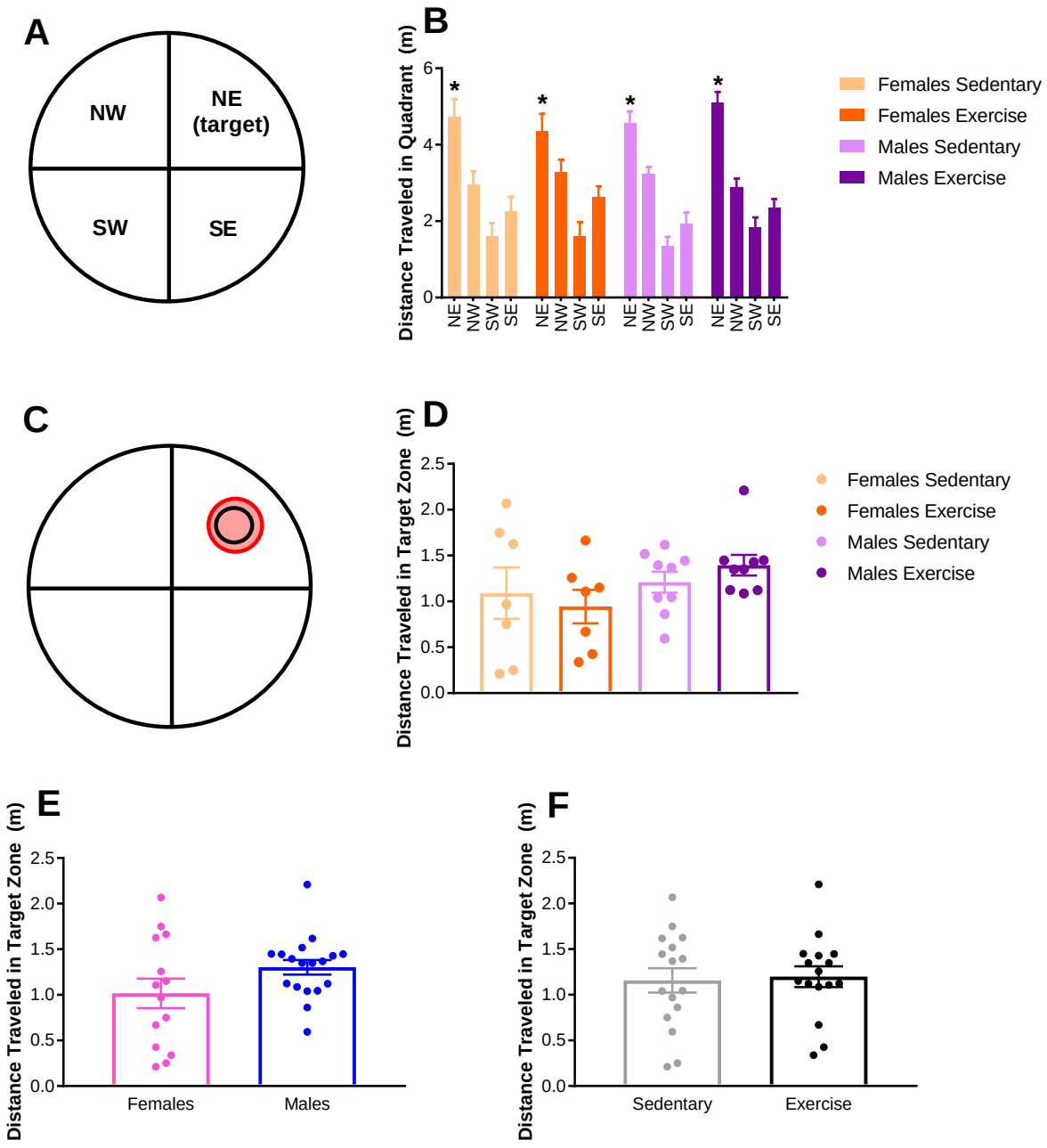

Figure 7. Distance traveled in the target quadrant and target zone during the probe trial. **A** Schematic of pool; target quadrant comprises 25% of total pool area. **B** All groups showed a significant preference for the target quadrant compared to any other quadrant. **C** Schematic of pool; target zone comprises 15% of the total pool area. **D** Looking at specifically the target zone in the first 30 seconds, there was no significant difference between groups. **E and F** There was also no significant main effect of sex or exercise on performance. Asterisk denotes significance ($p<0.05$).

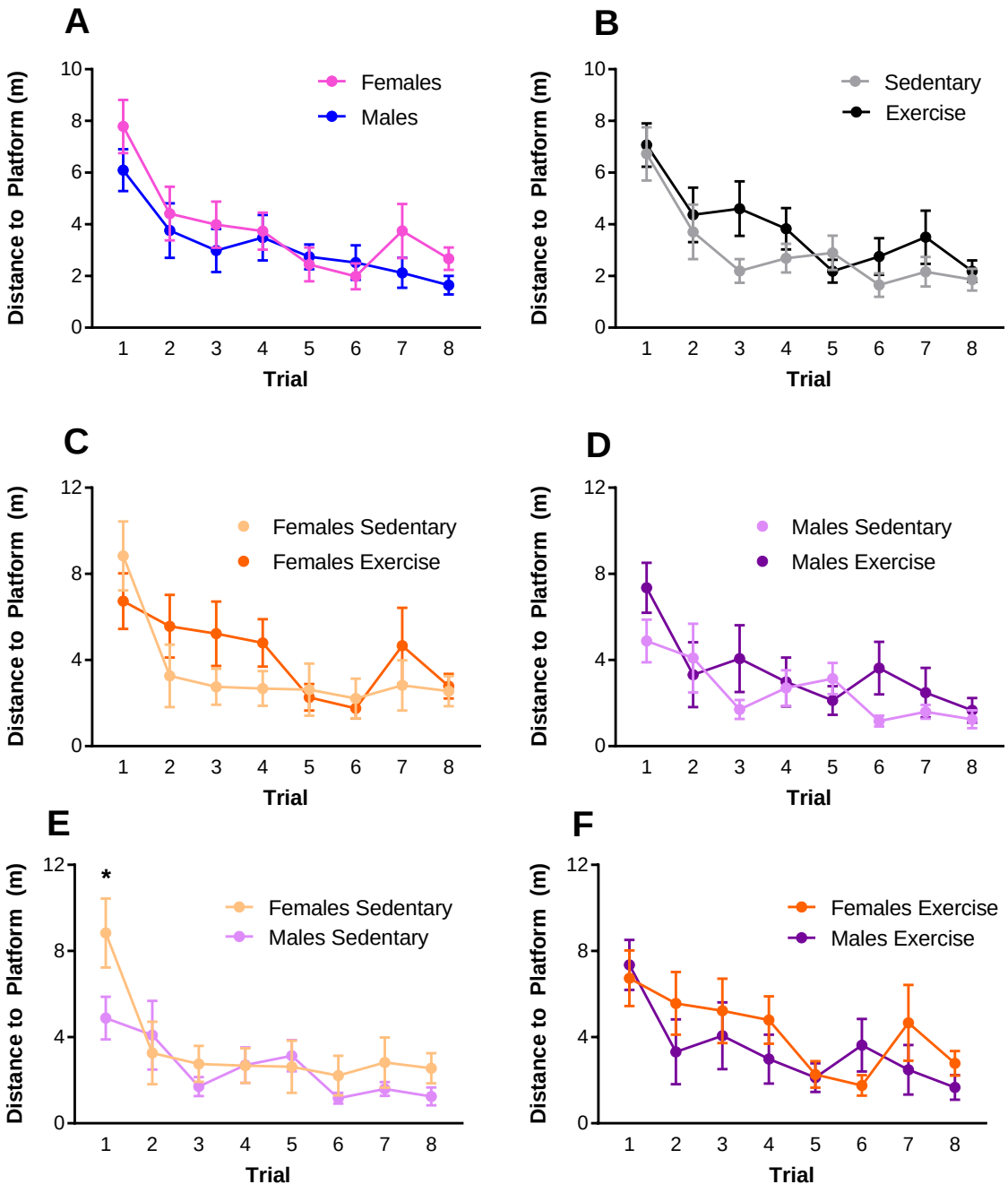

Figure 8. Distance to platform in the Morris water maze during the reversal learning stage. **A** There was no significant time by sex interaction. **B** There was also no significant exercise by time interaction. **C and D** There was no significant time by exercise interaction for either females or males. **E and F** There was a trend for an exercise by time by sex interaction, driven by a significant difference between the sedentary females and males on trial 1. Asterisk indicates significance ($p<0.05$).

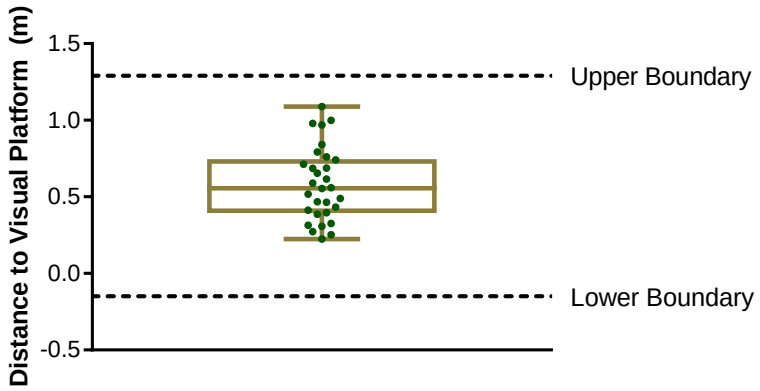

Figure 9. Visual platform control test. There were no outliers in the visual platform control test. Each green dot represents the average distance traveled to the platform across two visual control trials for each animal. The middle line of the box is the median value (0.557), the upper and lower boundaries of the box are Q3 (0.391) and Q1 (0.751), respectively. The whiskers extend to the maximum (1.089) and minimum (0.224) observed values. The upper and lower boundaries drawn on the graph indicate Q3 + (IQR*1.5) and Q1 - (IQR*1.5), respectively. No data points were observed beyond these boundaries.

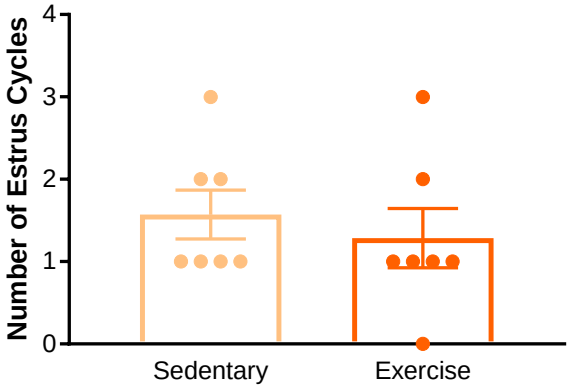

Figure 10. Number of estrous cycles during the last 9 days of the study. There was no significant difference in number of cycles between the sedentary and exercise groups (p>0.05).

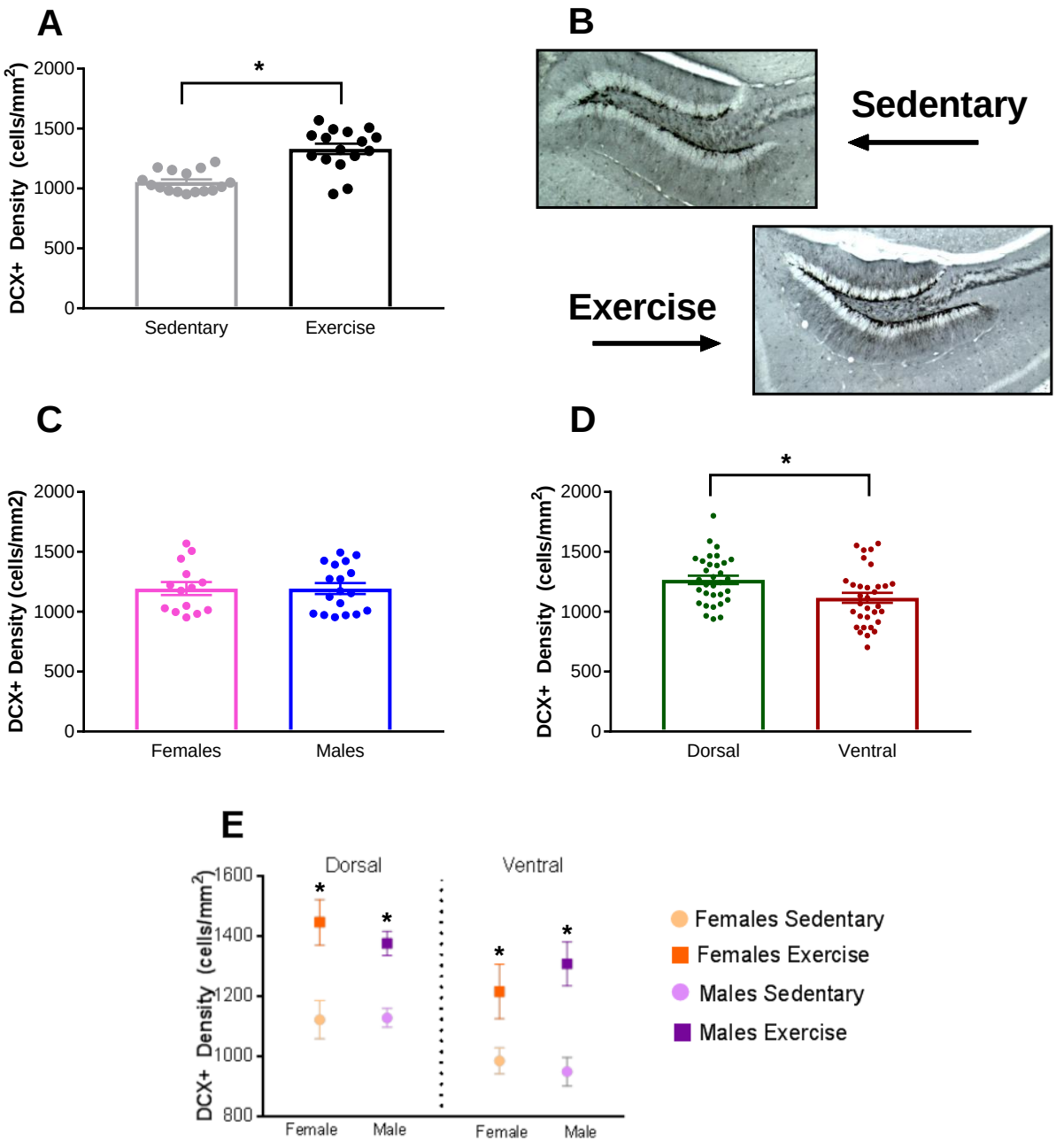

Figure 11. DCX+ density in the hippocampus. **A and B** Exercise increased DCX+ density in the hippocampus in both females and males. **C** Collapsed across treatment, females and males did not differ in hippocampal DCX+ density. **D** The dorsal hippocampus had significantly greater DCX+ density than the ventral hippocampus, but **E** There was no interaction between region (dorsal, ventral) and treatment or sex. Asterisk indicates significance ($p < 0.05$).

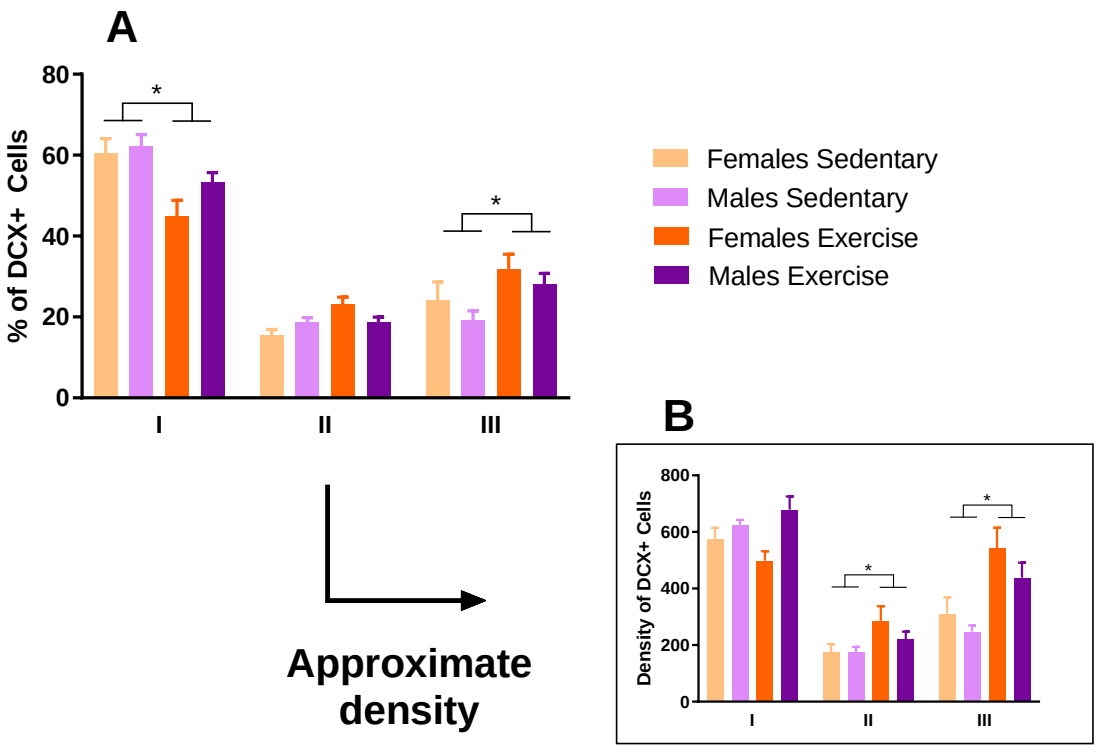

Figure 12. Exercise significantly increases Type 3 DCX+ cells. (**A**) Exercise increases the ratio of Type 3 to Type 1 DCX+ cells. (**B**) After converting percentages to approximate density, the significant effect of exercise on Type 1 cells was no longer observed. However, the density of Type 2 and Type 3 cells were significantly greater in exercise animals compared to sedentary animals. Asterisks indicate significance ($p<0.05$).

<u>There was a trend for running amount to be positively correlated with dorsal Type 3 DCX+ cells in females, but not males</u>

There was no significant correlation between DCX+ density and running amount in either sex in the dorsal hippocampus (females: $r = 0.41$, $p = 0.36$; males: $r = -0.04$, $p = 0.92$ (Figure 13A), nor in the ventral hippocampus (females: $r = 0.53$, $p = -0.29$; males: $r = -0.11$, $p = 0.78$ (Figure 13B). There was no significant correlation between dorsal Type 1 DCX cell density and running amount for either sex (females: $r = -0.17$, $p = 0.71$; males: $r = -0.04$, $p = 0.92$) (Figure 14A). While there was no significant correlation between dorsal Type 3 DCX, there was a trend for greater total running to be positively correlated with increased dorsal Type 3 DCX cell density for females ($r = 0.73$, $p = 0.06$), but not males ($r = -0.34$, $p = 0.37$) (Figure 14B). In the ventral hippocampus, females showed a non-significant trend of inverse correlation between Type 1 DCX cells and total running amount ($r = -0.75$, $p = 0.05$) (Figure 14C). No other significant

correlation between Type 1 or Type 3 cells and total running amount was observed for either males or females in the ventral hippocampus (females: r = 0.02, p = 0.97; males: r = 0.12, p = 0.97) (Figure 14D).

Correlations with Probe Trial Performance

Probe trial performance was not significantly correlated with total distance run for either females or males (females: r = -0.13, p = 0.79; males: r = -0.29, p = 0.49) (Figure 15A). Probe trial performance was significantly positively correlated with dorsal DCX+ cell density in males (r = 59; p = 0.02), but no correlation was observed in females (r = -0.11; p = 0.70) (Figure 15B). There was a significant positive correlation between dorsal Type 1 DCX cells and performance in the probe trial for males (r = 0.54, p = 0.03), but not females (r = 0.12, p = 0.70) (Figure 15C) There was no significant correlation between probe trial performance and the density of Type 3 DCX cells in the dorsal hippocampus for either females (r = -0.08, p = 0.80) or males (r = 0.17, p= 0.54) (Figure 15D).

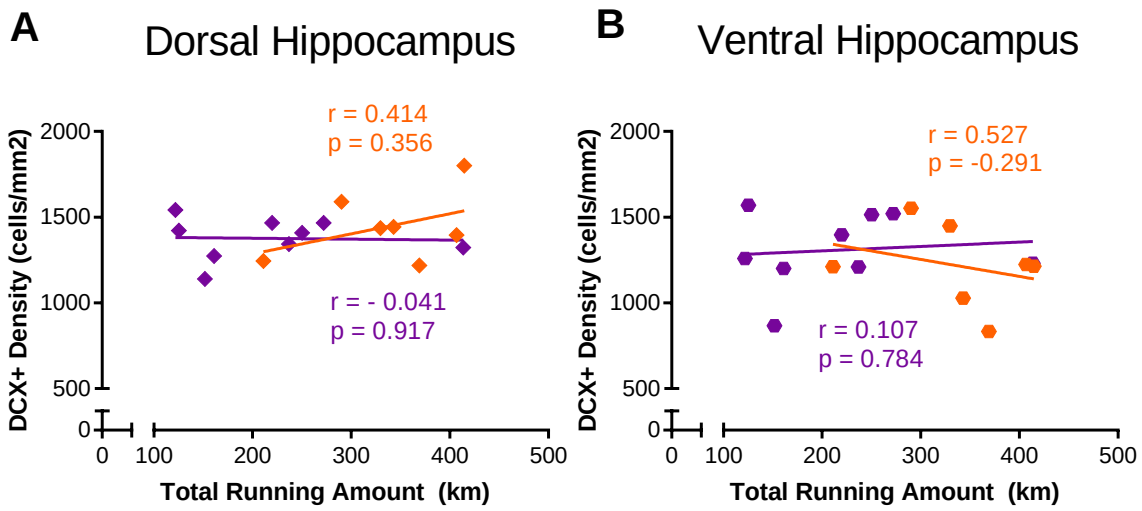

Figure 13. Correlations between total running amount and DCX+ cell density in the dorsal and ventral hippocampus. Orange represents females, purple represents males. **A** There was no significant correlation between running amount and DCX+ density in the dorsal region for either females or males. **B** There was also no significant correlation between running amount and DCX+ density in the ventral region for either females or males.

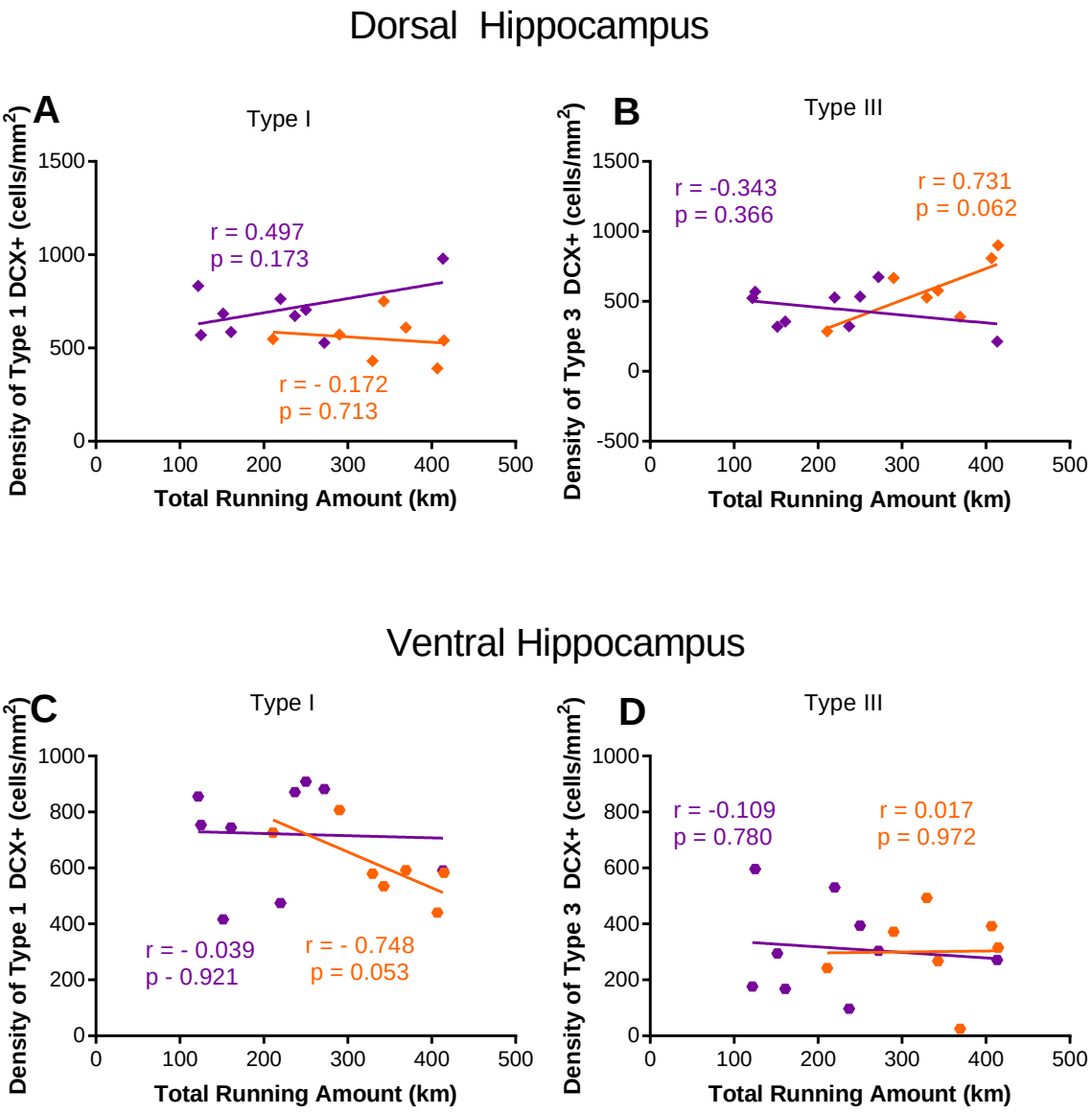

Figure 14. Correlations between total running distance and Type 1 or Type 3 DCX cell density in the dorsal and ventral hippocampus of females (orange) and males (purple). **A and B** There was a trend for increased running to be correlated with a greater density of Type 3 DCX cells in the dorsal hippocampus for females, but not males. No other trends or correlations were observed in the dorsal hippocampus for either sex. **C and D** There was a trend for increased running to be correlated with a decreased density of Type 1 DCX cells in the ventral hippocampus of females. No other trends or correlations were observed in the ventral hippocampus for either sex.

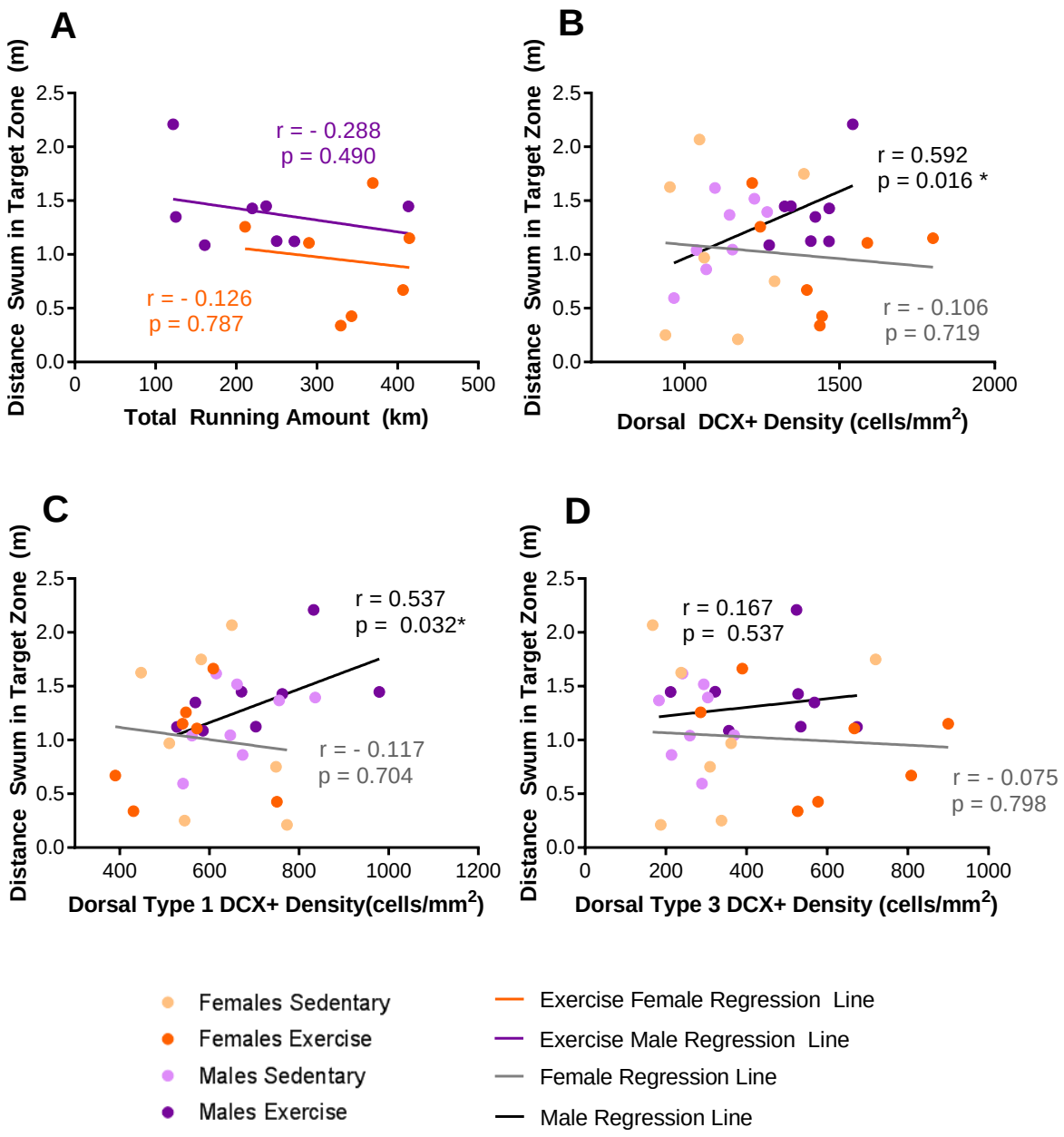

Figure 15. Correlations between probe trial performance and: total running amount (**A**), dorsal DCX density (**B**), dorsal Type 1 DCX+ cell density (**C**) and dorsal Type 3 DCX+ density (**D**). Probe trial performance was positively correlated with dorsal DCX+ density (**B**) and specifically dorsal Type 1 DCX+ density (**C**) in males, but not females. No other significant correlation was observed. Asterisk indicates significance ($p<0.05$).

Discussion

In this thesis project, I looked at sex-differences in the response of C57BL/6 mice to a 28 day running intervention. Specifically, I looked at the effect of the exercise intervention on neurogenesis, spatial learning and memory, body mass, and adrenal to body mass ratios (as a measure of stress). I found that females ran significantly more than males and that females showed a greater disturbance in running behaviour with the start of Morris water maze testing. 28 days of aerobic exercise significant increased neurogenesis in both the dorsal and ventral hippocampus of both sexes and all groups showed intact hippocampal-dependent learning and memory as measured in the Morris water maze. There was no significant effect of exercise or sex on spatial acquisition. All groups showed a significant preference for the target quadrant during the probe reference memory trial, and also showed the ability to learn a new platform location during the reversal learning stage. These findings will be described in further depth below, beginning with sex-differences in running behaviour and its resultant effects on body mass, followed by a discussion on the effect of exercise on neurogenesis, and finally a discussion regarding the role of neurogenesis in spatial learning and memory, with a focus on the role of the dorsal hippocampus.

In the current study, females ran a significantly greater distance over a 28-day period than males, a finding that is consistent with the literature (Wang et al., 1925; Hitchcock, 1925, Lightfoot et al., 2004; Clark et al., 2008). Wang et al. (1925) found that female rats ran an average of 6000-12,000 revolutions per day, while male rats only ran an average of 2000-8000 revolutions per day. Hitchcock (1925) also found that male rats ran on average only 56% of the distance traveled by female rats, a sex difference also seen in several strains of mice (Lightfoot et al., 2004; Clark et al., 2008). Interestingly, Lightfoot et al. (2004) showed that while females ran significantly farther than males, males spent an average of 15% more time running indicating that females ran at a significantly higher velocity. While there was a significant correlation between body mass and velocity for females, body mass exhibited a poor predictive fit for velocity (r^2=0.09), suggesting that body mass alone does not explain the sex-differences in running velocity. In the current study, females weighed significantly less than males throughout the entire duration of the study, though both males and females gained weight over the 28 days. Unfortunately, the wheel trackers used in this study did not measure velocity or time spent running, so it is not possible to make any direct comparisons to those findings, but it is possible that a similar phenomenon occurred in this current study as well.

In addition to running significantly greater distances than males, females also showed a greater disturbance in running behaviour at the start of Morris water maze testing. Running activity decreased in both females and males at the start of the water maze testing, but this drop was only significant in females. Clark et al. (2008) also showed that running significantly decreased with the start of behavioural testing, but sex differences were not mentioned. The specific mechanism of what drives the reduction in running behaviour following the start of behavioural testing has not been explicitly studied but is likely related to increased energy expenditure with swimming and thus a decreased drive for running in an attempt to maintain energy homeostasis. That Clark et al. (2008) did not find a sex-difference in the reduction of running following the start of water maze testing, while I did, could be potentially attributed to the fact that the females in my study was also subject to daily vaginal lavage, a potential stressor that could have interacted with the stress of water maze testing and resulted in a decreased desire for running. This hypothesis could be studied in future studies by including a control female group that is not subject to vaginal lavage. If the vaginal lavage stress exacerbates any other stress and thus decreases running behaviour, then it would be expected that this non-lavaged group would not show a significantly different drop in running distance compared to the male group.

Despite running greater distances than males, the exercise intervention had no effect on body mass for females. Previous research has shown that due to higher levels of estrogens, females have a greater percentage of body fat compared to males of the same mass. Interestingly, several studies have shown that during aerobic exercise, females preferentially oxidize fat for energy which would perhaps suggest that females should show a greater body mass reduction with exercise (Wu and O'Sullivan, 2011). However, during non-exercise periods, the female body reverts to a state of low fat oxidation in an effort to maintain and promote fat storage, an adaptation likely required to ensure sufficient energy during periods of pregnancy (Wu and O'Sullivan, 2011). Therefore, although females show a greater preference for fat use during exercise than males, this does not result in decreased body mass overall as the female body reverts to a preferentially fat-storing state during non-exercise periods. For males, the exercise group showed a trend towards lower body mass than the sedentary group, but this difference was apparent from the first time the animals were weighed, 7 days after the start of the exercise intervention. Because the animals were not weighed before the exercise intervention began, it is difficult to determine whether the exercise intervention caused a lower body mass in the exercise group, or whether this was simply a difference at baseline. Future studies should weigh

the animals before the exercise intervention and ensure that the animals of different body masses are distributed equally across both groups.

The findings that females run greater distances than males are important for interpreting voluntary aerobic exercise studies. Some studies have shown that neurogenesis is correlated with total distance run (Allen et al., 2001; Rhodes et al., 2003 in control mice). If females run more than males, it might be expected that females would show more neurogenesis than males simply because of having traveled greater distances, and not necessarily because of any physiological sex-differences in response to exercise. This relationship was not observed in the current study. Although females ran significantly more than males, both sexes showed similar increases in DCX+ cell density in the dentate gyrus. One possible explanation for this finding is that exercise may have hormetic effects on the brain, where more running beyond a certain point no longer leads to further neurogenesis. There is some evidence to support this explanation (Gradari et al., 2016). In a voluntary exercise intervention conducted on male mice, Kronenberg et al. (2006) found that the effects of exercise on hippocampal proliferation followed an inverted U-shaped curve. While short-term (3-10 days of running) increased proliferation in the dentate gyrus, these proliferative effects were no longer evident in male mice that had exercised for 32 days. The effects on immature neuron survival (as measured by DCX+ cells) showed a more gradual, but similar pattern. Mice that had exercised for 32 days showed significantly elevated DCX+ cells, but mice that underwent prolonged exercise (3-6 months) no longer showed sustained DCX+ elevation compared to non-runners. Further supporting this explanation, Rhodes et al. (2003) found that mice bred for high levels of running did not show higher levels of neurogenesis compared to wild-type (WT) C57BL6/J mice, despite running twice as much as WT mice. These findings suggest that while aerobic exercise increases neurogenesis, there seems to be a threshold beyond which further neurogenesis no longer increases despite further increases or continued higher-than-baseline levels of activity.

In addition to the fact that females consistently run greater distances than males, the second rationale for predicting sex differences in exercise-induced neurogenesis is that steroid hormones are on their own powerful regulators of adult hippocampal neurogenesis in rodents. A detailed review of the effect of sex hormones on neurogenesis is beyond the scope of this thesis but see Mahmoud et al., 2016 and Schoenfeld and Gould, 2012. for a more thorough review. Briefly, estradiol can initially increase proliferation (Tanapat et al., 1999; Barker and Galea, 2008), but repeated estradiol injections suppress new cell survival in females (Barker and

Galea, 2008). On the other hand, androgens have consistently shown to increase new cell survival (Ormerod and Galea, 2003; Spritzer and Galea, 2007).

There has been less research looking at the interaction between sex hormones and exercise in affecting neurogenesis. Though not directly investigating neurogenesis, Berchtold et al. (2001) looked at the interaction between estrogens and exercise in their effect on brain derived neurotrophic factor (BDNF) levels in the hippocampus. BDNF is a neuropeptide trophic factor that exerts its pro-growth and survival effects on the nervous system through the tropomyosin kinase B (trkB) receptor. Previous research has shown there to be a positive correlation between BDNF and neurogenesis (Neeper et al., 1996; Russo-Neustadt et al., 2004; Xu et al., 2004). Berchtold et al. (2001) confirmed past findings that estrogen loss results in decreased activity; OVX females ran significant less than their intact counterparts, though both groups showed increased running over 14 days. While aerobic exercise increased hippocampal BDNF in intact females, this effect was attenuated (though still significant) in females that had experienced short term (3 weeks) ovarian hormone deprivation, but no longer apparent in females that experienced long term (7 weeks) ovarian hormone deprivation. Although it can be argued that the decreased BDNF levels resulted from OVX-induced reductions in activity, it is worth noting that there was no difference in running amount between the short-term and the long-term ovarian hormone deprived mice, and the short-term ovarian hormone deprived mice still showed elevated BDNF levels while the long-term deprived mice did not. Thus, the reduction in BDNF cannot be solely attributed to decreased running. This suggests that exercise interacts with estrogens to increase BDNF in the hippocampus, and that long-term ovarian hormone deprivation makes the hippocampus no longer receptive to the effects of exercise. If using BDNF levels as a predictor of neurogenesis, ovarian hormone loss would have decreased adult hippocampal neurogenesis, a finding that is consistent with the literature (Tanapat et al., 1999; Banasr et al., 2004) and exercise would not have been able to increase neurogenesis in animals that were deprived of ovarian hormones for more than 3 weeks. This can be more explicitly studied in future research by following the same protocol of short term (3 week) and long term (7 week) ovarian hormone deprivation and looking at neurogenesis markers in tandem with BDNF levels. Though this extrapolation from BDNF to neurogenesis based on the research by Berchtold et al., (2001) is speculative, a study by Jin et al (2008) confirms that short-term ovarian hormone deprivation does not render the hippocampus unable to respond to exercise. In their study, OVX-mice underwent a 1-week aerobic exercise intervention after 2 weeks of ovarian hormone-deprivation and still showed exhibited exercise-induced neurogenesis compared to OVX-sedentary mice. These studies (Berchtold et al., 2001; Jin et

al., (2008) point to an interaction between estrogens and exercise that could drive sex-differences in neurogenesis in response to an exercise intervention.

The interaction between androgens and exercise on hippocampal neurogenesis is still a new area of research. Okamato et al. (2012) investigated the role of mild aerobic exercise in its ability to alter brain-derived levels of androgens and its effects on hippocampal neurogenesis. They found that castration reduced testosterone and DHT levels in the plasma and in the hippocampus. Aerobic exercise restored hippocampal, but not serum, DHT levels in both sham and castrated animal. Testosterone levels were not changed by exercise in either the hippocampus or serum. In accordance with the restoration of hippocampal DHT levels, they also found that the mRNA expression of 5α-reductase was increased with exercise in both the sham and castrated groups. AR expression also increased in both exercise groups compared to their sedentary counterparts. With regards to neurogenesis, Okamato et al. (2012) found that running was able to increase both proliferation and survival of new neurons in the hippocampus of intact males. However, if the animals were concurrently injected with the AR antagonist flutamide, running no longer increased survival of new neurons compared to their sedentary counterparts, though the positive effects of running on proliferation were still observed. This finding indicates that in the presence of testosterone, aerobic exercise increases survival of new neurons in the hippocampus through interactions with the AR, much like the survival promoting effects of DHT (Hamson et al., 2013). In a subsequent experiment, Okamato et al. (2012) also found that removal of the testes did not affect the ability of exercise to increase cell proliferation, even when castration was coupled with flutamide injections, indicating that the effects of running on proliferation are likely through an androgen-independent pathway. While castration alone did not prevent the running-induced increase in survival of new neurons, castration coupled with flutamide injections rendered the animal unresponsive to the neurogenesis cell survival effects of exercise. Not only does this finding agree with previous research that androgens are involved in increasing survival, not proliferation, of new hippocampal neurons (Ormerod and Galea, 2003; Spritzer and Galea, 2007), but it also suggests that brain-derived sources of androgens may be contributing to the neurogenic response to exercise.

I hypothesized that there would be a sex-difference in the effect of exercise on neurogenesis, but this hypothesis was rejected. Instead, the null hypothesis that there is no sex difference in the effect of exercise on neurogenesis was accepted. Greater running amounts in females did not translate to greater levels of neurogenesis compared to males. Additionally, females and males have different levels of endogenous sex hormones (both estrogens and androgens) and

although these hormones certainly affect neurogenesis on their own and in their interactions with exercise, these effects leveled out between females and males. One potential confounding factor is that females had a significantly greater adrenal to body mass ratio than males, a finding that has been observed previously (Bielohuby et al., 2007). Given that there was no effect of exercise, nor an exercise by sex interaction on the adrenal to body mass ratios, it is unlikely that the exercise intervention itself increased adrenal mass preferentially in females. Thus, the higher adrenal to body mass ratio in females likely reflects a baseline sex-difference. Adrenal mass can be used as a functional (Rubin et al., 1995) indicator of HPA-axis reactivity as chronic release of corticosterone ultimately leads to adrenal hypertrophy, and others have shown serum corticosterone to be positively correlated with adrenal to body mass ratio (Chan et al., 2014). Since chronic elevations of corticosterone suppress neurogenesis (Brummelte and Galea, 2010), it is possible that corticosterone attenuated potentially higher levels of neurogenesis induced by exercise in females than males. Future studies could explore this possibility by adrenalectomizing both males and females and seeing whether sex differences in the effect of exercise on neurogenesis become apparent in the absence of corticosterone release.

Looking at performance in the Morris water maze, all groups performed equally well on spatial acquisition over 5 days, significantly preferred the target quadrant during the probe trial, and learned the new platform location over 8 trials during the reversal learning stage. However, despite the increased neurogenesis observed in the exercise group, this did not translate to an improvement in spatial learning, memory, or reversal learning compared to the sedentary group. I will discuss this finding considering recent literature looking at the functional significance of neurogenesis and its proposed effects on behaviour.

Since neurogenesis occurs in the dentate gyrus of the hippocampus, a structure that is most known for its crucial involvement in learning and memory, one would predict that more cells would translate to improvements in this cognitive domain. While there have certainly been several studies suggesting a correlational relationship between neurogenesis and certain hippocampal-dependent learning and memory tasks (Kempermann and Gage, 2002 for spatial learning; Drapeau et al., 2003), others have found no correlation between the two (Merrill et al., 2003; Kempermann and Gage, 2002 for probe trial) (see Table 2 at end of discussion for more details). Further still, studies that have tried to elucidate a causal relationship found that impairing neurogenesis sometimes, but not always, leads to impairments in learning and memory (Dupret et al., 2008; Shors et al., 2002; Meshi et al., 2006; Jessberger et al., 2009; Martinez-Canabal et al., 2013) (see Table 3 at end of discussion for more details). In my study,

exercise significantly increased neurogenesis in both females and males to an equal extent. Despite increased neurogenesis with exercise, the exercise and sedentary groups performed equally well on the spatial acquisition, probe trial, and reversal learning tests in the Morris water maze. I will discuss the results of three subtests of the Morris water maze in turn.

In my study, all animals learned to find the hidden platform quickly, with a sharp drop in distance to platform between day 1 and 2, and a more gradual drop between day 3 and 4. A review by Garthe and Kempermann (2013) noted that there have been inconsistencies in determining to what extent spatial acquisition is affected by neurogenesis. For example, some studies have found that impairing neurogenesis does not affect spatial learning performance in the Morris water maze (Shors et al. 2002; Snyder et al. 2005; Meshi et al. 2006; Jessberger et al. 2009; Martinez-Canabal et al., 2013 in adult animals), while others observed worsened spatial learning in the absence of neurogenesis (Dupret et al., 2008; Garthe et al., 2009; Martinez-Canabal et al., 2013 in young animals). The reasons underlying these discrepancies are not yet fully clear, but can likely be attributed to, at least in part, by differences in species, strain, sex, age, behavioural testing paradigms, and neurogenesis depletion protocols (Garthe and Kempermann, 2013). Thus, my findings agree with some, but not all, of the literature surrounding the role of neurogenesis on spatial learning in the Morris water maze.

In my study, there were also no significant differences in performance between groups in the probe trial. Again, the reports on the effects and requirements of neurogenesis on spatial reference memory have been equivocal. Some studies show that animals with impaired neurogenesis perform more poorly than controls (Dupret et al., 2008, Snyder et al., 2005 for longer-term memory; Jessberger et al., 2009) while have shown neurogenesis deficient animals to be unimpaired on probe trial performance (Shors et al. 2002; Snyder et al., 2005 for shorter-term memory; Meshi et al., 2006). One crucial component of Morris water maze testing that must be considered when interpreting results on memory function is the delay between the learning phase and the probe trial. There appears to be converging evidence that hippocampal neurogenesis is required for long-term, but not necessarily short-term reference memory. Shors et al. (2002) showed that impairing neurogenesis did not affect performance in a probe trial conducted 24-hours after the spatial learning phase. Using low levels of irradiation to impair neurogenesis, Snyder et al. (2005) also found that these animals showed normal short term (1 week) memory, and additionally found that their long-term (2 week and 4 week) memory was significantly impaired. Jesseberger et al. (2009) also found that neurogenesis impairment significantly impaired long-term (2, 4, 8 week) memory. Together, these findings suggest that as

the task demands increase (in this case, by increased time between the learning and test phase), the role of hippocampal neurogenesis becomes more important. My experiment tested animals in the probe trial 24 hours after the learning phase and accordingly, there were no differences in performance observed between groups. Future studies could give a second probe trial at a later time point (2-4 weeks after the learning phase) to determine whether the exercise-induced increase in neurogenesis is beneficial for long-term memory.

In my study, all animals underwent a reversal learning task where the hidden platform was moved from the NE quadrant to the SW quadrant and the animals had to learn the new location over 8 trials. All groups were able to learn the new location as shown by a significant decrease in path length across trials. However, there were no differences in performance between groups. The reversal learning task tests both executive function, as it looks at the ability of the animal to learn a new rule while forgetting the old one, and hippocampal-dependent learning as it requires that the animal learn a new spatial cognitive map. Importantly, this task is presumably able to target the new hippocampal cells specifically as it challenges the hippocampus to distinguish between two very similar, yet slightly different, contexts in a process known as pattern separation. Pattern separation is a process that has been attributed to the dentate gyrus initially in computational models (Treves and Rolls, 1994), and more recently in animal studies (Clelland et al., 2009; Sahay et al., 2011). Indeed, the anatomy and the connection of the dentate gyrus within the hippocampus make it an ideal candidate for functioning as a pattern separator (Deng et al., 2010). The entorhinal cortex (EC) receives input from all other cortical areas and relays this information to the granule cells of the dentate gyrus, which then relays this information to the CA3 hippocampal area. Although the EC receives a large amount of information, the DG has 5-10 times more neurons than the EC. This means that inputs with many overlapping features in the EC can still be encoded separately in the DG and thus be conveyed as two distinct inputs for later recall. In the case of the Morris water maze reversal learning trials, much of the task was overlapping with the initial learning phase – the room and cues were identical, and the general task was the same. However, after the first (or first few) trials, the mouse would have to learn to distinguish the location of the platform in the new location from that of the old location, despite all the otherwise identical spatial inputs. Having more neurons in the dentate gyrus would theoretically be helpful for this as it would promote greater pattern separation. In my thesis study, there were no differences across groups in distance to platform for the reversal learning phase, so it is unclear whether the increased neurogenesis improved pattern separation. It is possible that all mice were able to ‘pattern separate’ and learn the new location easily because they were young and healthy with no

cognitive dysfunction. It is possible that neurogenesis would improve performance on spatial learning tasks in older mice who are showing signs of cognitive decline and/or neurogenesis. Indeed, Garthe et al. (2009) found that male mice with impaired neurogenesis were not able to learn a new platform location in the Morris water maze reversal task. Thus, future research could use older mice who naturally show reduced levels of neurogenesis to determine if there are interactions between age, exercise, neurogenesis, sex, and reversal learning. Future research could also administer a second probe trial after the reversal learning task to see whether all the mice could in fact remember the new location after a certain delay, or whether they preserved in remembering the initial location. Granted, this would not necessarily indicate level of hippocampal pattern separation functioning and could also indicate impairment in executive (i.e. set-shifting), but it would provide a more thorough picture of the behavioural effects that occur due to exercise-induced neurogenesis.

In my study, I had hypothesized that dorsal neurogenesis would be correlated with performance in the memory test of the Morris water maze. The hippocampus is a functionally heterogenous structure (Moser and Moser, 1998). An increasing amount of research is shows functional differences between the dorsal and ventral hippocampus in rodents. The dorsal region is particularly important for spatial learning and memory. Lesions of the dorsal hippocampus cause severe impairments on Morris water maze acquisition (Bannerman et al., 1999; Moser et al., 1995; Pothuizen et al., 2004) while ventral lesions do not (Bannerman et al., 1999), unless the ventral lesion is very large (Moser et al., 1995). On the other hand, the ventral hippocampus appears to be more involved in the emotional and stress associated components of memory (Bannerman et al., 1999). Therefore, I hypothesized that neurogenesis in the dorsal region would be correlated with performance on the probe trial in a sex-dependent manner. This hypothesis was accepted as dorsal neurogenesis was correlated with increased performance in the probe trial in males, but not females.

In an exercise study on neurogenesis, Vivar et al. (2016) found that the dorsal hippocampus had a greater number of new cells, irrespective of exercise intervention, than the ventral region, a result that was consistent with my own findings in this thesis project. They also found that one month of voluntary running increased neurogenesis by 3-fold in the dorsal and middle, but not the ventral dentate gyrus. In contrast, my results showed that one month of voluntary running increased neurogenesis in the dorsal and the ventral dentate gyrus in both sexes. This discrepancy may be in part due to the different ways we categorized the hippocampal regions. Vivar et al. (2016) split the hippocampus into three regions – dorsal, middle, and ventral – based

on Bregma coordinates, where the dorsal region was − 1.24 mm to − 2.16 mm from Bregma, the middle region was − 2.36 mm to − 2.96 mm from Bregma, and the ventral region was − 3.16 mm to − 4.12 mm from Bregma. In the case of my study, the hippocampus was distributed into these regions based on slightly different coordinates based on the Mouse Brain Atlas (Paxinos and Franklin, 2001). The dorsal region was − 0.94mm to − 2.70mm from Bregma, the intermediate/middle was − 2.92mm to − 3.40mm from Bregma, and ventral region was − 3.64mm to − 3.88mm from Bregma. Additionally, I grouped the intermediate and ventral sections together to ensure sufficient statistical power, as there were fewer intermediate and ventral sections than dorsal sections. Therefore, that Vivar et al. (2016) did not find that exercise increased neurogenesis in the ventral region while I did, could perhaps be attributed to the fact that I included a comparatively more dorsal region (− 2.92 mm from Bregma vs. – 3.16 mm from Bregma in Vivar et al. (2016)) in the ventral analysis. The inconsistency across the literature in designating specific coordinates for the dorsal and ventral hippocampal regions likely leads to differences in findings. For example, a study by Bednarczyk et al. (2009) found that voluntary exercise increases both proliferation and survival equally across the entire hippocampus (from −1.00mm to −3.50mm from Bregma), while a study by Piatti et al. (2011) found exercise to increase neuronal maturation only in the ventral hippocampus (dorsal: −0.9mm to -1.8mm, intermediate/ventral: −2.7mm to −3.8mm; region between −1.8mm and −2.7mm from Bregma not specified). Studies on the effects of environmental enrichment, which often contain a voluntary exercise component, also showed inconsistent effects. One study showed increased proliferation in both regions (dorsal: − 1.06mm to -2.06mm from Bregma, ventral: −3.08mm to −3.80mm from Bregma) with increased survival only in the dorsal region (Tanti et al., 2012), while another showed increased proliferation and survival in both the dorsal and the ventral hippocampus (Bregma coordinates not specified) (Tashiro et al., 2007). Future studies looking at the effects on exercise on specific regions of the hippocampus should continue to indicate the Bregma coordinates investigated in order to compare and understand potentially discrepancies between findings.

Given the emerging role of dorsal hippocampus in spatial learning and memory, I had hypothesized that there would be a relationship between exercise-induced dorsal neurogenesis and performance in the Morris water maze. Voluntary exercise increased neurogenesis in both the dorsal and the ventral hippocampus, but was not correlated with distance run for either sex. However, the amount of dorsal neurogenesis was significantly correlated with probe trial performance for males, but not females. Specifically, there was a correlation between the density of Type 1 DCX+ cells and probe trial performance in males. Categorization of DCX+ into

three types was introduced by Plumpe et al. (2007). Type 1 DCX+ cells have a round cell body with no (or a very short) process, and this type of cell still has regenerative properties, but no longer expresses the same proteins as the glial-like stem cells (such as nestin or Sox2) Thus, Type 1 DCX+ cells can theoretically be used as a marker of new cell proliferation. Type 2 DCX+ cells have a medium process that may or may not reach the molecular layer of the hippocampus. Type 3 DCX+ cells have a strong process that branches in the molecular layer, but do not yet express NeuN, a marker of mature neurons. The transition from Type 1 through Type 3 DCX+ cells reflects maturation of the cell. That probe trial performance was correlated with Type 1, but not Type 3, DCX+ density in males is a finding that is somewhat unexpected and open to interpretation. In mice, new adult generated neurons are activated by surrounding GABAergic neurons and are not yet integrated into the hippocampal circuit (reviewed in Deng et al., 2010). At two weeks, these cells begin to grow processes with the dendrites growing towards the molecular layer, and the axon growing towards the CA3 region, and receive excitatory GABAergic synaptic input from local interneurons (reviewed in Deng et al., 2010). During this time, the new cells have increased excitability compared to older granule cells. At around 3-4 weeks, the new neurons become integrated into the local hippocampal circuit, making appropriate pre-synaptic connections with EC cells and post-synaptic connections with CA3 hippocampal pyramidal cells (reviewed in Deng et al., 2010). Additionally, a species-comparison study conducted by Snyder et al. (2009) found that mice show continued DCX+ expression for the first 3 weeks after adult neuronal birth, with a significant drop in the expression levels when the new cells reach 4 weeks of age. Combined, these findings suggest that the Type 1 DCX+ cells are not yet involved in the hippocampal circuitry, so it is unclear what relevance they have in behaviour and why expression of these specific cells correlates with probe trial performance for males. Nonetheless, I had hypothesized that dorsal neurogenesis would be correlated with probe trial performance in a sex-dependent manner. This hypothesis was accepted, as dorsal neurogenesis was correlated with improved probe trial reference memory in males, but not females.

My thesis study adds to an ever growing and, often conflicting, body of research on the effects of exercise on neurogenesis and resultant effects on cognition. While my study showed a sex-difference only in the correlation between Type 1 DCX+ cells and spatial memory, it is likely that the Morris water maze protocol in this study was simply not difficult enough to see more subtle effects of exercise on cognition. Therefore, future studies must continue to use both males and females, as the two sexes respond to interventions, stressors, and experimental manipulations differently or with different levels of intensity. Additionally, the functional significance of new

neurons is an area of active research, with much of this literature pointing to a role of these new neurons in pattern separation. Behavioural tasks must be designed in such a way that the new neurons are targeted directly. For example, the Morris water maze is a commonly used test spatial learning and memory in rodents. The level of difficulty depends on the number of days and trials given to learn the task, but also on the nature of the cues around the room. Fewer, larger, and less complex cues make the task easier and the rodents do not have to necessarily employ the new neurons to successfully navigate through the maze. While researchers explicitly state the paradigm of testing (i.e. number of days, trials), the cues surrounding the maze are less often described or visualized. Therefore, future studies should explicitly describe the cues used, and ideally also include a figure showing the setup of the room. This would allow others to more closely replicate the study design and would help minimize the discrepancies observed between studies.

Table 2. Selected studies looking at the correlation between adult hippocampal neurogenesis and spatial learning and memory in the Morris water maze.

Paper	Animals (species, strain, sex, age)	Neurogenesis protocol	Visual platform	Learning Protocol	Memory Protocol	Correlation
Kempermann and Gage, 2002	**Species:** Mice **Strain:** Strain comparison: C57BL/6J, DBA2/J and BXD substrains (2, 5, 6, 8, 12, 18, 19, 25, 28, 29, 30) **Age and Sex:** not mentioned	**Markers:** BrdU, NeuN 10d of BrdU injections started 10d after behavioural tests ended. **Prolif:** 1 day after last injection. **Survival:** 4 weeks after last injection	Yes, on day 6, following learning and probe trial. 4 trials of 40s.	4 days x 6 trials/day, intertrial interval of 10s. Starting points varied daily. Each trial 40s.	24 hr after learning stage - 60 second probe trial.	Correlation between survival (BrdU/NeuN) and learning. No correlation for memory.
Drapeau et al., 2003	**Species:** Rats **Strain:** Sprague-Dawley **Age:** 3 and 20 months **Sex:** Males	**Markers:** BrdU, Ki67, NeuU 5d of BrdU injections started 21d after behavioural tests ended. **Prolif:** BrdU stain 1 day after last injection, Ki67 **Survival:** BrdU stain 3 weeks after last injection, NeuN	Yes, for 2 consecutive days following the acquisition stage.	3 days x 4 trials/day, intertrial interval of 30s. Starting point varied randomly each day. Each trial 90s.	N/A	Correlation between proliferation (BrdU and Ki67) and learning for older mice. No correlation for younger mice.
Merrill et al., 2003	**Species:** Rats **Strain:** Fischer **Age:** 2 and 21 months on arrival **Sex:** Females	**Markers:** BrdU 5d of BrdU injections started immediately after behavioural tests ended. **Survival:** BrdU stain 10d after last injection	Yes, for 2 consecutive days following testing.	**Multiple trial place learning:** 10 days x 2 trials/day, intertrial interval of 30s. Starting points chosen randomly at beginning of test day. Each trial 90s	**Multiple trial place learning:** Probe trial "after day 10 of training" – unclear if immediately after or 24h after. 60s probe trial.	No correlation between cell survival (BrdU) and learning or memory.

Table 3. Selected studies looking at the causal relationship between impairment of neurogenesis and spatial learning and memory in the Morris water maze.

Paper	Animals (species, strain, sex, age)	Method of neurogenesis impairment	Visible platform?	Learning protocol	Memory protocol	Result of neurogenesis impairment on learning and memory
Dupret et al., 2008	**Species:** Mice **Strain:** transgenic, derived from C57BL6/J **Sex:** Males **Age:** 2-3 months	Induction of apoptosis specifically in neural precursor cells using transgenic mice	Yes, on day following probe trial.	8 x 3 trials/day, intertrial interval of 5 min. Each trial 60s.	60s probe trial "upon completion of the training" – unclear if immediately or 24h after (only for hippocampus dependent)	Neurogenesis impaired animals showed impaired learning and no preference for target quadrant (impaired memory)
Shors et al., 2002	**Species:** Rats **Strain:** Sprague-Dawley **Sex:** Males **Age:** 220-250g (adult)	14 days of injections of methylazoxymethanol acetate (MAM), an antimitotic agent	No / not mentioned.	4 days x 4 trials/day, intertrial interval of 60s. Each trial 60s.	60s probe trial 24h after training	Neurogenesis impaired animals showed normal spatial acquisition and probe trial performance (memory) performance
Meshi et al., 2006	**Species:** Mice **Strain:** 129v/Ev **Sex:** Females **Age:** 10 weeks	Low dose irradiation to the head on day 15, 19, 22 (5 gy irradiation per exposure)	Yes, 2 days before spatial learning (2 days x 3 trials/day)	5 days x 3 trials/day, intertrial interval of 45 minutes. Each trial 120s.	Immediately after last training session. 60s probe trial.	Neurogenesis impaired animals showed normal spatial acquisition and probe trial performance.

Paper	Animals (species, strain, sex, age)	Method of neurogenesis impairment	Visible platform?	Learning protocol	Memory protocol	Result of neurogenesis impairment on learning and memory
Jessberger et al., 2009	**Species:** Rats **Strain:** Sprague-Dawley **Sex:** Males **Age:** 7-8 weeks	Intrahippocampal injections of WNT knockdown vector	Somewhat. Only if the animal could not find hidden platform during spatial acquisition.	7 days x 4 trials/day, but each day also began with a single probe trial. Intertrial interval not specified. 60 seconds hidden platform and then if not sound, 60 seconds visual platform.	60s probe after 2, 4, and 8 week delay.	Neurogenesis impaired animals showed normal spatial acquisition. Animals with high levels of WNT knockdown showed impairments in all probe trials compared to low-knockdown and controls.
Martinez-Canabal et al., 2013	**Species:** Mice **Strain:** 129Svev x C57BL/6 cross **Sex:** Not mentioned **Age:** 1 month (juvenile), 2 month (adult), 11 month (middle-aged)	Injections of temozolomide, an anti-mitotic agent that leads to apoptosis of diving cells.	No / not mentioned.	6 days x 3 trials/day. Intertrial interval not specified. Each trial 60s.	Several probe trials – before training on days 1, 3, 5 of spatial acquisition. Final probe trial on day 7, 24h after last learning trial.	All groups learned the location of hidden platform, but significant effect of treatment. The effect of TMZ on learning impairment was more pronounced in juvenile than adult or middle-age.
Snyder et al., 2005	**Species:** Rats **Strain:** Long Evans **Sex:** Males **Age:** 40 days old	2 consecutive days of 10 Gy gamma irradiation to the head	No / not mentioned.	6 days x 8 trials/day. Intertrial interval of 5 min. Each trial 60s.	30s probe trials at 1, 2, 4 weeks after training.	Animals with impaired neurogenesis showed normal spatial acquisition. Animals with impaired neurogenesis showed normal probe trial performance at 1 week post training, but impaired memory at 2 and 4 weeks post training.

Paper	Animals (species, strain, sex, age)	Method of neurogenesis impairment	Visible platform?	Learning protocol	Memory protocol	Result of neurogenesis impairment on learning and memory
Garthe et al., 2009	**Species:** Mice **Strain:** C57BL/6 **Sex:** Females **Age:** 6-8 week old	Temozolomide injections for 3 consecutive days/week for 4 weeks.	No / not mentioned.	3 days x 6 trials/day. Intertrial interval of 30 min. Each trial 120s.	Probe trial, but not explicitly stated how long after last training session	Neurogenesis impaired animals eventually learned platform location, but slower than controls. Neurogenesis impaired animals showed intact memory during the probe trial.

References

Allen, D. M., van Praag, H., Ray, J., Weaver, Z., Winrow, C. J., Carter, T. A., ... & Gage, F. H. (2001). Ataxia telangiectasia mutated is essential during adult neurogenesis. *Genes & development*, *15*(5), 554-566.

Baker, L. D., Frank, L. L., Foster-Schubert, K., Green, P. S., Wilkinson, C. W., McTiernan, A., ... & Duncan, G. E. (2010). Effects of aerobic exercise on mild cognitive impairment: a controlled trial. *Archives of neurology*, *67*(1), 71-79.

Banasr, M., Hery, M., Brezun, J. M., & Daszuta, A. (2001). Serotonin mediates oestrogen stimulation of cell proliferation in the adult dentate gyrus. *European Journal of Neuroscience*, *14*(9), 1417-1424.

Bannerman, D. M., Yee, B. K., Good, M. A., Heupel, M. J., Iversen, S. D., & Rawlins, J. N. P. (1999). Double dissociation of function within the hippocampus: a comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. *Behavioral neuroscience*, *113*(6), 1170.

Barha, C. K., Davis, J. C., Falck, R. S., Nagamatsu, L. S., & Liu-Ambrose, T. (2017a). Sex differences in exercise efficacy to improve cognition: A systematic review and meta-analysis of randomized controlled trials in older humans. *Frontiers in neuroendocrinology*.

Barha, C. K., Falck, R. S., Davis, J. C., Nagamatsu, L. S., & Liu-Ambrose, T. (2017b). Sex differences in aerobic exercise efficacy to improve cognition: a systematic review and meta-analysis of studies in older rodents. *Frontiers in Neuroendocrinology.*

Barker, J. M., & Galea, L. A. M. (2008). Repeated estradiol administration alters different aspects of neurogenesis and cell death in the hippocampus of female, but not male, rats. *Neuroscience*, *152*(4), 888-902.

Bednarczyk, M. R., Aumont, A., Décary, S., Bergeron, R., & Fernandes, K. J. (2009). Prolonged voluntary wheel-running stimulates neural precursors in the hippocampus and forebrain of adult CD1 mice. *Hippocampus*, *19*(10), 913-927.

Berchtold, N. C., Kesslak, J. P., Pike, C. J., Adlard, P. A., & Cotman, C. W. (2001). Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. *European Journal of Neuroscience*, *14*(12), 1992-2002.

Bielohuby, M., Herbach, N., Wanke, R., Maser-Gluth, C., Beuschlein, F., Wolf, E., & Hoeflich, A. (2007). Growth analysis of the mouse adrenal gland from weaning to adulthood: time-and gender-dependent alterations of cell size and number in the cortical compartment. *American Journal of Physiology-Endocrinology and Metabolism*, *293*(1), E139-E146.

Brummelte, S., & Galea, L. A. (2010). Chronic high corticosterone reduces neurogenesis in the dentate gyrus of adult male and female rats. *Neuroscience*, *168*(3), 680-690.

Cameron, H. A., & Mckay, R. D. (2001). Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *Journal of Comparative Neurology*, *435*(4), 406-417.

Chan, M., Chow, C., Hamson, D. K., Lieblich, S. E., & Galea, L. A. (2014). Effects of chronic oestradiol, progesterone and medroxyprogesterone acetate on hippocampal neurogenesis and adrenal mass in adult female rats. *Journal of neuroendocrinology*, *26*(6), 386-399.

Clark, P. J., Brzezinska, W. J., Thomas, M. W., Ryzhenko, N. A., Toshkov, S. A., & Rhodes, J. S. (2008). Intact neurogenesis is required for benefits of exercise on spatial memory but not motor performance or contextual fear conditioning in C57BL/6J mice. *Neuroscience*, *155*(4), 1048-1058.

Clelland, C. D., Choi, M., Romberg, C., Clemenson, G. D., Fragniere, A., Tyers, P., ... & Bussey, T. J. (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science*, *325*(5937), 210-213.

Colcombe, S., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychological science*, *14*(2), 125-130.

Cotman, C. W., Berchtold, N. C., & Christie, L. A. (2007). Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends in neurosciences*, *30*(9), 464-472.

Creer, D. J., Romberg, C., Saksida, L. M., van Praag, H., & Bussey, T. J. (2010). Running enhances spatial pattern separation in mice. *Proceedings of the National Academy of Sciences*, *107*(5), 2367-2372.

Deng, W., Aimone, J. B., & Gage, F. H. (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory?. *Nature Reviews Neuroscience*, *11*(5), 339-350.

Drapeau, E., Mayo, W., Aurousseau, C., Le Moal, M., Piazza, P. V., & Abrous, D. N. (2003). Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. *Proceedings of the National Academy of Sciences*, *100*(24), 14385-14390.

Dupret, D., Revest, J. M., Koehl, M., Ichas, F., De Giorgi, F., Costet, P., ... & Piazza, P. V. (2008). Spatial relational memory requires hippocampal adult neurogenesis. *PloS one*, *3*(4), e1959.

Eadie, B. D., Redila, V. A., & Christie, B. R. (2005). Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *Journal of Comparative Neurology*, *486*(1), 39-47.

Erickson, K. I., & Kramer, A. F. (2009). Aerobic exercise effects on cognitive and neural plasticity in older adults. *British journal of sports medicine*, *43*(1), 22-24.

Esteban-Cornejo, I., Tejero-Gonzalez, C. M., Sallis, J. F., & Veiga, O. L. (2015). Physical activity and cognition in adolescents: A systematic review. *Journal of Science and Medicine in Sport*, *18*(5), 534-539.

Fanselow, M. S., & Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures?. *Neuron*, *65*(1), 7-19.

Fischer, T. J., Walker, T. L., Overall, R. W., Brandt, M. D., & Kempermann, G. (2014). Acute effects of wheel running on adult hippocampal precursor cells in mice are not caused by changes in cell cycle length or S phase length. *Frontiers in neuroscience*, *8*.

Garthe, A., Behr, J., & Kempermann, G. (2009). Adult-generated hippocampal neurons allow the flexible use of spatially precise learning strategies. *PloS one*, *4*(5), e5464.

Garthe, A., & Kempermann, G. (2013). An old test for new neurons: refining the Morris water maze to study the functional relevance of adult hippocampal neurogenesis. *Frontiers in neuroscience*, *7*.

Gradari, S., Pallé, A., McGreevy, K. R., Fontán-Lozano, Á., & Trejo, J. L. (2016). Can Exercise Make You Smarter, Happier, and Have More Neurons? A Hormetic Perspective. *Frontiers in neuroscience*, *10*.

Hamson, D. K., Wainwright, S. R., Taylor, J. R., Jones, B. A., Watson, N. V., & Galea, L. A. M. (2013). Androgens increase survival of adult-born neurons in the dentate gyrus by an androgen receptor-dependent mechanism in male rats. *Endocrinology*, *154*(9), 3294-3304.

Hausmann, M., Slabbekoorn, D., Van Goozen, S. H., Cohen-Kettenis, P. T., & Güntürkün, O. (2000). Sex hormones affect spatial abilities during the menstrual cycle. *Behavioral neuroscience*, *114*(6), 1245.

Hillman, C. H., Erickson, K. I., & Kramer, A. F. (2008). Be smart, exercise your heart: exercise effects on brain and cognition. *Nature reviews neuroscience*, *9*(1), 58-65.

Hitchcock, F. A. (1925). Studies in vigor. *American Journal of Physiology--Legacy Content*, *75*(1), 205-210.

Holschneider, D. P., Yang, J., Guo, Y., & Maarek, J. M. (2007). Reorganization of functional brain maps after exercise training: importance of cerebellar–thalamic–cortical pathway. *Brain research*, *1184*, 96-107.

Jakubs, K., Nanobashvili, A., Bonde, S., Ekdahl, C. T., Kokaia, Z., Kokaia, M., & Lindvall, O. (2006). Environment matters: synaptic properties of neurons born in the epileptic adult brain develop to reduce excitability. *Neuron*, *52*(6), 1047-1059.

Jessberger, S., Clark, R. E., Broadbent, N. J., Clemenson, G. D., Consiglio, A., Lie, D. C., ... & Gage, F. H. (2009). Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learning & memory*, *16*(2), 147-154.

Jin, J., Jing, H., Choi, G., Oh, M. S., Ryu, J. H., Jeong, J. W., ... & Park, C. (2008). Voluntary exercise increases the new cell formation in the hippocampus of ovariectomized mice. *Neuroscience letters*, *439*(3), 260-263.

Kempermann, G., & Gage, F. H. (2002). Genetic determinants of adult hippocampal neurogenesis correlate with acquisition, but not probe trial performance, in the water maze task. *European Journal of Neuroscience*, *16*(1), 129-136.

Kronenberg, G., Bick-Sander, A., Bunk, E., Wolf, C., Ehninger, D., & Kempermann, G. (2006). Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus. *Neurobiology of aging*, *27*(10), 1505-1513.

Lightfoot, J. T., Turner, M. J., Daves, M., Vordermark, A., & Kleeberger, S. R. (2004). Genetic influence on daily wheel running activity level. *Physiological genomics*, *19*(3), 270-276.

Lou, S. J., Liu, J. Y., Chang, H., & Chen, P. J. (2008). Hippocampal neurogenesis and gene expression depend on exercise intensity in juvenile rats. *Brain research*, *1210*, 48-55.

Mahmoud, R., Wainwright, S. R., & Galea, L. A. (2016). Sex hormones and adult hippocampal neurogenesis: regulation, implications, and potential mechanisms. *Frontiers in neuroendocrinology*, *41*, 129-152.

Martinez-Canabal, A., Akers, K. G., Josselyn, S. A., & Frankland, P. W. (2013). Age-dependent effects of hippocampal neurogenesis suppression on spatial learning. *Hippocampus*, *23*(1), 66-74.

Meeusen, R., & De Meirleir, K. (1995). Exercise and brain neurotransmission. *Sports Medicine*, *20*(3), 160-188.

Merrill, D. A., Karim, R., Darraq, M., Chiba, A. A., & Tuszynski, M. H. (2003). Hippocampal cell genesis does not correlate with spatial learning ability in aged rats. *Journal of Comparative Neurology*, *459*(2), 201-207.

Meshi, D., Drew, M. R., Saxe, M., Ansorge, M. S., David, D., Santarelli, L., ... & Hen, R. (2006). Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. *Nature neuroscience*, *9*(6), 729-731.

Moser, M. B., Moser, E. I., Forrest, E., Andersen, P., & Morris, R. G. (1995). Spatial learning with a minislab in the dorsal hippocampus. *Proceedings of the National Academy of Sciences*, *92*(21), 9697-9701.

Moser, M. B., & Moser, E. I. (1998). Functional differentiation in the hippocampus. *Hippocampus*, *8*(6), 608-619.

Neeper, S. A., Gómez-Pinilla, F., Choi, J., & Cotman, C. W. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain research*, *726*(1), 49-56.

Okamoto, M., Hojo, Y., Inoue, K., Matsui, T., Kawato, S., McEwen, B. S., & Soya, H. (2012). Mild exercise increases dihydrotestosterone in hippocampus providing evidence for androgenic mediation of neurogenesis. *Proceedings of the National Academy of Sciences*, *109*(32), 13100-13105.

Olson, A. K., Eadie, B. D., Ernst, C., & Christie, B. R. (2006). Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus*, *16*(3), 250-260.

Ormerod, B. K., & Galea, L. A. M. (2003). Reproductive status influences the survival of new cells in the dentate gyrus of adult male meadow voles. *Neuroscience letters*, *346*(1), 25-28.

Paxinos, G., Franklin, K. B., & Franklin, K. B. J. (2001). The mouse brain in stereotaxic coordinates. 2nd.

Piatti, V. C., Davies-Sala, M. G., Espósito, M. S., Mongiat, L. A., Trinchero, M. F., & Schinder, A. F. (2011). The timing for neuronal maturation in the adult hippocampus is modulated by local network activity. *Journal of Neuroscience*, *31*(21), 7715-7728.

Plümpe, T., Ehninger, D., Steiner, B., Klempin, F., Jessberger, S., Brandt, M., ... & Kempermann, G. (2006). Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC neuroscience*, *7*(1), 77.

Pothuizen, H. H., Zhang, W. N., Jongen-Rêlo, A. L., Feldon, J., & Yee, B. K. (2004). Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within-subject, within-task comparison of reference and working spatial memory. *European Journal of Neuroscience*, *19*(3), 705-712.

Redila, V. A., & Christie, B. R. (2006). Exercise-induced changes in dendritic structure and complexity in the adult hippocampal dentate gyrus. *Neuroscience*, *137*(4), 1299-1307.

Rhodes, J. S., Van Praag, H., Jeffrey, S., Girard, I., Mitchell, G. S., Garland Jr, T., & Gage, F. H. (2003). Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. *Behavioral neuroscience*, *117*(5), 1006.

Rubin, R. T., Phillips, J. J., Sadow, T. F., & McCracken, J. T. (1995). Adrenal gland volume in major depression: increase during the depressive episode and decrease with successful treatment. *Archives of general psychiatry*, *52*(3), 213-218.

Russo-Neustadt, A. A., Alejandre, H., Garcia, C., Ivy, A. S., & Chen, M. J. (2004). Hippocampal brain-derived neurotrophic factor expression following treatment with reboxetine, citalopram, and physical exercise. *Neuropsychopharmacology*, *29*(12), 2189.

Sahay, A., Scobie, K. N., Hill, A. S., O'carroll, C. M., Kheirbek, M. A., Burghardt, N. S., ... & Hen, R. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature*, *472*(7344), 466-470.

Schoenfeld, T. J., & Gould, E. (2012). Stress, stress hormones, and adult neurogenesis. *Experimental neurology*, *233*(1), 12-21.

Shors, T. J., Townsend, D. A., Zhao, M., Kozorovitskiy, Y., & Gould, E. (2002). Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus*, *12*(5), 578-584.

Sibley, B. A., & Etnier, J. L. (2003). The relationship between physical activity and cognition in children: a meta-analysis. *Pediatric exercise science*, *15*(3), 243-256.

Snyder, J. S., Hong, N. S., McDonald, R. J., & Wojtowicz, J. M. (2005). A role for adult neurogenesis in spatial long-term memory. *Neuroscience*, *130*(4), 843-852.

Snyder, J. S., Choe, J. S., Clifford, M. A., Jeurling, S. I., Hurley, P., Brown, A., ... & Cameron, H. A. (2009). Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice. *Journal of Neuroscience*, *29*(46), 14484-14495.

Spalding, K. L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H. B., ... & Possnert, G. (2013). Dynamics of hippocampal neurogenesis in adult humans. *Cell*, *153*(6), 1219-1227.

Speisman, R. B., Kumar, A., Rani, A., Foster, T. C., & Ormerod, B. K. (2013). Daily exercise improves memory, stimulates hippocampal neurogenesis and modulates immune and neuroimmune cytokines in aging rats. *Brain, behavior, and immunity*, *28*, 25-43.

Spritzer, M. D., & Galea, L. A. (2007). Testosterone and dihydrotestosterone, but not estradiol, enhance survival of new hippocampal neurons in adult male rats. *Developmental neurobiology*, *67*(10), 1321-1333.

Swain, R. A., Harris, A. B., Wiener, E. C., Dutka, M. V., Morris, H. D., Theien, B. E., ... & Greenough, W. T. (2003). Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat. *Neuroscience*, *117*(4), 1037-1046.

Tanapat, P., Hastings, N. B., Reeves, A. J., & Gould, E. (1999). Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *Journal of Neuroscience*, *19*(14), 5792-5801.

Tanti, A., Rainer, Q., Minier, F., Surget, A., & Belzung, C. (2012). Differential environmental regulation of neurogenesis along the septo-temporal axis of the hippocampus. *Neuropharmacology*, *63*(3), 374-384.

Tashiro, A., Makino, H., & Gage, F. H. (2007). Experience-specific functional modification of the dentate gyrus through adult neurogenesis: a critical period during an immature stage. *Journal of Neuroscience*, *27*(12), 3252-3259.

Taupin, P. (2007). BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls,

Ter Horst, J. P., De Kloet, E. R., Schächinger, H., & Oitzl, M. S. (2012). Relevance of stress and female sex hormones for emotion and cognition. *Cellular and molecular neurobiology*, *32*(5), 725-735.

Thompson, C. L., Pathak, S. D., Jeromin, A., Ng, L. L., MacPherson, C. R., Mortrud, M. T., ... & Puchalski, R. B. (2008). Genomic anatomy of the hippocampus. *Neuron*, *60*(6), 1010-1021.

Toni, N., & Schinder, A. F. (2016). Maturation and functional integration of new granule cells into the adult hippocampus. *Cold Spring Harbor perspectives in biology*, *8*(1), a018903.

Treves, A., & Rolls, E. T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus*, *4*(3), 374-391.

Van Praag, H., Kempermann, G., & Gage, F. H. (1999a). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature neuroscience*, *2*(3), 266-270.

Van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999b). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences*, *96*(23), 13427-13431.

Van Praag, H., Shubert, T., Zhao, C., & Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *Journal of Neuroscience*, *25*(38), 8680-8685.

Van Praag, H. (2008). Neurogenesis and exercise: past and future directions. *Neuromolecular medicine*, *10*(2), 128-140.

Vivar, C., Peterson, B. D., & van Praag, H. (2016). Running rewires the neuronal network of adult-born dentate granule cells. *Neuroimage*, *131*, 29-41.

Wang, G. H., Richter, C. P., & Guttmacher, A. F. (1925). Activity studies on male castrated rats with ovarian transplants, and correlation of the activity with the histology of the grafts. *American Journal of Physiology--Legacy Content*, *73*(3), 581-599.

Warburton, D. E., Nicol, C. W., & Bredin, S. S. (2006). Health benefits of physical activity: the evidence. *Canadian medical association journal*, *174*(6), 801-809.

Wu, B. N., & O'Sullivan, A. J. (2011). Sex differences in energy metabolism need to be considered with lifestyle modifications in humans. *Journal of nutrition and metabolism*, *2011*.

Xu, H., Luo, C., Richardson, J. S., & Li, X. M. (2004). Recovery of hippocampal cell proliferation and BDNF levels, both of which are reduced by repeated restraint stress, is accelerated by chronic venlafaxine. *The pharmacogenomics journal*, *4*(5), 322-331.Zhang, C. L., Zou, Y., He, W., Gage, F. H., & Evans, R. M. (2008). A role for adult TLX-positive neural stem cells in learning and behaviour. *Nature*, *451*(7181), 1004