Cognitively-demanding physical activity enhances resilience to anxiety and females are more resilient in some environments

by Stephanie A Dudok

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University of Manitoba

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ABSTRACT

Physical activity induces plastic changes in the brain to enhance resilience to stress and anxiety, but unknown is whether quality of the activity matters. The present study examined whether cognitively-demanding physical activity differentially promotes resilience to anxiety, compared with regular or no physical activity. Subjects were randomly assigned to an experimental condition over four weeks: A- physical activity, B- cognitively-demanding physical activity, and C- sedentary. Three behaviour tests were administered to elicit anxiety-like responses. Group A displayed less feeding suppression in a novel environment, an effect moderated by sex. Group B displayed fewer anxiety-like behaviours compared to sedentary controls in an elevated plus maze. Females displayed fewer anxiety-like behaviours than males in light-dark boxes whether or not they were physically active. These findings demonstrate physical activity enhances resilience to the behavioural consequences of anxiety, particularly when activity is enriched with cognitive elements. Sex differences exist, with resilience dependent on environment.

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Stephanie A Dudok

Dedication

To the 36 precious ones,

your sacrifice is honoured

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CHAPTER 1: INTRODUCTION

The central nervous system, including the brain, possesses a special ability to show reversible functional and morphological changes in response to internal and external experiences. Brain plasticity can be driven in part by external stimuli including physical activity, cognitive activity such as learning, and stress. Plastic changes may contribute to adaptive or maladaptive behaviours. Resilience is an adaptive response to external experiences of stress and contributes to lessening symptoms of anxiety. Recruiting resilience would have many benefits to long-term health of the brain. Physical activity is beneficial to physical and mental health and wellness, and facilitates neuroprotective and neuroplastic processes to enhance resilience. The aim of this study was to examine whether voluntary physical activity promotes resilience to anxiety, and to determine any differential effects between cognitively-demanding physical activity and regular physical activity.

People who exercise regularly live longer, are healthier and more productive, and are more likely to avoid illness and injury. Clinical trials have shown regular exercise is effective in the prevention and treatment of many pathologies including cardiovascular disease (e.g. Jolliffe et al., 2001), diabetes (e.g. Boulé et al., 2001), cancer (e.g. Friedenreich & Orenstein, 2002; Kruk, 2007), and stroke (e.g. Wendel-Vos et al., 2004). Although less studied, regular exercise is also beneficial to mental health and wellness, likely because of adaptive changes induced in the brain. Physical activity prevents, delays onset, and alleviates symptoms of several mental disorders, including depression (e.g. Farmer et al., 1988), post-traumatic stress disorder (e.g. Manger & Motta, 2005), obsessive compulsive disorder (e.g. Brown et al., 2007), and dementias (e.g. Santana-

Sosa, 2008). Physical activity is associated with lower rates of mood and anxiety disorders (e.g. Goodwin, 2003; Strohle 2008; Zschucke et al., 2013), has been linked to psychological well-being (e.g. Barnes, 1992; Atlantis et al., 2004), and has been shown to play some role in recovery from physical and psychological trauma and stress (e.g. Rees et al., 2012; Emery et al., 2005). For adolescents, frequent physical activity and participation in sports is associated with lower levels of anxiety and depressive symptoms, as well as increases in self-reports of well-being (McMahon et al., 2017). Daily physical activity also reduces the severity of many impairing symptoms of attention deficit hyperactivity disorder (ADHD) in young children (Smith et al, 2013). Sagatun (2007) and colleagues discovered boys who had been physically active in their mid-teens were less likely than others to report depressive and other negative emotional symptoms or peer problems in early adulthood, such as social impairment with family or friends. Likewise, Colman (2014) and colleagues found physically active Canadian adolescents were at less risk of becoming depressed following stressful employment or life experiences as adults. Physical activity appears to have some protective benefit leading to adaptive changes in the brain, which help prevent the development and expression of mental disorders and their symptoms.

Physical activity is any bodily movement requiring energy expenditure and using skeletal muscles, whereas exercise, which is also physical activity, is deliberate and planned. Regular physical activity leads to long-lasting adaptations in the brain that alter responses to external events. These brain adaptations are evident in regular exercisers even after just one aerobic session (e.g. Lulic et al., 2017). Adaptations can occur because the brain's structure and function are not fixed, but rather shift and mold with experience.

Brain plasticity refers to the morphological and functional changes that take place within the brain. Brain plasticity involves structural alterations including changes in cortical thickness (Diamond et al., 1967; Diamond et al., 1981), cortical volume (Rosenzweig et al., 1962), dendritic length (Kolb et al., 2003), dendritic spine density (Globus et al., 1973), synapse formation (Greenough et al., 1985a), re-organization of existing cells and neuron size increases (Diamond et al., 1967), and modification of glial cells (Salois & Smith, 2016). These changes occur as a result of experience. Links between anatomical brain changes and observable behaviours exist. Structural changes occurring in the brain due to plasticity result in corresponding behavioural changes associated with implicated brain regions. For example, Jacobs et al. (2003) investigated the relationship between cortical dendrite systems and cognitive functioning, including verbal behaviours. The researchers discovered increasing levels of learning through formal education results in corresponding increases in dendritic length. The investigators concluded dendritic make-up can be an effective indicator of one's personal experience, including vocational behaviours and activities. These findings provide an example of external events leading to long-lasting adaptations in the brain.

Physical activity and long-term exercise are neuroprotective, facilitating brain plasticity and enhancing functioning. In humans, even a single session of aerobic exercise has the capacity to induce short-term neuroplasticity (i.e. for at least 30 minutes) in areas of the brain including the motor cortex (Perini et al., 2016) and visual cortex (Lunghi & Sale, 2015; Perini et al., 2016). Indeed, many different brain regions and brain structures are influenced by physical activity and exercise, with many different corresponding molecular and cellular pathways mediating effects. Specifically, physical activity

markedly influences the long-term integrity of the medial temporal lobe brain structure known as the hippocampus, by influencing the expression of genes and stimulating the process of neurogenesis, including cell proliferation and cell survival, and vascularization (e.g. van Praag et al., 1999; 2005). Physical activity enhances long-term potentiation in the dentate gyrus and stimulates the production, or uptake, of growth factors (e.g. van Praag et al., 1999; Trejo et al., 2001), including insulin-like growth factor (IGF) (Carro et al., 2000) and brain-derived neurotrophic factor (BDNF) (Neeper et al., 1995). Growth factors are important regulators of brain cell production and differentiation. Physical activity, specifically in the form of running, also activates neurotransmitters, including noradrenaline, dopamine and serotonin in the hippocampus (Chaouloff et al., 1987; Soares et al., 1999). These neurotransmitters influence brain plasticity and neurogenesis, as well as cognition (van Praag, Kempermann, & Gage, 1999). Indeed, increased proliferation and survival of neurons in the hippocampus is thought to mediate learning, memory, and other psychological and cognitive outcomes, including resilience.

Cognitive activity, such as learning, changes the brain and can induce plasticity. When rodents learn a new motor skill, such as traversing a runway, neurons in the motor cortex reorganize. Evidence of re-organization in the motor cortex include expansion of dendritic fields (Greenough et al, 1985b), synaptogenesis (Kleim et al., 1996), and alterations in synapse morphology and strength (Federmeier et al., 2002). Motor tasks can induce changes in mRNA and protein expression, which influence and regulate neural plasticity (e.g. Kleim, 1996; Irwin et al, 2000). Neural changes underlying the learning and execution of complex motor tasks lead to experience-dependent reorganization of the motor cortex, which notably persist over time (Kami et al., 1995). Learning a new motor

skill, even if it involves physical activity, differs from basic types of exercise, such as running. Learning a new skill requires more practice, persistence and cognitive involvement.

The acquisition and maintenance of skills requires cognitively-driven executive control, and neuronal changes in the brain may be more profound after extensive learning, or mastery, of a skill as compared to the early skill acquisition period (Kleim, 2004). Motor skill learning can, thus, be used as an effective means of enriching the impact of physical activity performed. Quality of activity alters the outcomes of physical activity. Matthis et al. (2018) examined human gait manipulation in response to changes in terrain (e.g. a flat packed-earth trail versus an extremely rocky dry creek bed). Walkers engaged cognitive processes (e.g. attention, planning, navigation, etc.) to a greater extent on the rocky terrains as they had to constantly identify visually the location of safe footholds, control foot placements, and adjust gait accordingly. In contrast, flat terrain walkers maintained a preferred gait and only occasionally engaged with their environment visually. The motor learning task for rodents used by Derksen et al. (2007) was remarkably comparable, consisting of a runway with multiple upended dowels, which rodents traverse on top of. It should be the case rodents executing the dowel motor task experience what it is like for humans to navigate an extremely rocky dry creek bed, requiring significant cognitive engagement. In contrast, unconstrained environments, such as walking or running on unchanging terrain, are energetically optimal and much less cognitively-demanding (Matthis et al., 2018). In a rodent model, an unconstrained environment should be equivalent to running in a cage wheel. Thus, for rodents, running

in a wheel and performing a dowel motor task can be considered good models of regular physical activity and cognitively-demanding physical activity, respectively.

Physical activity is being explored empirically for its function in enhancing resilience. Like learning, memory, and brain rehabilitation and repair, resilience depends on the plastic brain's ability to shift and mold with experience. Resilience involves adaptive recovery of capacity, health and well-being following adverse experiences, and resilient individuals experience fewer detrimental outcomes in both physiological and psychological dimensions following exposure to stressful experiences. Both human and animal studies have contributed evidence of the ability for physical activity to enhance resilience to stress and anxiety. Fulk and colleagues (2004) tested the effects of chronic physical activity on anxiety-like behaviours in rodents. Rodents ran regularly on treadmills over the course of ten weeks before undergoing behavioural tests in environments designed to evoke anxiety-induced responses. Regular runners showed reduced anxiety-like behaviours on both tests relative to non-runners, suggesting physical activity promoted resilience. Additional rodent studies examining the therapeutic effects of physical activity have demonstrated running, even for as little as three weeks, is effective in preventing or reversing the physiological and behavioural consequences of stress and anxiety (Greenwood et al., 2005; 2007; Lee et al., 2015; Sciolino et al., 2012; 2015). Rodents who undergo regular physical activity also seem to respond to stressors with an overall reduction of stress, reflected by lower plasma corticosterone concentrations, as compared to sedentary rodents (e.g. Hoffman, 2015). Human studies too, utilizing self-reports of anxiety, have demonstrated a resilience effect associated with regular physical activity (e.g. De Mello et al., 2013; Stubbs et al., 2017). Physical activity

induces positive mood and acts as an anxiolytic, reducing negative feelings such as frustration, nervousness, and irritability. The effect of resilience is especially apparent following stressful experiences. Childs & de Wit (2014) found greater decreases in positive affect following a stressful experience in sedentary individuals, relative to participants who reported regular exercise. When men and women reported on their mood following a social stress test, the non-exercisers' mood declined, as compared to before test administration. Regular exercisers were able to maintain baseline positive feelings, such as friendliness and elation, despite exposure to the same stressful environment. In summary, the literature supports physical activity as an important promotor of resilience to stress and anxiety. Though mechanisms are not yet well understood, physical activity renders humans less prone to experiencing anxiety symptoms and animals less prone to expressing anxiety-like behaviours.

Although most empirical evidence has examined the quantity of physical activity in promoting resilience to stress, anxiety or symptoms of mental disorders, little has been studied with respect to the quality of physical activity. Greenwood et al. (2013) discovered resilience to stress and anxiety occurs whether or not exercise is forced or voluntary, but there exists a research gap when considering the role mental and cognitive enrichment plays. This research gaps exists even though physical and cognitive activity have many neural mechanisms in common, influencing brain plasticity and neurogenesis in similar ways. Investigation into the relationship between cognition, cognitive impairment and mood has been growing, due in large part to exponential growth in the population of older adults within Canada and globally, and the associated age-related reduction in physical and psychological resilience. Evidence now demonstrates even mild

cognitive impairment is associated with people experiencing more prevalent symptoms of depression and anxiety as compared to people with normal cognitive function (Yates, Clare & Woods, 2016). Hogan (2005) found participation in both cognitive and physical activity, and the combination, produces broad physical and psychological resilience to illness, disease and disorder in aging adults and has, thus, been promoted for both interventional and rehabilitative purposes. It is possible the combination of physical and cognitive activity can provide a mechanism to prevent or reverse anxiety-induced behavioural responses.

Like physical activity and learning, stress and anxiety also have an effect on the brain and can influence plasticity. In the short term, these changes may lead to adaptive behaviours. For example, the stressful experience of coming face to face with a predator could evoke a protective flight response. If, however, the danger passes yet the behavioural state persists along with, or due to, changes in neural structure and circuitry, these changes and corresponding emotional states may become maladaptive. In humans, changes as a result of stressful or anxiety-inducing situations may lead to compromised mental functioning and mental disorders (Perry et al., 1995). Acute and chronic stress has the potential to induce an imbalance of function in neural circuitry that affects cognition, decision making, anxiety and mood, and can increase expression of anxiety and fear-related behaviours.

The hippocampus, pre-frontal cortex and amygdala are each areas of the brain associated with learning, memory, goal-directed activity, emotional regulation and resilience (reviewed in McEwan, 2010). Stress-induced structural changes in the hippocampus, previously noted as a malleable brain structure capable of transforming in

response to experience, include dendritic remodeling and atrophy of dendrites (Magariños et al., 1997; McKittrick et al., 1996; Sousa et al, 2000), suppression of neurogenesis and cell survival (Gould et al., 1997), dentate gyrus shrinkage and inhibition of long-term potentiation in the dentate gyrus (Pham et al, 2003). Thus, stress-induced structural changes in the hippocampus are largely opposite to the changes induced by physical and cognitive activity. Stress-induced changes are associated with increased expression of depressive and anxiety-like behaviours, and even cognitive impairment (e.g. impaired learning and memory), in contrast to resilience associated with physical and cognitive activity. Both acute and chronic stress also cause functional and structural changes in the prefrontal cortex and amygdala. Dendritic shortening, in response to stress, occurs in the prefrontal cortex (Radley et al., 2005), whereas dendritic growth occurs in the amygdala and orbitofrontal cortex (e.g. Vyas et al, 2002; Yi et al., 2017). The amygdala is a region of the brain particularly associated with anxiety and anxiety-induced behaviours (Conrad et al., 1999). Amygdala hypertrophy can increase the strength and connectivity of neurons, leading to an increased propensity for responding or behaving with anxiety. Vyas and colleagues (2002) found associations between dendritic remodeling in the amygdala and increased anxiety-like behaviours in rodents following exposure to chronic stress. The findings by these investigators suggest certain types of stressful environments may lead to behavioural manifestations of the emotional (i.e. anxious, fearful) states of subjects.

Klumpers and colleagues (2017) made an important distinction between anxiety and fear, which are often and mistakenly considered to be the same emotional state at the neural level. The researchers established that different neurocircuitry is involved in

defensive responses to threat anticipation compared to acute confrontation. Although there are large overlaps in function and connectivity, the authors report threat anticipation is associated with the brain's forebrain region (i.e. the bed nucleus of the striata terminalis), whereas acute confrontation is associated with the amygdala. These findings point toward the likelihood of differences in the expression of behaviours, too, that are primarily anxiety driven and those fear-induced. This distinction is especially important for animal studies, as it suggests assessing anxiety via anxiety-like responses in nonhumans is possible.

Human studies of physical activity induced resilience to anxiety have largely relied on self-reported outcome measures (e.g. De Mello et al., 2013; Childs & De Wit, 2014; Stubbs et al., 2017). Yet, a meta-analysis on the anxiolytic effects of exercise (Wipli and colleagues, 2008) reported reliance on these measures can produce a source of response bias. Animal models provide an alternative method to further the scientific understanding of anxiety resilience. Techniques invoking threat anticipation, such as those exploiting an approach-avoidance conflict, rather than acute confrontation, such as the presence of an aggressive stranger, are likely to produce a result that closely mimics human anxiety. The findings of Klumpers and colleagues (2017) provide evidence that models exploring anxiety through use of behaviour tests should rely on threat anticipation rather than any acute confrontation (e.g. confrontation with an unknown, aggressive stranger). Accordingly, animal models using threat anticipation, such as the elevated plus maze and light-dark boxes, can be considered a good representation of anxiety.

Animal research provides an opportunity to examine effects and mechanisms in a highly controlled environment, which allows for the experimental manipulation of

physical activity. Yet, accurately measuring anxiety-like responses in animals without self-report is challenging. Physiological measures (e.g. heart rate) can be used, but these measures are difficult and complex to obtain in a small rodent, and reliability can be affected by variables such as weight and other factors. Thus, behavioural investigation techniques are commonly used.

Though no single measure reliably mirrors an equivalent to human anxiety by itself, validated behavioural tests can be used to evaluate anxiety-like behaviours in rodents. These tests are designed to elicit behaviours mimicking anxiety responses in humans (e.g. Fulk, 2004). With rodents, environments which produce a strong approach-avoidance conflict are validated methods for assessing anxiety responses and the mechanisms underlying anxiety-induced behaviours. Such environments inhibit characteristic behaviours normally observed in a rodent such as exploration, social investigation, and consumption of palatable foods. The elevated plus maze, light-dark box, and novelty suppressed feeding tests are three such environments, each a representation of threat anticipation. Each of these tests can be used to assess rodents' spontaneous exploratory and avoidant behaviours.

The elevated plus maze is one of the most widely used tests for assessing anxiety-like responses in rodents. The elevated plus maze was first proposed by Handley & Mithani (1984) and has been validated by numerous researchers since (e.g. File et al, 1990). An elevated plus maze is based on the natural threat aversion of rodents for open and elevated areas. Rodents in an elevated plus maze tend to display typical behavioural reactions: avoidance of the open arms or escape from an open arm into a closed, presumably perceived as safer, arm. Entering more frequently, and staying longer, in the

open arms of the elevated plus maze can be used as indices of greater resilience to elevated, open space-induced anxiety. Studies (e.g. Bourin, 2015; Braun et al., 2011) examining the anti-anxiety effects of pharmacological agents and steroid hormones have demonstrated rodents treated with agents to enhance resilience, such as anxiolytic drugs, show considerably less open area avoidance. In contrast, untreated rodents avoid exposed areas of an elevated plus maze, preferring instead the enclosed areas.

Rodents tend to naturally avoid brightly illuminated areas, preferring darker ones. The light-dark box test is another validated method to assess anxiety-like responses in rodents, introduced even before the elevated plus maze. Developed by Crawley & Goodwin (1980), the light-dark box test is based on a rodent's innate threat aversion to brightly illuminated areas. Light-dark environments make use of the natural aversion of rodents towards open and illuminated spaces. Resilient rodents are more likely to explore the illuminated area and show considerably less light-box avoidance. More frequent entries, and duration spent, in the light box can be used as indices of greater resilience to illuminated, open space-induced anxiety.

Highly stressed rodents will resist consuming food they normally find palatable, a behaviour called hyponeophagia. One the oldest studies chronicling anxiety-related feeding suppression was conducted decades ago. Hall (1934) was the first to report hyponeophagia in rodents, a behaviour generated by anxiety-like states associated with a novel environment. Bodnoff et al. (1988) validated the novelty suppressed feeding test. The novelty suppressed feeding test creates a conflict between a rodent's natural tendency to consume palatable food and the rodent's threat aversion to novel, unprotected, central locations. Rodents who take a long time to initiate palatable food intake, or who consume

very little of the palatable food, are considered to be expressing characteristic anxiety-induced behaviour.

Although physical and cognitive activity alike induce plasticity, they have so far been studied only separately for their individual effects in promoting resilience. The blend of cognitive and physical activity has not been studied for its combined role in promoting resilience to anxiety. It would be valuable to know whether individuals who participate in physical activity with cognitive components, such as learning, navigation, and attention, are more resilient to the behavioural consequences of stress and anxiety. Well controlled *in vivo* comparisons between subjects engaging in cognitively-demanding physical activity versus regular or no physical activity are necessary to determine if quality of activity is important for enhancing resilience to stress and anxiety.

The objectives of the present study were to examine whether physical activity promotes resilience to anxiety, and to examine cognitively-demanding physical activity in contrast to regular physical activity, to determine differential effects on resilience. Resilience would be measured indirectly by measuring anxiety-like behaviours directly. To fulfill these objectives, I used an adult rat test of anxiety and compared two levels of physical activity (one with cognitive components including learning, navigation and attention, and one primarily movement-based) and a sedentary control. I expected subjects who were physically active prior to undergoing mildly stressful behavioural tasks, designed to elicit anxiety-like responses, to demonstrate greater resilience, measured indirectly through the expression of fewer anxiety-like behaviours. I predicted the existence of a positive relationship between physical activity and anxiety resilience. I hypothesized subjects participating in four weeks of physical activity prior to undergoing

the novelty suppressed feeding, elevated plus maze, and light-dark box tests would enter more frequently and stay longer in the stress-inducing areas of each environment, as compared to the sedentary group. I also predicted the effect would be larger for the cognitively-demanding physical activity group relative to the regular or no physical activity groups. Correct predictions would provide evidence that cognitively-demanding physical activity promotes resilience to a greater extent than basic physical activity lacking cognitive components. In this way, I expected results would show it is advantageous to combine the plasticity-inducing effects of both physical and cognitive activity to maximally enhance resilience.

CHAPTER 2: MANUSCRIPT

ABSTRACT

Physical activity plays a role in inducing plastic changes in the brain. These changes have been shown to enhance resilience to stress and anxiety, however, there has been limited investigation as to whether the quality of activity matters. The present study examined whether participation in cognitively-demanding physical activity differentially promotes resilience to anxiety, compared with participation in regular or no physical activity. Thirty-six male (n=18) and female (n=18) Long-Evans rats were randomly assigned to one of three experimental conditions, in which they were maintained over four weeks: A – physical activity, B – cognitively-demanding physical activity, and C – sedentary control. Subjects in Group A ran in cage wheels for 30 min/day increasing to 60 min/day. Subjects in Group B performed a complex motor task for 20 trials per day followed by 30min/day of cage wheel running. Control subjects did not participate in any physical activity but were removed from cages and handled once daily. Subsequently, all subjects underwent three behaviour tests designed to elicit anxiety-like responses: novelty-supressed feeding, elevated plus maze, and light-dark environment. Subjects in Group A displayed less feeding suppression in the novelty-supressed feeding test and this relationship was moderated by sex. Subjects in Group B displayed fewer anxiety-like behaviours compared to sedentary controls in the elevated plus maze, entering more frequently into open arms. Female subjects displayed fewer anxiety-like behaviours than males in the light-dark box, whether or not they had been physically active. These findings demonstrate physical activity does enhance resilience to the behavioural consequences of anxiety, and that resilience is greater when the activity is enriched with cognitive elements.

These findings provide evidence of sex differences on anxiety resilience, with impacts dependent on aspects of the environment.

INTRODUCTION

Physical activity contributes to healthy bodies, but it also has a positive impact on brain anatomy and physiology, which may promote resilience. Anxiety is a common response to events occurring in our lives and environments, particularly when those events cause us to feel threatened or endangered. Though the subjective feeling of anxiety is familiar to most, the consequences of stressful events go beyond the affective and can change the structure and function of the brain. Brain plasticity, which involves functional and morphological changes, can be driven in part by internal and external experiences. External experiences include physical activity, cognitive activity and stress. Plastic changes may contribute to adaptive or maladaptive behaviours. Resilience is an adaptive response to external experiences of stress and contributes to lessening symptoms of anxiety. Recruiting resilience would have many benefits to long term brain health.

Physical and cognitive activity are beneficial to mental health and wellness, and facilitate neuroprotective and neuroplastic processes to enhance resilience in similar ways. Resilience involves adaptive recovery of capacity following adverse experiences and resilient individuals are more likely to return quickly to baseline physiological and behavioural states following exposure to stress (Childs & de Wit, 2014; Oken et al., 2015). Both physical and cognitive activity play some role in enhancing resilience to the behavioural and physiological consequences of stress and anxiety as a result of the plastic changes they induce (Stubbs et al., 2017; Brenes et al., 2016; Anderson-Hanley, 2012).

As such, it is likely physical and cognitive activity combined induce integrated plastic changes to promote anxiety resilience to a greater extent than either does alone.

Physical activity promotes brain plasticity and influences key elements that contribute to plasticity, such as insulin-like growth factor (IGF) (Carro et al., 2000) and brain-derived neurotrophic factor (BDNF) (Neeper et al., 1995). Growth factors are important regulators of brain cell production and differentiation. Physical activity, specifically in the form of running, also activates neurotransmitters in the hippocampus, such as noradrenaline, dopamine and serotonin (Chaouloff et al., 1987; Soares et al., 1999). These neurotransmitters influence brain plasticity and neurogenesis, as well as cognition (van Praag, Kempermann, & Gage, 2000). Rodents who run regularly show reduced anxiety-like behaviours on tests of anxiety relative to non-runners, suggesting physical activity promotes resilience (e.g. Fulk et al., 2004). Studies examining the therapeutic effects of exercise have demonstrated that three or more weeks of wheel running is effective in preventing or reversing the physiological and behavioural consequences of stress and anxiety (e.g. Greenwood et al., 2005; 2007; 2012; Lee et al., 2015; Sciolino et al., 2012; 2015). In addition, rodents who undergo regular physical activity also seem to respond to stressors with an overall reduction of stress, reflected by lower plasma corticosterone concentrations, as compared to sedentary rodents (e.g. Hoffman, 2015). Running is an ideal form of exercise for examining the effects of physical activity on anxiety resilience in rodents.

Learning, including motor learning and skill mastery, is a cognitively-demanding activity that induces plastic changes in the brain too. Neural changes underlying the learning and execution of complex motor tasks lead to experience-dependent

reorganization of the motor cortex, which notably persist over time (Kami et al., 1995). Evidence of re-organization in the motor cortex include expansion of dendritic fields (Greenough et al, 1985b), synaptogenesis (Kleim et al., 1996), and alterations in synapse morphology and strength (Federmeier et al., 2002). Learning a new motor skill, even if it involves physical activity, differs from basic types of exercise, such as running. Learning a new skill requires more practice, persistence and cognitive involvement. The acquisition and maintenance of skills requires cognitively-driven executive control, and neuronal changes in the brain may be more profound after extensive learning, or mastery, of a skill as compared to the early skill acquisition period (Kleim, 2004). Motor skill learning can, thus, be used as an effective means of enriching the impact of physical activity performed.

The quality of physical activity that maximally promotes resilience to anxiety remains largely unexamined. We wanted to know whether quality of physical activity matters, and if challenging the mind along with the body would maximize the brain's plastic potential to promote resilience. By examining resilience indirectly through direct measures of anxiety-like behaviours, the objective of the present study was to examine whether physical activity promotes resilience to the behavioural consequences of anxiety. A secondary objective was to determine whether participation in cognitively-demanding physical activity, incorporating motor learning and skill mastery, differentially promotes resilience to anxiety, as compared to regular physical activity (i.e. running) or no physical activity. We predicted rodents who participated in physical activity prior to undergoing behavioural tasks designed to induce anxiety would demonstrate greater resilience relative to sedentary controls, and we expected this effect would be largest for the cognitively-

demanding physical activity group. We expected the resilience of subjects in the cognitively-demanding physical activity group to be most enhanced, relative to other subjects.

METHODS

Subjects

Subjects (n=36) were 18 male and 18 female Long-Evans rats (Charles River, QC). Subjects were 52 – 66 days old at the beginning of pre-training, considered equivalent in age to young human adults. Animals had to be physically mature enough to successfully train on cognitively-demanding tasks requiring complex learning and motor skills. Subjects were housed in plastic cages with wood chip bedding, paired with another member of the same group and sex. Subjects had access to food and water *ad libitum*, except when undergoing experimental protocols at which point they only had free access to water when in the running wheel cages. Subjects were maintained in a temperature and humidity controlled room on a 12-hr light-dark cycle, with lights turned on at 7 a.m. daily. All procedures took place during the light cycle. Subjects (n=12/group) were randomly assigned to one of three groups: (A) physical activity, (B) cognitively-demanding physical activity, and (C) sedentary. Subjects in experimental Group A ran in cage wheels and subjects in experimental Group B executed a complex motor task in additional to running in cage wheels. Control subjects in Group C did not participate in any physical activity, but were removed from cages and handled to correspond with the experimental groups. The experiment was carried out in accordance with the Canadian Council of Animal Care guidelines and ethics of the study were approved by the University of Manitoba Animal Care Committee (protocol #F17-021).

Apparatus

Physical activity: Running wheels

Four rodent cages with attached running wheels were used as apparatus for regular physical activity. The overall dimensions of the cages were 20cm x 48cm x 28cm and the wheels were 35cm high with diameters measuring 9.5 cm. The wheels each had a Tico731 rotation counter attached, which digitally recorded each full wheel revolution.

Cognitively-demanding physical activity: Runways

Runways were used as apparatus for cognitively-demanding physical activity. Runway 1, a flat 184cm x 5cm beam, was used during the pre-training phase. Runway 2, used during the training phase, was also 184cm x 5cm in length but consisted of a number of upended dowels (0.3 cm diameter), designed to match learning tasks carried out in previous studies in this lab (Derksen et al, 2007; Larson et al, 2007). Dowels, spaced 2cm apart, protruded vertically from Runway 2 at various angles. A stand with a small platform (10-20cm) at each end served as starting and ending points during both pre-training and training phases**.** The stand elevated both runways 70cm from the ground.

Novelty Suppressed Feeding

The apparatus for the novelty suppressed feeding test consisted of an open and illuminated wooden box measuring 61cm x 64cm x 61cm. A total of five Hershey's milk chocolate Chipits purchased from Real Canadian Superstore in Winnipeg, Manitoba were placed in the center of the open field in small plastic laboratory dishes assigned to each subject for use in the test of hyponeophagia.

Elevated Plus Maze

The elevated plus maze apparatus was made of painted wood and was comprised of two open arm platforms and two closed arm platforms in the configuration of a plus (+) sign. Each open arm platform measured 50cm x 9cm across from each other and perpendicular to two closed arm platforms measuring 50cm x 9cm. The closed arm platforms each had a high (24cm) opaque wall fully enclosing the arm on the three vertical sides. The central junction of the elevated plus maze measured 9cm x 9cm. The entire apparatus was elevated 75cm above the floor and stationed in the middle of the experimental room.

Light-Dark Boxes

The light-dark box apparatus consisted of two Plexiglas© compartments connected by a 7cm x 5cm door. One compartment, called the light box, measured 31cm x 31cm and was brightly lit with transparent sides and a transparent lid. The second half of the apparatus, called the dark box, measured 31cm x 31cm, and was fully enclosed and dark.

Procedure

Physical Activity Protocol Group A

All subjects in Group A pre-trained to run on cage wheels as adaptation for three consecutive days, during which time they were allowed to explore the apparatus and undergo one running trial per day lasting no more than 15 minutes. Following the three-day pre-training phase, subjects were conditioned to run on the cage wheels voluntarily for a maximum of 30 minutes daily, five days per week, during week 1 of the study. Running

increased to a maximum of 60 minutes daily for the remaining weeks of the study (weeks 2 – 4) for five days per week during weeks 2-3, and four days per week during week 4. Subjects received two rest days following pre-training and each consecutive five days of training.

Physical Activity Protocol Group B

All subjects in Group B pre-trained on Runway 1 (flat beam) as adaptation for three consecutive days, during which time they were prompted to traverse the distance of the beam for ten trials lasting no more than 15 minutes in total. Subjects were encouraged to continue moving forward when needed with a gentle push on the area where the tail meets the body. Each subject was given a maximum of one minute of rest between trials. Following the pre-training phase, subjects in Group B were trained on Runway 2 (dowel beam as per Derksen et al., 2007 and Larson et al., 2007), for a maximum of 30 minutes daily, five days per week during week 1, to ensure acquisition and, subsequently, maintenance of motor skill learning. Subjects were prompted to transverse Runway 2 for a maximum of twenty trials per day with a maximum of 2 minutes of rest in between trials. Although subjects were habituated with the goal of the task due to the pre-training phase, the animals were occasionally encouraged to mount the dowels or continue moving forward with a touch to the tail or side of the body as was necessary. During the remainder of the study (weeks 2 – 4) subjects in Group B executed the Runway 2 task for a maximum of 30 minutes daily followed by a maximum of 30 minutes of cage wheel running, five days per week during weeks 2-3, and four days per week during week 4. Subjects received two rest days following pre-training and each consecutive five days of training. The physical activity protocol for subjects in Group B was designed to provide physical activity

comparable in amount and intensity to subjects in Group A during week 1, as well as subsequent weeks 2 – 4.

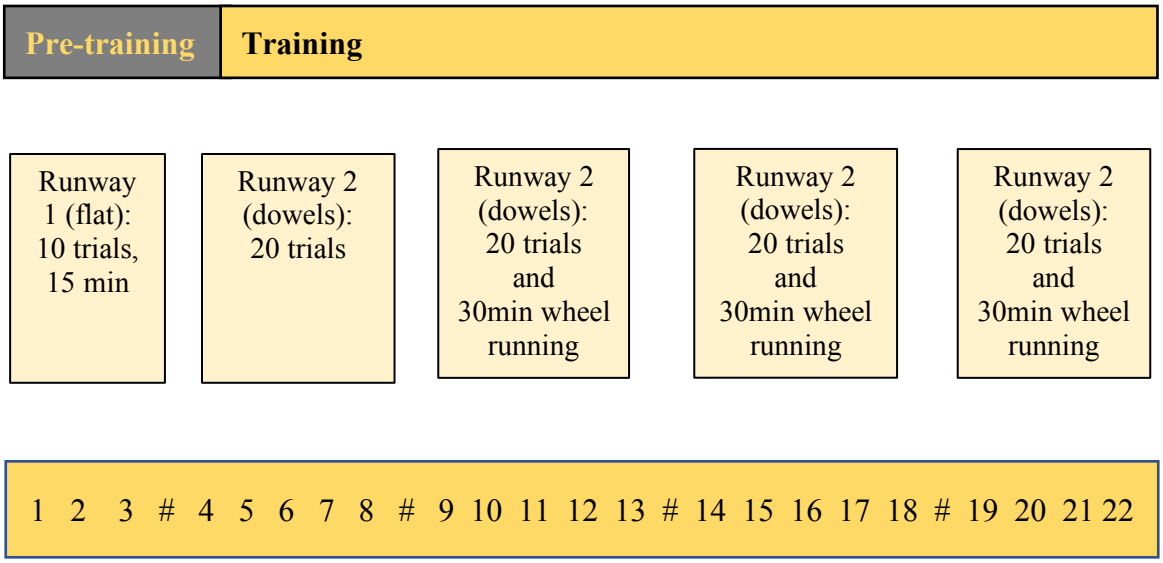

Figure 1. The cognitively-demanding physical activity protocol for subjects in Group B during the pre-training and training phases, by experimental day. A # indicates when subjects had two consecutive rest days consisting of no physical activity.

Behaviour Test 1: Novelty Suppressed Feeding

Twenty-four hours after the last day of training, subjects underwent the novelty suppressed feeding test for hyponeophagia behaviour. Subjects were placed once in the center of the novel, open field apparatus for a total of five minutes. The number of chocolate chips consumed were counted and recorded by an observer. Latency to consume chocolate chips was recorded by a video-tracking system. A blind observer later assessed the latency measure using the recorded video and a Fisher scientific stopwatch to obtain latency times. Behaviour Test 1 was repeated a second time approximately forty-eight hours later.

Behaviour Test 2: Elevated Plus Maze

Twenty-four hours after the last day of training, subjects underwent a behavioural test using the elevated plus maze. The elevated plus maze task did not require pre-training and was simple enough for subjects to navigate. Each subject was placed once in the center of the elevated plus maze at the juncture where the four arms overlap. Subjects were placed facing an open arm and were given free roaming access for a total of five minutes (as per Hoffman, 2015; Cohen, 2008). Behaviours were recorded by video camera and later coded and assessed by a blind observer who used a Fisher scientific stopwatch for timed measures. The following behaviours were assessed: number and proportion of open arm entries, number of closed arm entries, total exploration (number of entries into all arms), time spent in open arms, and latency to enter open arm. As per Hoffman and colleagues (2015), arm entry was defined as entering an arm with all four paws. An anxiety index score was also calculated as described in previous studies investigating physical activity and anxiety resilience *(Figure 2)*. Higher scores corresponded with more anxiety-like behaviours. Behaviour Test 2 was repeated approximately forty-eight hours later.

$$\text{anxiety score} = 1 - \frac{\left[\frac{\text{total time in open arms}}{5\text{ min}}\right] + \left[\frac{\text{number of open arm entries}}{\text{total exploration}}\right]}{2}$$

Figure 2. Anxiety index score calculation. Adapted from "Early post-stressor intervention with high-dose corticosterone attenuates posttraumatic stress response in an animal model of posttraumatic stress disorder," by H. Cohen, M.A. Matar, D. Buskila, D., Z. Kaplan and J. Zohar, 2008, *Biological Psychiatry*, *64*, p. 711.

Behaviour Test 3: Light-Dark Boxes

Twenty-four hours after the last day of training, subjects were placed in the light-dark box apparatus and assessed for anxiety-induced behavioural responses. The light-dark box test did not require any prior training. Subjects were placed in the dark box and given free roaming access for a total of five minutes. Behaviours were recorded by video camera and later coded and assessed by a blind observer who used a Fisher scientific stopwatch for timed measures. The following behaviours were assessed: number of light box entries, number of dark box entries, total exploration (transitions) between boxes, time spent in light box, and latency to enter light box. Box entry was defined as entering a box with all four paws. Behaviour Test 3 was repeated approximately forty-eight hours later.

Statistical Analysis

Data were analyzed using a two-factor between-subjects analysis of variance for each measure across the entire battery of behaviour tests. Factors were defined as Group (physical activity, cognitively-demanding physical activity, sedentary) and Sex (male, female). Data were further analyzed using a one-factor within-subjects analysis of variance for the elevated plus maze test and light-dark box test, with the single factor defined as Day (Test Day 1, Test Day 2). Statistical significance was indicated by $p < 0.05$. Significant interactions were followed by Tukey's LSD post-hoc analyses to further determine differential effects. Using Cohen's convention, we considered $\eta^2_p = 0.01 – 0.06$ a small effect size, $\eta^2_p = 0.06 – 0.14$ a medium effect size, and 0.14 or greater a large effect size. Grubbs' test was performed to determine if extreme values were statistically significant outliers. Detected outliers, however, were not removed from the data as we considered all

data to constitute an accurate account of the expected variation of subjects' behavioural expressions of anxiety.

RESULTS

Results: Novelty Suppressed Feeding

There was a significant main effect of group on the number of chocolate chips consumed. Subjects in Group A consumed more chocolate chips compared to the other groups $F(2, 30) = 7.32$, $p < 0.05$, $\eta^2_p = 0.33$. There was a significant interaction between group and sex on this measure $F(2,30) = 3.69$, $p < 0.05$, $\eta^2_p = 0.20$, with post-hoc analysis revealing the increased consumption in Group A to be dependent on male subjects.

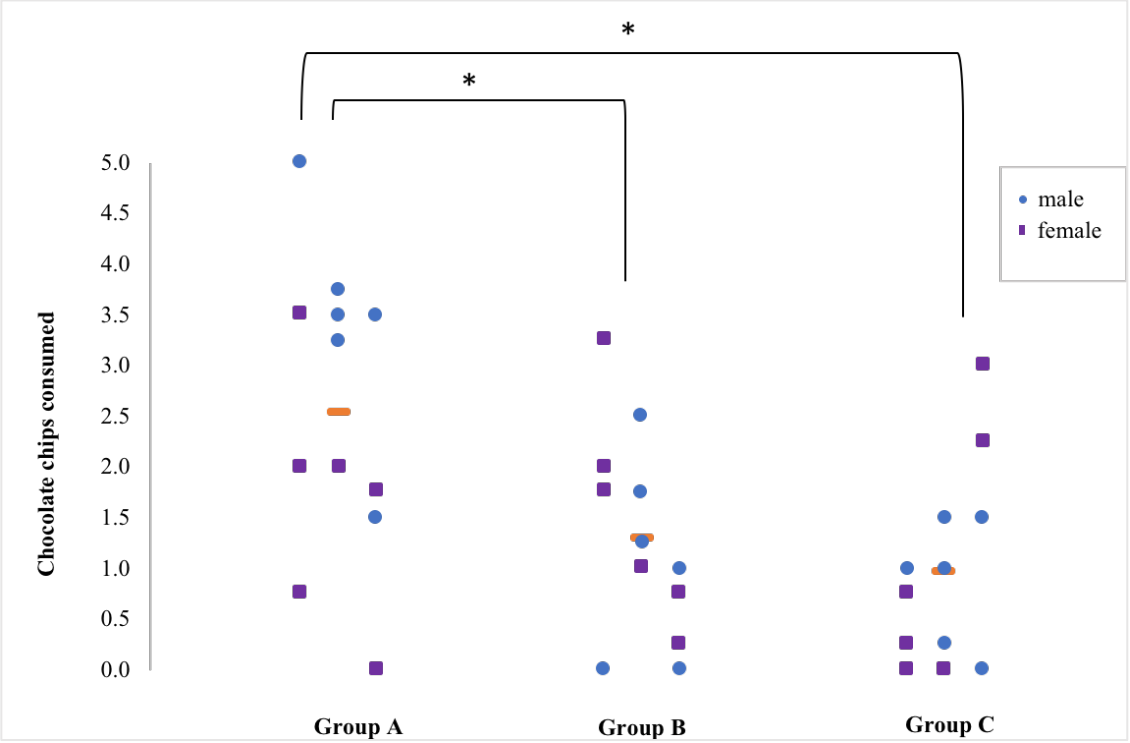

Figure 3. Novelty Suppressed Feeding test results. This figure illustrates the number of palatable food pieces (chocolate chips) consumed, by group. Purple squares represent female subjects and blue dots represent male subjects. Orange lines represent the mean number of chocolate chips consumed. An * indicates $\alpha<0.05$.

There were no main effects or interaction effects found for the other dependent variable, latency to consume chocolate chips.

Results: Elevated Plus Maze

There was a significant main effect of group on total entries into open arms $F(2,30) = 4.99$, $p < 0.05$, $\eta^2_p = 0.25$, with post-hoc analysis demonstrating subjects in Group B entered more frequently into the open arms compared with subjects in Group C. No interaction effects were found.

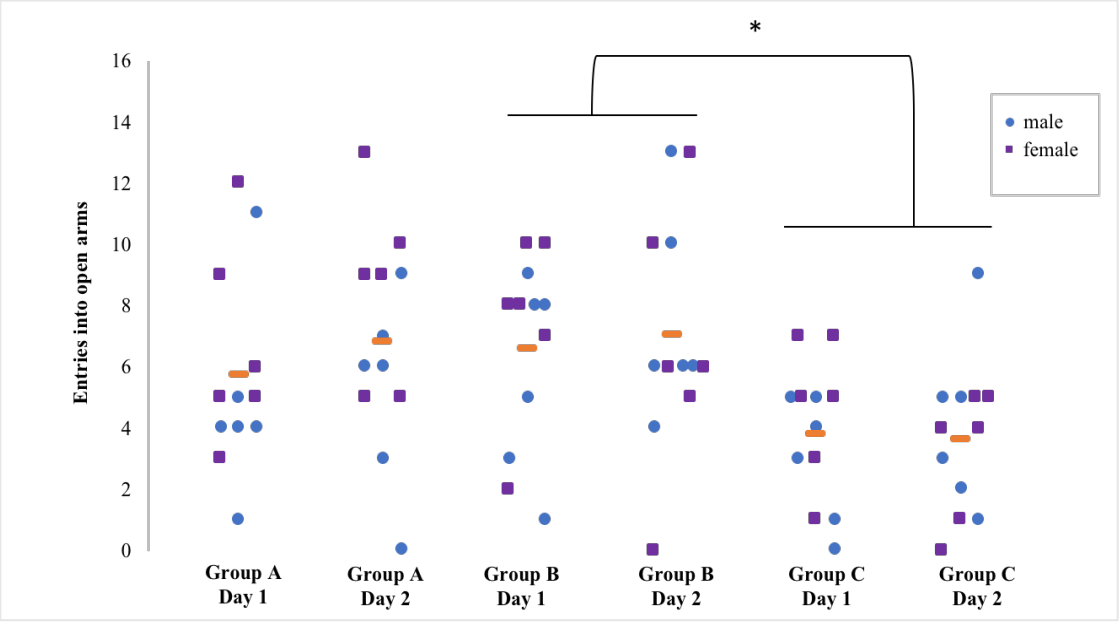

Figure 4. Elevated Plus Maze test results. This figure depicts the number of entries into open arms of the elevated plus maze, by group and day. Purple squares represent female subjects and blue dots represent male subjects. Orange lines represent the mean number of entries into open arms. An * indicates $\alpha<0.05$.

A main effect of day was found for total exploration (entries into open and closed arms combined), $F(1,30) = 13.74$, $p < 0.05$, $\eta^2_p = 0.31$, and for entries into closed arms, $F(1,30) = 24.93$, $p < 0.05$, $\eta^2_p = 0.45$. Subjects explored all arms more frequently, and entered more frequently into the closed arms, during day 2 testing. No interaction effects were found for these measures.

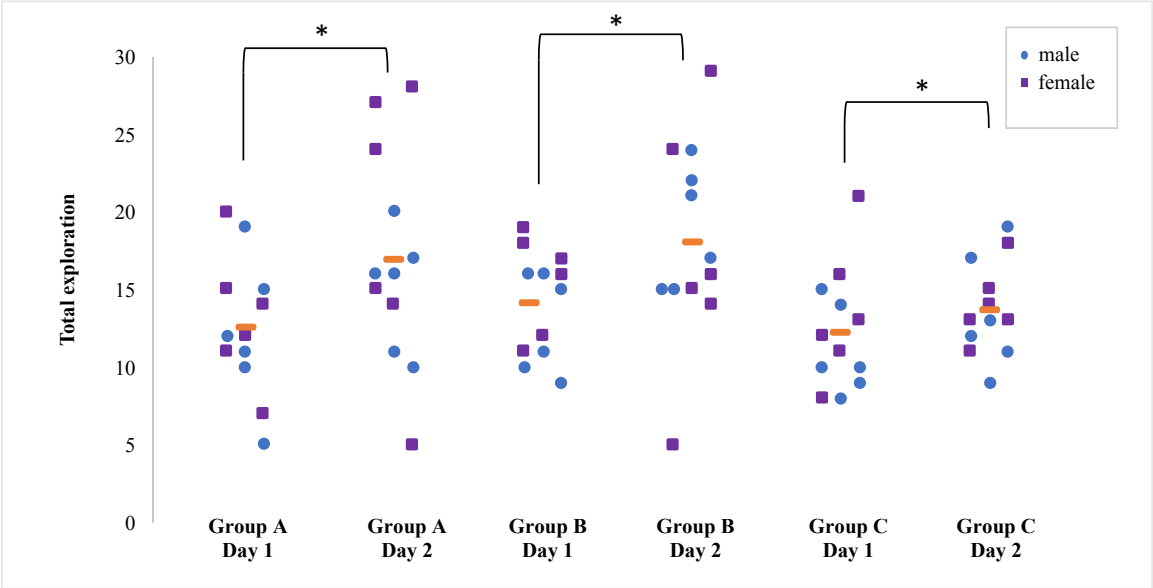

Figure 5. Elevated Plus Maze test results. This figure depicts the mean total exploration (entries into open and closed arms combined) into all arms of the elevated plus maze, by group and day. Purple squares represent female subjects and blue dots represent male subjects. Orange lines represent the mean total exploration. An * indicates $\alpha<0.05$.

A main effect of day was found for proportion of entries into the open arms, $F(1,30) = 4.39$, $p < 0.05$, $\eta^2_p = 0.13$. As a proportion, subjects entered more often into the open arms during day 1 testing compared to day 2. An outlier was detected in Group B Day 2, $Z = 2.61$, $p < .05$.

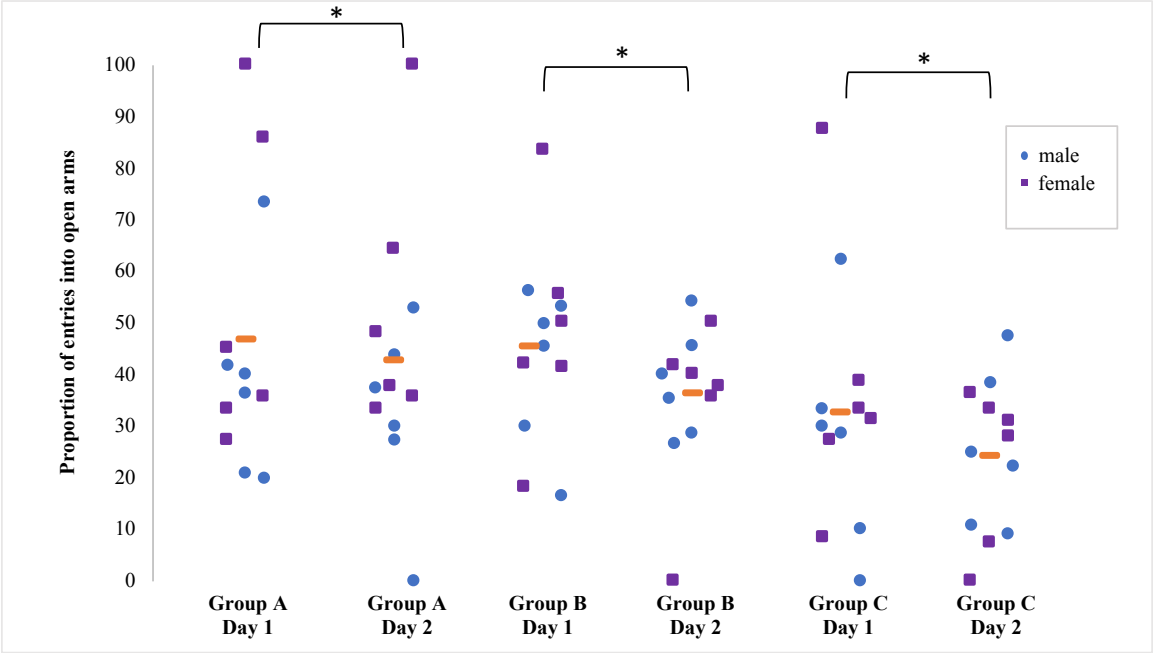

Figure 6. Elevated Plus Maze test results. This figure depicts the number of entries made into open arms of the elevated plus maze as a proportion of all entries, by group and day. Purple squares represent female subjects and blue dots represent male subjects. Orange lines represent the mean proportion of entries into open arms. An * indicates $\alpha<0.05$.

There were no main effects or interaction effects found for total time spent in open arms, latency to enter open arm, or for the anxiety index score.

Results: Light-Dark Boxes

There was a significant main effect of sex on total entries into the light box, with females entering the light box more often than males $F(1,30) = 9.86$, $p < 0.05$, $\eta^2_p = 0.25$. There was also a significant main effect of day $F(1, 30) = 22.98$, $p < 0.05$, $\eta^2_p = 0.43$, with subjects entering the light box less often on their first day of testing compared to their second. A significant interaction for group and day was also found, $F(2,30) = 4.16$, $p < 0.05$, $\eta^2_p = 0.22$. Post-hoc analysis revealed the increased entries into the light box from Test Day 1 to Test Day 2 depended on subjects in Group A and Group C, but not on

subjects in Group B, where entries into the light box remained stable across both test days.

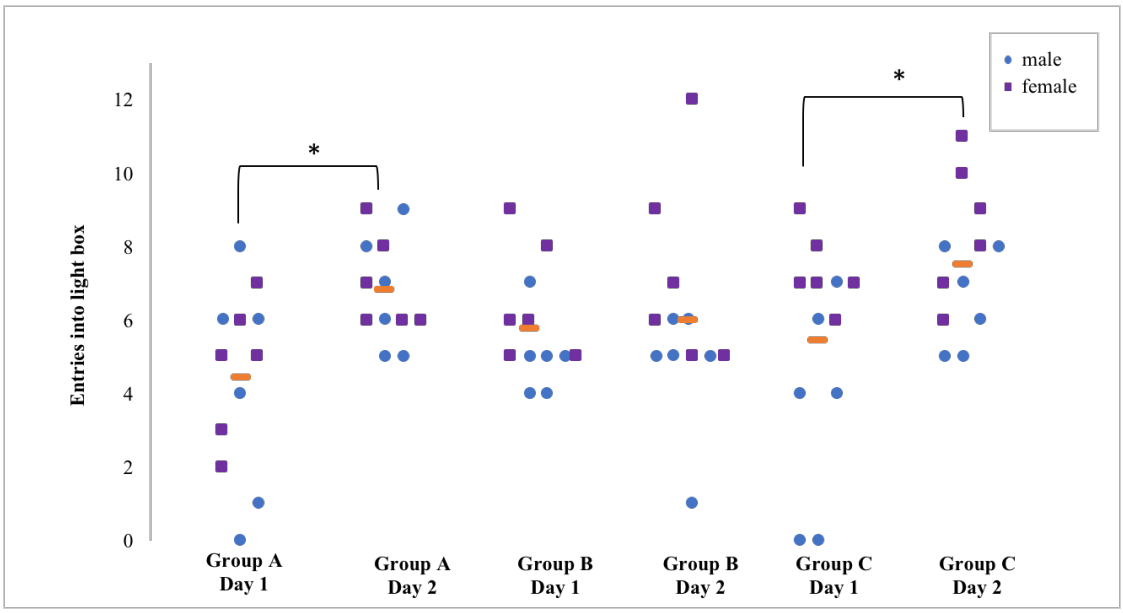

Figure 7. Light-Dark Box test results. This figure illustrates the total number of entries into the light box, by group and day. Purple squares represent female subjects and blue dots represent male subjects. Orange lines represent the mean number of entries into the light box. An * indicates $\alpha<0.05$.

There was a significant main effect of sex on latency, with females taking less time to enter the light box for the first time than males $F(1,30) = 4.558$, $p < 0.05$, $\eta^2_p = 0.13$. Subjects also took significantly more time to enter the light box for the first time on Test Day 1 compared with Test Day 2 $F(1, 30) = 8.498$, $p < 0.05$, $\eta^2_p = 0.22$. An outlier was detected in Group A Day 1, $Z = 2.46$, $p < .05$, and Group B Day 2, $Z = 2.81$, $p < .05$. There were no interaction effects found for latency to enter the light box.

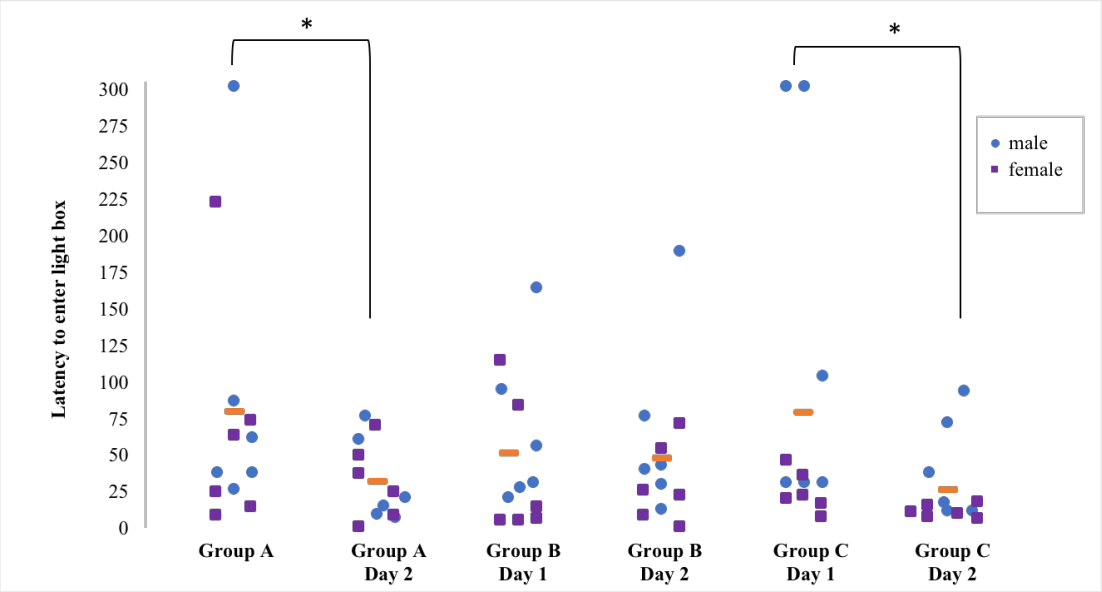

Figure 8. Light-Dark Box test results. This figure depicts subjects' latency to enter the light box for the first time, by group and day. Purple squares represent female subjects and blue dots represent male subjects. Orange lines represent mean latency. An * indicates $\alpha<0.05$.

There was a significant main effect of sex on total transitions between boxes, with females transitioning between the light and dark boxes more often than males $F(1,30) = 8.66$, $p < 0.05$, $\eta^2_p = 0.22$. A significant main effect of day was also found, $F(1, 30) = 31.23$, $p < 0.05$, $\eta^2_p = 0.51$, with subjects transitioning between boxes less often on Test Day 1 compared to Test Day 2. A significant interaction for group and day was also found, $F(2,30) = 4.33$, $p < 0.05$, $\eta^2_p = 0.22$, with post-hoc analysis revealing that increased transitioning between boxes from Test Day 1 to Test Day 2 depended on subjects in Group A and Group C, but not on subjects in Group B where transitioning remained stable across both test days. An outlier was detected in Group B Day 2, $Z = 2.42$, $p < .05$.

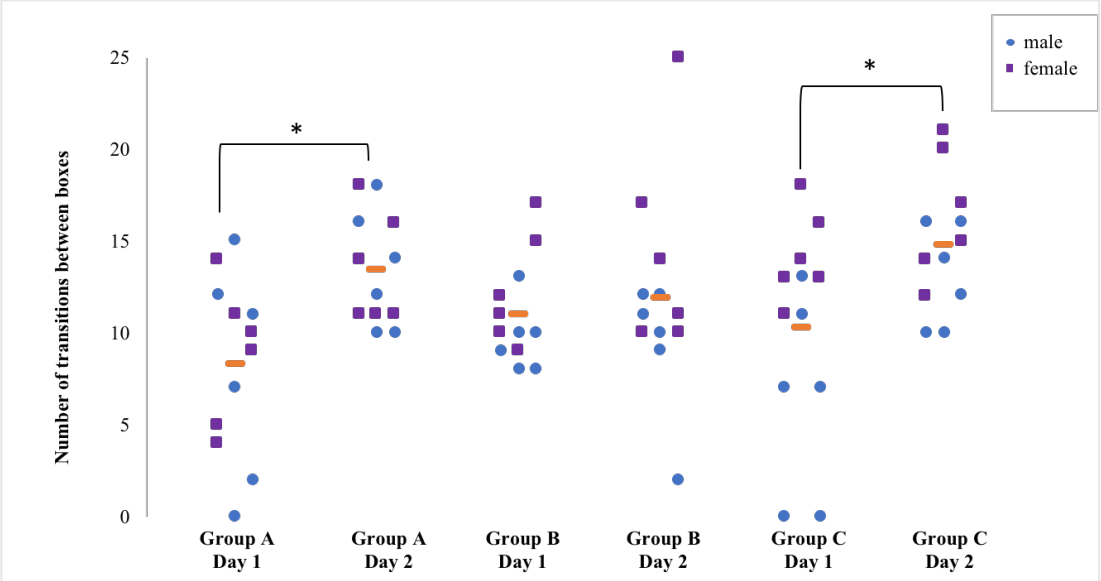

Figure 9. Light-Dark Box test results. This figure illustrates total number of transitions between the light and dark box, by group and day. Purple squares represent female subjects and blue dots represent male subjects. Orange lines represent the mean number of transitions. An * indicates $\alpha<0.05$.

There was a significant main effect of sex on total entries into the dark box, $F(1,30) = 7.39$, $p < 0.05$, $\eta^2_p = 0.20$, with females entering the dark box more often than males, and a significant main effect of day $F(1, 30) = 38.58$, $p < 0.05$, $\eta^2_p = 0.56$, with subjects entering the dark box less often on their first day of testing compared to their second. A significant interaction for group and day was also found, $F(2,30) = 4.16$, $p < 0.05$, $\eta^2_p = 0.22$. Post-hoc analysis revealed the increased entries into the dark box from Test Day 1 to Test Day 2 depended on subjects in Group A and Group C, but not on subjects in Group B, where entries into the dark box remained stable across both test days.

There was a significant main effect of day on total time in light box $F(1, 30) = 13.39$, $p < 0.05$, $\eta^2_p = 0.31$, with subjects spending less time in the light box on their first day of testing compared to their second. A significant interaction for group and day was also found, $F(2,30) = 4.11$, $p < 0.05$, $\eta^2_p = 0.22$, with post-hoc analysis revealing that the

increased length of time spent in the light box from Test Day 1 to Test Day 2 depended on subjects in Group A and Group C, but not on subjects in Group B, where time spent in the light box remained stable across both test days.

DISCUSSION

Our study compared physically active and sedentary subjects to determine if physical activity promotes greater resilience to anxiety-like behaviours. By analyzing anxiety-like behaviours directly, we were able to examine resilience in an indirect way. Responses to the behaviour tests used in this study are likely to reflect the way subjects typically respond to anxiety-inducing environments, including variations. For this reason, and because of the tightly controlled nature of the study, detected outliers were not removed from the data. Consistent with our first hypothesis, subjects who participated in physical activity displayed fewer indices of anxiety when placed in a novel environment, as demonstrated by increased consumption of palatable food. This relationship was moderated by the subject's sex. Male subjects who participated in four weeks of regular physical activity (i.e. running) suppressed their feeding to a lesser extent than all other subjects, including females in the same group as well as both sexes in the other two groups. Contrary to our second hypothesis, resilience was not greater in the cognitively-demanding group relative to the other groups.

Our second hypothesis was supported by the elevated plus maze test. Male and female subjects who underwent cognitively-demanding physical activity displayed fewer anxiety-like behaviours compared to controls who did not perform any physical activity. With no significant group differences in total exploration of the maze, the difference in

number of open arm entries was driven by the combined, and notably large, effect of physical and cognitive activity. This finding suggests cognitively-demanding physical activity can rescue some negative behavioural responses associated with an environment capable of inducing anxiety. A larger study beyond the scope of our current investigation would be required to determine if this enhanced resilience is a direct result of the combination of cognitive and physical activity, or if cognitive activity on its own produces the same result.

Contrary to our first and second hypotheses, resilience to anxiety was not greater in physically active experimental groups relative to controls in the light-dark box test. Physical activity had no effect on anxiety-like behaviours in this behavioural test, even when cognitively enriched. Although neither of our hypotheses were supported by the light-dark box test, this test did produce some unpredicted, but noteworthy, results. Female subjects displayed fewer anxiety-like behaviours in this environment, whether or not they had been physically active. There were significant differences between sexes in number of light box entries, latency to enter the light box, number of dark box entries and total box transitions. Females avoided the anxiety-inducing compartment and suppressed their exploratory behaviour less than males, and were, thus, more resilient to the anxiety-inducing aspects of this particular environment. When compared, male subjects demonstrated significantly more anxiety-like behaviours. Three male subjects did not even enter the anxiety-inducing compartment throughout their first day of testing, avoiding the light box completely. This finding aligns with research supporting the existence of behaviour-setting components of mood, including stress and anxiety, which are differentially regulated in males and females by exercise (Munive et al., 2016). We suggest

further exploration of this sex-dependent phenomenon and its underlying mechanisms is warranted, especially given our large effect sizes and because these results were not replicated in the other tests of behaviour. Indeed, our results in the novelty-suppressed feeding test show an opposite effect, as it was physically active males, not females, who demonstrated greater resilience in this specific environment compared to all other subjects. Likewise, the results we obtained in the light-dark boxes were not replicated in the elevated plus maze. Johnston and File (1990) found sex-dependent reduced aversion to anxiety-inducing compartments of the maze, indicating females may experience less anxiety. Though our findings in the elevated plus maze did not support such sex-differences, we did find evidence of sex-differences in the light-dark boxes. Our findings point to the possibility the novelty-suppressed feeding environment, the elevated plus maze, and light-dark environment test different aspects of anxiety, and that each of these environments affect males and females uniquely. Our findings also indicate males and females express anxiety-like behaviours differently, depending on both the environment and on the behavioural demands of the task.

We discovered a second unpredicted result in the light-dark box test. Subjects in Group B, who underwent cognitively-demanding physical activity, did not alter their behaviours from Test Day 1 to Test Day 2. Subjects in Group B responded almost identically on Test Day 2 as during their initial exposure, entering into and spending roughly equivalent amounts of time in the light box. In contrast, Subjects in Groups A and C entered more frequently and spent more time in the light box on Test Day 2, relative to Test Day 1. Group B's anxiety-like behaviours remained constant, with no indication of increased resilience. In contrast, Subjects in Groups A and C showed signs of enhanced

resilience, demonstrated by increased exploration, on Test Day 2 compared to Test Day 1. Our findings indicate subjects in Groups A and C behaved as if they had less anxiety, but nonetheless were completely unfamiliar with, or unhabituated to, their environment. Habituation, one of the simplest forms of non-associative learning, is the waning of a response as a result of repeated exposure to a novel stimulus, generally resulting is less exploratory behaviours by animal subjects. One explanation for this occurrence could be that regular exercisers and sedentary controls did not remember their environments as well. It is possible that anxiety interfered with the ability of subjects in Group A and C, but not Group B, to either store or retrieve environmental information in memory. The cognitively-demanding physical activity, Group B subjects participated in, may have lessened anxiety interference on memory. In this way, it is possible that participation in cognitively-demanding physical activity improved long-term memory of the light-dark environment. In the absence of results derived from specific tests of memory, however, it is difficult to conclusively decipher whether or not this cognitive function was affected by participation in cognitively-demanding physical activity.

Another possible explanation for the observed results is subjects in the cognitively-demanding groups found the light-dark box less interesting to explore. It could be the case these subjects were already used to being in an environment they found challenging, as a result of their experimental condition, and found the light-dark box less interesting and stimulating than the other groups. The literature suggests interest is tied to attention, and that selective attention to a stimulus leads to behavioural outcomes including investigation, exploration and interaction with the stimulus. In contrast, boredom arises when tasks are not challenging enough and an animal or person is unable to exercise skills they possess

on their environment (Meehan & Mench, 2007; Clark, 2011). Cognitively-demanding physical activity may have supplied subjects in Group B with greater cognitive challenge, over four weeks, compared to other groups in our study. This previous exposure to cognitive challenges could mean the group's stable activity levels across test days is a signal of boredom in the light-dark box environment. As activity (i.e. exploration) and lack thereof, can be associated with cognitive engagement and boredom alike depending on context, it is difficult to conclusively decipher if lack of interest or boredom is an adequate explanation of our findings without further investigation.

The results of our study suggest promising directions for future work. There are various ways to explore the human experience of anxiety in rodents. The behaviour test battery administered in our study is highly reliable based on extensive use of, particularly, the elevated plus maze and light-dark boxes in the literature, as validated models of anxiety. Our study is highly reliable in providing evidence for situational and environmentally-induced anxiety behaviours. In humans, however, anxiety is influenced by factors other than environment, which can trigger characteristic anxiety-induced behaving, including genetics (e.g. Binelli et al., 2013), personality and temperament (e.g. Clark et al., 1994), and personal history (e.g. Handa, 2008). The results of our study should encourage future investigators to explore additional tests to determine the effect of physical activity on other types of anxiety (i.e. induced by factors beyond environment), especially through concurrent rodent and human studies. As pre-training measures were not obtained to account for individual subject differences, our future research will include measures of variance between pre-training and post-training measures of anxiety. In doing so, we expect to capture the interaction effects of physical activity and individual differences, such

as anxiety sensitivity or personality traits like pessimism and optimism, which we expect may modulate anxiety resilience.

Our study design required subjects in Group B to learn a new motor skill (traversing a dowel runway) and then execute the same task multiple times over four weeks. Based on observation, this approach may have been limited as repeatedly executing a motor learning task did not appear to be cognitively-demanding once mastery inevitably occurred, after approximately 1-2 weeks. A better method to include in our future work will ensure cognitive components are consistently present throughout the entirety of a physical activity training phase. These future investigations will incorporate ongoing learning through successive mastery of increasingly complex physical activity tasks (e.g. tasks requiring ongoing or new learning, memorization, navigation, and decision making) so that we can better understand the combined effects of physical and cognitive activity on anxiety. Following administration of an anxiety test battery, we expect subjects exposed to increasingly complex cognitive activities in combination with physical activity (i.e. cognitively-demanding physical activity) to demonstrate resilience on more of the anxiety measures.

CONCLUSION

Physical and cognitive activity have typically been examined separately for their individual effects in promoting resilience to stress and anxiety, despite both being plasticity-inducing stimuli. The present study supports quality of physical activity as important promotor of resilience. Plastic changes induced by physical activity result in adaptive behaviours that promote resilience to anxiety-induced behaviours, most profoundly when physical activity is cognitively-demanding. Furthermore, females are

more resilient to anxiety induced by a light-dark environment, displaying fewer anxiety-like behaviours compared to males regardless if they have been physically active or not. The results of this study contribute to our understanding of the role physical activity plays in promoting resilience to the behavioural consequences of anxiety-inducing environments and adds to our knowledge of the different ways physical activity may moderate anxiety in males and females. The results of this study also provide insights into strategies for preventing anxiety-induced behaviours and suggest prevention strategies need to be tailored to specific tasks and environments.

CHAPTER 3: SIGNIFICANCE AND FUTURE DIRECTIONS

The goal of this research was to understand the effect of physical activity on anxiety directly, and on resilience indirectly. This research provides some evidence that physical activity promotes resilience to anxiety-like responding in rodents, and physical activity with cognitive components significantly and positively promotes resilience to a greater extent than regular physical activity (i.e. running). This study has broadened our understanding of the role physical activity plays in enhancing resilience to the behavioural consequences of anxiety-inducing environments and adds to our knowledge of the influencers of brain plasticity. Furthermore, these findings contribute to our understanding of strategies available to prevent, or even reverse, anxiety-induced behavioural responses in certain environments. My findings contribute to the literature on the behavioural consequences of brain plasticity and have a direct influence on researchers interested in brain plasticity, stress, anxiety, resilience, cognition, learning, and the protective properties of physical activity and exercise.

Several future directions have emerged from what I have learned through executing this experiment. If given the opportunity, there are directions I would follow in subsequent studies. In my experimental design, I investigated the effect of physical activity on anxiety-like responding by having subjects learn a new motor skill (to traverse a dowel runway) and then execute the same task multiple times over weeks. This approach was limited as repeatedly executing a learned task ceased to be a cognitively-demanding activity once mastery inevitably occurred. Based on observation, this mastery likely occurred during weeks 1-2. In future studies, I would be sure to account for mastery in any experimental design. Ongoing learning through successive mastery of

increasingly complex physical activity tasks (i.e. cognitively-demanding physical activity that includes tasks requiring ongoing learning, memorization, navigation, and decision making) would ensure cognitive components are consistently present throughout the entirety of a physical activity training phase.

If granted with the opportunity, I would incorporate a cognitive control group in addition to a physical activity control group in order to determine if any differential effects are truly due to the combination of physical and cognitive activity, or if cognitive activity alone is responsible for enhanced resilience. One Group B subject especially highlighted the importance of such a control group. Subject #29, though he appropriately executed the cognitively-demanding dowel task, nonetheless refused to voluntarily run on a cage wheel throughout the course of my study. As a result, this particular subject participated in much less physical activity overall than others in Group B (though still participated in the cognitively enriched motor task). Observing this subject's data derived from the three behaviour tests, it is apparent that he performed differently than other subjects, demonstrating greater indices of anxiety on nearly all measures. Observationally, his resilience to anxiety was low, demonstrated by a propensity for anxiety-like behaviours. The Grubbs' test too indicated that he was a statistically significant outlier (e.g. *Figure 8).* It would be prudent to test if these results would be replicated in a group of subjects through addition of a fourth experimental condition (i.e. cognitive activity control group) in the future. A cognitive control group would help me understand if the results I obtained in the present study are a direct result of the combination of cognitive and physical activity, or if cognitive activity on its own produces the same resilience effect.

It is possible a dose response exists for physical activity and anxiety resilience. A short-coming of my study is the lack of investigation into a potential dose response. Moving forward, I would look at the differential effects of different levels of physical activity (e.g. none, low, moderate and high) based on the amount of voluntary activity subjects elect to undergo. For example, this could include classifying exercisers based on the number of wheel rotations completed per session, or overall, likely amounting to a difference in kilometers of running based on observations of my collected data.

Finally, through undertaking this study, I learned about the importance of taking into account individual differences. Although I found several outliers in my data, I retained each value to reflect the diversity in responses that I expected would be seen in the population, largely due to individual differences. One difficulty that arises when it comes to promoting resilience to stress and anxiety is that many protective factors are uncontrollable and, thus, are not amenable to change, such as personality traits. Such factors are often referred to as individual differences. Though participation in physical activity is an exception to this and, as a result, I was able to manipulate it, I suggest future studies of a similar nature should also investigate the effect of individual differences on anxiety resilience. Just like humans, animals too are highly individual and differ in personality, motivation, aptitude and characteristic behaving. For example, Drozd et al (2017) found pessimistic rodents are more vulnerable to stress induced motivational deficits as compared to optimistic ones, and that pessimism in rodents is linked with higher vulnerability to stress-induced anhedonia. Optimism may be protective. As my present research was designed to examine one independent variable only, the potential for attributing effects to only one factor and missing others is large. In the future, I would

ensure my study designs account for individual differences. I would also ensure an experimental design would include measures of variance between pre-training and post-training measures as a way to capture individual subject differences. Assessing variance between pre-training, or baseline, and post-training measures of anxiety would be an experimental technique I would employ in future studies, in order to examine within-subject effects to further account for individual differences.

In conclusion, throughout this study I assessed behavioural responses in subjects who differed in quality of physical activity. The results of behavioural tests showed greater resilience among the physically active groups, relative to sedentary controls, and this effect was greatest when physical activity was cognitively enriched. Responses to the behaviour tests used in this study may reflect the way subjects typically respond to stressful situations and anxiety-inducing environments. Responses suggest that physically active individuals may be more resilient to the behavioural consequences of anxiety, especially when the physical activity involves the mind and not just the body. In at least some environments, it also appears females are more resilient than males, regardless of physical activity level. Although it has long been known that physical activity is good for our bodies, the results of this study emphasize it is good for our minds as well.

APPENDIX A

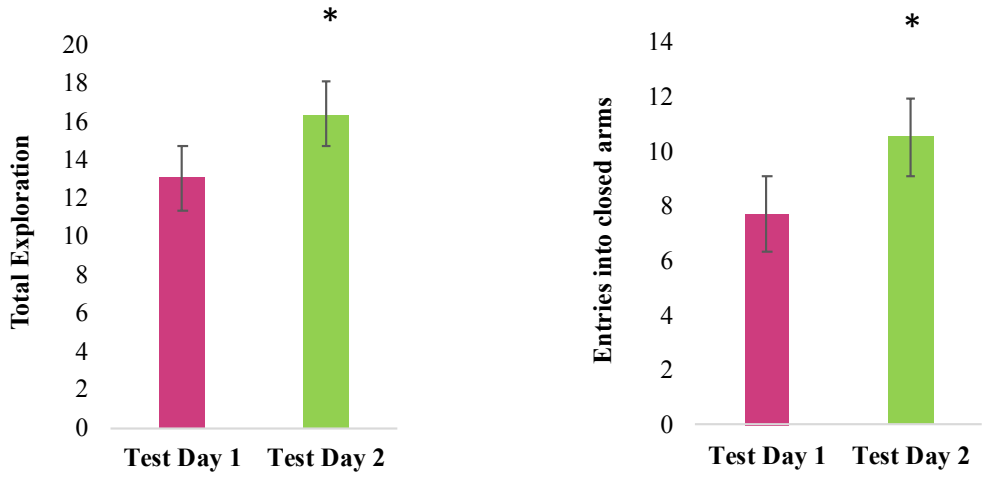

Figure 10. Elevated Plus Maze test results (collapsed across treatment conditions). This figure depicts the mean total exploration (number of entries into open and closed arms combined) into all arms of the elevated plus maze, and mean number of entries into the closed arms, by day. Error bars represent standard errors. An * indicates $\alpha<0.05$.

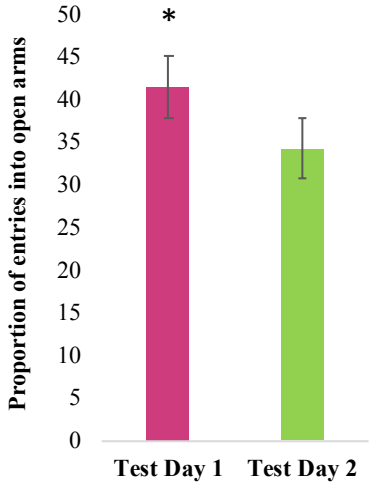

Figure 11. Elevated Plus Maze test results (collapsed across treatment conditions). This figure depicts the mean number of entries made into open arms of the elevated plus maze as a proportion of all entries, by day. Error bars represent standard errors. An * indicates $\alpha<0.05$.

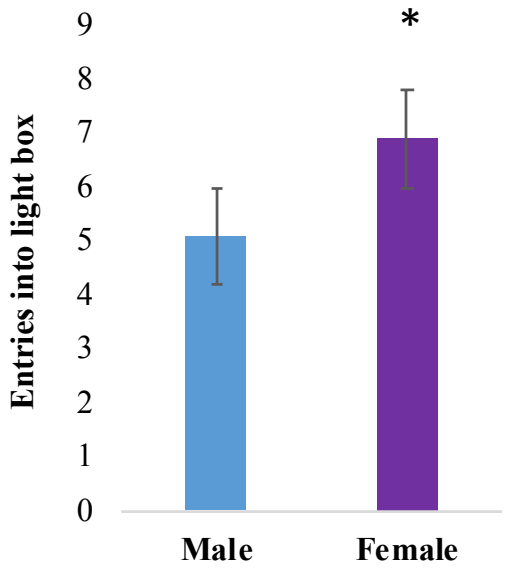

Figure 12. Light-Dark Box test results (collapsed across treatment conditions). This figure depicts mean number of entries made into the light box, by sex. Error bars represent standard errors. An * indicates $\alpha<0.05$.

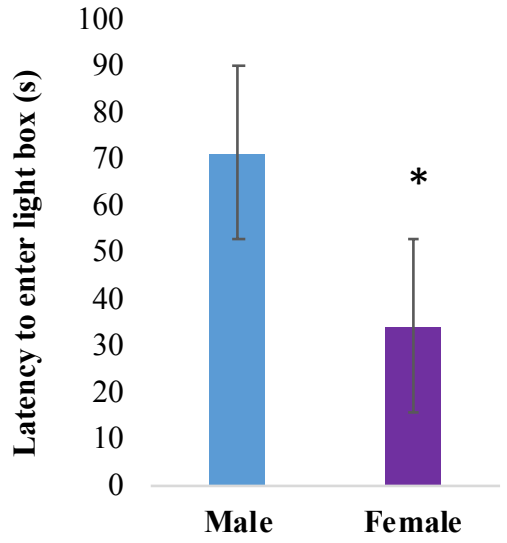

Figure 13. Light-Dark Box test results (collapsed across treatment conditions). This figure depicts mean latency to enter the light box for the first time, by sex. Error bars represent standard errors. An * indicates $\alpha<0.05$.

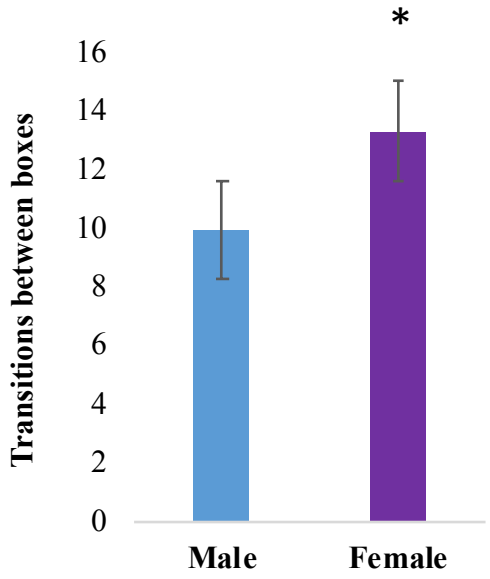

Figure 14. Light-Dark Box test results (collapsed across treatment conditions). This figure illustrates the mean number of transitions between the light and dark box, by sex. Error bars represent standard errors. An * indicates $\alpha<0.05$.

APPENDIX B

Table 1

Summary of the results of the novelty suppressed feeding behaviour test

		df	*F*	p	η^2_p
a. Number of chocolate chips consumed	Group	2	7.32	< .05	0.33
	Sex	1	1.91	> .05	0.04
	Group * Sex	(2, 1)	3.69	< .05	0.20
b. Latency to consume chocolate chips	Group	2	1.95	> .05	0.11
	Sex	1	0.01	> .05	0.01
	Group * Sex	(2, 1)	1.06	> .05	0.07

Table 2

Summary of the results of the elevated plus maze behaviour test

		df	*F*	p	η^2_p
a. *Number of entries into open arms*	Group	2	4.99	< .05	0.25
	Sex	1	1.79	> .05	0.06
	Group * Sex	(2, 1)	0.72	> .05	0.05
	Day	1	0.68	> .05	0.02
	Day * Group	(1, 2)	0.39	> .05	0.03
	Day * Sex	(1, 1)	1.24	> .05	0.04
	Day*Group* Sex	(2,30)	1.46	> .05	0.09

b. *Total exploration*	Group	2	1.70	> .05	0.10
	Sex	1	1.12	> .05	0.04
	Group * Sex	(2, 1)	0.19	> .05	0.01
	Day	1	13.74	< .05	0.31
	Day * Group	(1, 2)	1.01	> .05	0.06
	Day * Sex	(1, 1)	0.53	> .05	0.02
	Day*Group* Sex	(2,30)	1.43	> .05	0.09
c. *Number of entries into closed arms*	Group	2	0.26	> .05	0.02
	Sex	1	0.10	> .05	0.01
	Group * Sex	(2, 1)	0.15	> .05	0.01
	Day	1	24.93	< .05	0.45
	Day * Group	(1, 2)	1.00	> .05	0.06
	Day * Sex	(1, 1)	---	> .05	---
	Day*Group* Sex	(2,30)	0.70	> .05	0.04
d. *Proportion of entries into open arms*	Group	2	2.77	> .05	0.16
	Sex	1	1.73	> .05	0.06
	Group * Sex	(2, 1)	0.84	> .05	0.05
	Day	1	4.39	< .05	0.13
	Day * Group	(1, 2)	0.21	> .05	0.01
	Day * Sex	(1, 1)	0.80	> .05	0.03
	Day*Group* Sex	(2,30)	0.73	> .05	0.05
e. *Total time spent in open arms*	Group	2	1.482	> .05	0.09
	Sex	1	0.95	> .05	0.03
	Group * Sex	(2, 1)	1.17	> .05	0.07

	Day	1	3.48	> .05	0.10
	Day * Group	(1, 2)	0.02	> .05	0.01
	Day * Sex	(1, 1)	2.75	> .05	0.08
	Day*Group* Sex	(2,30)	0.84	> .05	0.05
f. *Latency to enter open arm*	Group	2	1.68	> .05	0.10
	Sex	1	2.22	> .05	0.07
	Group * Sex	(2, 1)	0.13	> .05	0.01
	Day	1	4.03	> .05	0.12
	Day * Group	(1, 2)	0.04	> .05	0.01
	Day * Sex	(1, 1)	0.19	> .05	0.01
	Day*Group* Sex	(2,30)	0.86	> .05	0.05
g. *Anxiety index score*	Group	2	1.84	> .05	0.11
	Sex	1	1.66	> .05	0.05
	Group * Sex	(2, 1)	1.00	> .05	0.06
	Day	1	3.11	> .05	0.09
	Day * Group	(1, 2)	0.08	> .05	0.01
	Day * Sex	(1, 1)	0.85	> .05	0.03
	Day*Group* Sex	(2,30)	0.49	> .05	0.03

Table 3

Summary of the results of the light-dark box behaviour test

		df	*F*	p	η^2_p
a. *Number of entries into light box*	Group	2	0.74	> .05	0.05
	Sex	1	9.86	< .05	0.25
	Group * Sex	(2, 1)	1.63	> .05	0.10
	Day	1	22.98	< .05	0.43
	Day * Group	(1, 2)	4.16	< .05	0.22
	Day * Sex	(1, 1)	0.18	> .05	0.01
	Day*Group* Sex	(2,30)	1.73	> .05	0.10
b. *Latency to enter light box*	Group	2	0.04	> .05	0.01
	Sex	1	4.56	< .05	0.13
	Group * Sex	(2, 1)	0.92	> .05	0.06
	Day	1	8.50	< .05	0.22
	Day * Group	(1, 2)	1.68	> .05	0.10
	Day * Sex	(1, 1)	1.81	> .05	0.06
	Day*Group* Sex	(2,30)	1.09	> .05	0.07
c. *Number of transitions between boxes*	Group	2	0.68	> .05	0.04
	Sex	1	8.66	< .05	0.22
	Group * Sex	(2, 1)	1.68	> .05	0.10
	Day	1	31.23	< .05	0.51
	Day * Group	(1, 2)	4.33	< .05	0.22
	Day * Sex	(1, 1)	0.50	> .05	0.02
	Day*Group* Sex	(2,30)	2.48	> .05	0.14

d. *Number of entries into dark box*	Group	2	0.62	> .05	0.04
	Sex	1	7.39	< .05	0.20
	Group * Sex	(2, 1)	1.69	> .05	0.10
	Day	1	38.58	< .05	0.56
	Day * Group	(1, 2)	4.16	< .05	0.22
	Day * Sex	(1, 1)	0.98	> .05	0.03
	Day*Group* Sex	(2,30)	3.22	> .05	0.18
e. *Total time spent in light box*	Group	2	0.34	> .05	0.02
	Sex	1	1.16	> .05	0.04
	Group * Sex	(2, 1)	0.07	> .05	0.01
	Day	1	13.39	< .05	0.31
	Day * Group	(1, 2)	4.11	< .05	0.22
	Day * Sex	(1, 1)	0.52	> .05	0.02
	Day*Group* Sex	(2,30)	0.29	> .05	0.02

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