THE INFLUENCE OF AEROBIC EXERCISE ON HIPPOCAMPAL STRUCTURE AND FUNCTION IN MALE ADOLESCENTS

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CERTIFICATE OF APPROVAL

This is to certify that the PhD dissertation of

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Table of Contents

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Abstract

A hallmark of adolescence is dramatic neurodevelopmental and cognitive change. It is also a time when a number of psychological disorders emerge, such as substance abuse and depression, which are accompanied by several features including learning and memory deficits. Thus, it is essential to understand what lifestyle factors influence typical brain development as a means to help foster better prevention and treatment programs for psychopathology during adolescence. Animal and human aging studies suggest that aerobic exercise has beneficial effects on the brain and subsequent learning and memory. However, little research exists on how exercise affects the human adolescent brain. Thus, the goal of this dissertation project was to examine the association between aerobic exercise and learning and memory and its brain basis in human adolescents. I hypothesized that aerobic fitness would relate to larger hippocampal volumes, better learning and memory performance, as well as enhancements in learning-related neural circuitry.

To this end, I examined how aerobic fitness related to verbal and spatial learning and memory in 34 male adolescents, ages 15 to 18 years. Furthermore, I collected structural and functional magnetic resonance imaging (MRI) data on this same sample of adolescents to explore how exercise related to hippocampal volume and learning-related neural circuitry. Results showed that aerobic fitness, as measured by VO_2 peak, relates to enhanced spatial learning (as measured by performance changes) on a virtual Morris Water Task, as well as larger hippocampal volumes; however, statistical requirements for the hippocampus to mediate the relationship between aerobic fitness and learning were not met. To determine the influence of exercise on neural functioning, brain activity during the encoding of new verbal associative memories was collected and compared

between 17 higher (HA) and 17 lower (LA) aerobically active male adolescents. Memory performance was similar on this task between HA and LA youth. During successful memory encoding, both groups activated the left prefrontal cortex and left hippocampus, and showed deactivation of default mode regions. However, compared to HA youth, LA adolescents showed less deactivation in default mode brain regions and also recruited the right prefrontal cortex and hippocampus when encoding information that was later remembered. Taken in the context of the vast literature of normative brain activity seen during memory encoding in adults, I suggest that these results may reflect atypical default mode network function in LA individuals. Furthermore, I argue that the recruitment of right hemisphere homologues (i.e. right prefrontal cortex and hippocampus) may represent a compensatory neural mechanism in LA youth, which may allow for them to obtain similar task performance to their HA peers. Together, these studies suggest that the exogenous factor of aerobic exercise influences spatial learning, hippocampal structure, and the neural basis of memory encoding during adolescence.

Chapter 1. Introduction

Adolescent brain development and cognition

Adolescence is the transitional period between childhood and adulthood during which significant neurodevelopmental and cognitive changes occur. Neuroimaging studies have shown that maturation of gray matter brain volume shows an inverted U-shaped pattern, with increases seen across childhood and peaking around early adolescence (Giedd et al. 1999a; Giedd 2004). This developmental trajectory, however, varies greatly by brain region. Sensory and motor cortices develop early on, while regions subserving higher cognitive functions, like the prefrontal cortex, undergo considerable maturation during the adolescent years (Sowell et al. 2004). Contrary to gray matter, white matter volume continues to increase with age into adulthood (Giedd et al. 1999a; Giedd et al. 1999b). In addition to cortical changes, subcortical regions, such as the hippocampus, also undergo significant development during this time. Specifically, hippocampal development continues for both males and females until adulthood (Giedd et al. 1996b). Furthermore, distinct changes in hippocampal subregions are seen across adolescence. The posterior subregion, including cornu ammonis field 3 and the dentate gyrus (see hippocampus anatomy description below), increases with age, while the anterior head decreases in volume with development (Gogtay et al. 2006). These volumetric changes in gray and white matter are likely to reflect synaptic pruning and increased myelination. In typical development, this maturation is associated with more efficient processing and coincides with significant changes in cognition, including better learning and memory (Casey et al. 2000; Sowell et al. 2001; Shaw et al. 2006). Together, these processes attest to the adaptive capability and the enormous plasticity of the teenage brain.

Given that the brain is still undergoing remodeling, the adolescent period is thought to be an especially sensitive period for environmental factors to impart their maximal effects on brain and behavior (Andersen 2003; Masten 2004; Marco et al. 2011). For this reason, it has been of great interest to the field of adolescent psychology and neuroscience to identify environmental factors that may influence normative, healthy adolescent brain development and cognition. This is especially of interest, as adolescence is also a time in which a number of psychiatric disorders and symptoms begin to materialize, including depression, substance abuse, and schizophrenia (Angold et al. 1999; American's Children: Key National Indicators of Well-Being 2009), which are associated with atypical brain structure, function, and cognitive deficits (Campbell et al. 2004; MacMaster and Kusumakar 2004; Nagel et al. 2005; de Castro-Manglano et al. 2011). Thus, by expanding our knowledge of exogenous factors that may influence typical brain structure and function in adolescents, we may ultimately be able to develop better youth-based prevention and treatment programs for psychopathology. Once identified, environmental and behavioral factors could be used to help facilitate positive adaptations in learning and memory, which could have implications for helping individuals achieve both academic and occupational success.

The goal of the current dissertation is strongly in line with the impetus in the fields of adolescent psychology and neuroscience to understand how teens' experience and their environment relate to brain and behavior. Specifically, this dissertation was aimed toward understanding if exercise relates to hippocampal structure and function in adolescents. Aerobic exercise was specifically chosen, as it has been shown to be a potent environmental factor that affects brain and behavior in a number of mouse models of psychopathology, including alcohol abuse and depression (Crews et al. 2004; Marais et al. 2009). Moreover, the reason for examining the influence of aerobic

fitness on hippocampal structure and function was two-fold. First, as previously mentioned the psychiatric disorders that emerge during adolescence are associated with deficits, including impairments in learning and memory and altered hippocampal structure and function (Campbell et al. 2004; MacMaster and Kusumakar 2004; Nagel et al. 2005; de Castro-Manglano et al. 2011). Thus, understanding what environmental experiences may benefit learning and memory capabilities and associated brain substrates is important for creating better prevention and treatment strategies aimed at addressing these disorders in adolescents. Secondly, while little research has been conducted with human adolescents, both animal and human literature strongly suggests that aerobic exercise affects the hippocampus (van Praag 2008; Erickson et al. 2009; van Praag 2009; Chaddock et al. 2010a; Erickson et al. 2011) — making it a reasonable first target in determining if exercise may benefit the human adolescent brain.

Learning and memory

Learning is a long-term change in behavior that results from previous experience. Memory is the representation of this learned behavior, while remembering, or retrieval, is the recovery of information from a memory store (Domjan 2003). Memory can further be broken down into short-term memory and long-term memory (Cowan 2008). Short-term memory is the ability to hold a limited capacity of information active in mind and lasts in the order of seconds, whereas long-term memory is the retention of vast information that lasts minutes, days, weeks, months, or even years. The transfer of information from short to long-term memory is known as memory consolidation (Domjan 2003).

Beyond these basic definitions, it has become increasingly clear that there are multiple forms of long-term memory which are subserved by distinct brain systems. Two-distinct classifications are declarative and non-declarative memory (Cohen and Squire

1980). Declarative memory (also referred to as explicit memory) is that of conscious recollection of information. This type of memory can be further broken down into conscious recollection of facts and knowledge (known as semantic memory), or autobiographical events (also known as episodic memory)(Tulving 1972). These types of declarative memory can be assessed by conventional tests of conscious retrieval, such as recall or recognition. In contrast, non-declarative memory is a term given to classify the wide array of memory processes that can be assessed without conscious retrieval (Cohen and Squire 1980). Also known as implicit memory, this includes procedural memory, which reflects skill-based (motor, perceptual, etc) learning and habits, as well as priming and simple classical conditioning. Non-declarative memories are therefore assessed through performance rather than conscious retrieval.

Distinct brain systems have been shown to subserve these different types of long-term memory (For review see Squire 1992a). Studies in rodents, non-human primates, and humans have shown that declarative memory depends on the hippocampus (see below), whereas non-declarative memories do not rely on this structure (Squire 1992b). Beyond the hippocampus, a number of cortical structures interact with the hippocampus and are important for learning new declarative information (Eichenbaum 2000a). Sensory cortices and association areas are needed for processing stimuli to be encoded by the hippocampus. The prefrontal and parietal cortices have also been shown to be important for declarative memories, as they are important for attention, organization, and use of various strategies needed to successfully encode and retrieve information. Lastly, other limbic brain regions also affect learning and memory behavior through motivation and emotional processes. Specifically, emotional arousal can enhance memory formation via stimulating stress hormones in the adrenal glands and activating noradrenaline and acetylcholine release in the amygdala, which can

influence memory consolidation (For review see Ferry and McGaugh 2000). The ventral striatum (including the nucleus accumbens) receives input from the amygdala and the hippocampus, as well as dopaminergic input from the ventral tegmental area. In addition to also being involved in emotional affect, like the amygdala, this region is also important in outcome prediction learning, and subserves motivational processing for goal-directed behaviors, such as learning new information (For review see Pennartz et al. 2011).

In contrast to the cortico-hippocampal networks seen for declarative memory, cortical areas interact with the dorsal striatum (caudate nucleus and putamen) to support procedural memories, such as skill learning and habits (For review see Packard and Knowlton 2002). Specifically, cortico-basal ganglia projections have been found between the striatum and cortex and these fronto-cortical-striatal loops are important for motor and stimulus-response learning. In addition, the cerebellum has also been shown to be important for motor learning for procedural tasks (For review see Saywell and Taylor 2008), as well as eyeblink and other simple forms of timing-based conditioning (Thompson and Steinmetz 2009; Freeman and Steinmetz 2011).

The hippocampus

The hippocampal formation is comprised of multiple subregions, including the cornu ammonis fields (CA3, CA2, CA1) and the dentate gyrus (DG), subiculum, presubiculum, parasubiculum (Andersen et al. 2007). Neocortical neurons provide input into the hippocampus via the shallow layers of the entorhinal cortex (EC). Among the EC afferents, a number of unidirectional, excitatory afferents project to the DG; this is the beginning of the major hippocampal pathway known as the perforant path. Granule cells of the DG then project their axons, known as the mossy fibers, to make contact with CA3 pyramidal cells. CA3 efferents, also known as the schaffer collaterals, then project to the CA1 subregion, and CA1 neurons, in turn, make excitatory connections with cells in the

subiculum. Neurons from the subiculum project to a number of regions, including the presubiculum, parasubiculum, retrosplenial cortex, and back to the deep layers of the EC. The EC efferents project primarily to the cortical structures via the perirhinal and parahippocampal cortices. While synapses of the perforant path are excitatory, using glutamate as their neurotransmitter, a number of inhibitory (GABAergic) interneurons are also found within the hippocampal formation (Andersen et al. 2007).

The hippocampus and declarative memory

The hippocampus plays a vital role in declarative learning and memory formation (Milner 1972; Squire 1982; Squire 1992b; Squire 2004). Evidence for hippocampal involvement in memory processes originally emerged from both human and animal lesion studies. Hippocampal damage in humans has been shown to result in memory impairments, including retrograde and anterograde amnesia (Milner 1972; Rempel-Clower et al. 1996). For example, neuropsychological assessment of the infamous amnesic patient HM provided strong evidence that the medial temporal lobe was important for declarative memory. After undergoing a bilateral medial temporal lobe resection as an attempt to reduce severe epilepsy, HM was unable to create new declarative memories (anterograde amnesia), but was fully capable of learning non-declarative memory tasks (Milner 1962). He also displayed severe retrograde amnesia, although he could remember remote events that occurred prior to his 15th birthday (Milner 1962). Studying HM provided evidence that the medial temporal lobe, including the hippocampus, was important for encoding new declarative information, and played a time-limited role in memory storage.

Although his surgery removed a large portion of the medial temporal lobe, which made it difficult to clarify what structures were responsible for HM's impairments, animal

studies have since confirmed the importance of the hippocampus in acquiring and retrieving declarative memories. Bilateral lesions in animals have been shown to impair learning and memory, including object discrimination and delayed non-match-to-sample tasks (Squire 1992b), as well as spatial memory (Morris et al. 1982). Furthermore, retrograde amnesia studies in non-human primates were able to replicate that the hippocampus is important for initial memory storage, but ultimately reduces its role in memory storage as time passes (Zola-Morgan and Squire 1990). Thus, with consolidation, there is reorganization of memory storage. That is, over time the more permanent memory is stored outside the hippocampus, in the cortex (Squire 1992b).

Recent advances of neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), have provided further evidence that the hippocampus is engaged during encoding of new declarative information as well as retrieval (Schacter and Wagner 1999; Davachi and Wagner 2002; Reber et al. 2002; Greicius et al. 2003; Giovanello et al. 2004; Binder et al. 2005; Karlsgodt et al. 2005; Chua et al. 2007). In terms of retrieval, the hippocampus is activated along with the prefrontal cortex; although this pattern is moderated by type of recollection (e.g. greater prefrontal activation for recall versus recognition tasks)(Okada et al. 2012). A more in-depth review of the functional circuitry of learning and memory, as determined by fMRI can be found below (see sub-section on Functional Neural Circuitry of Learning and Memory and Chapter 3's *Introduction* section).

The hippocampus role in memory formation may include binding new associations. Animal physiology research has shown that hippocampal neurons respond to relations between stimuli (associations), most notably spatial, but also including non-spatial, relationships (Squire 1994; Eichenbaum 2000b; Eichenbaum 2003). Again, human fMRI studies have provided further evidence for this idea, as hippocampal

activation is especially robust during associative learning and memory (Killgore et al. 2000; Davachi and Wagner 2002; Binder et al. 2005). Beyond associative encoding, the hippocampus is thought to be especially important in spatial memory and navigation. Hippocampal neurons have been shown to have unique place fields, as they activate in response to specific locations within space (O'Keefe and Dostrovsky 1971). Together, these characteristics have led to the idea that the hippocampus may help with creating and maintaining a cognitive map of the environment (O'Keefe 1991).

Taken together, it has become increasingly clear that the hippocampus subserves declarative learning and is involved in memory retrieval. The hippocampus's role in these processes spans a number of modalities, and includes binding both spatial and non-spatial information together to form new memories.

Aerobic exercise

Exercise is physical activity that is planned, structured, repetitive, and is purposely performed to allow for improvement or maintenance of physical fitness (Docherty 1996). *Aerobic exercise* is defined as sustained activity that stimulates heart and lung function, resulting in improved oxygen transport to the body's cells, and includes activities such as running, walking, swimming, and cycling (Armstrong and Welsman 2007). *Aerobic fitness* has, thus, been defined as the "ability to deliver oxygen to the muscles and generate energy during exercise" (Armstrong and van Mechelen 2008).

The assessment and interpretation of an individual's aerobic fitness can be challenging, as a number of methods exist, each with their own strengths and limitations. In the animal literature, aerobic exercise is usually studied in rodents by giving the animal access to a running wheel. Studies can then compare rodents who did or did not

have access to a running wheel, as well as how the duration, or training, directly relates to the dependent variable of interest (Pietropaolo et al. 2008). The changes seen in the dependent variable are interpreted as a result of *aerobic exercise*.

Compared to animal studies, it is much harder to accurately quantify aerobic exercise and aerobic fitness in humans. One common way to assess exercise in humans is to use self-report methods, such as a questionnaire, to determine the type and frequency of exercise (Armstrong and van Mechelen 2008). The strength of this method is that it is low in cost and subject burden, whereas the limitations of this method includes that it is purely subjective, and may be influenced by the opinion and perception of the participant. While self-reports of frequency and duration of aerobic activity levels are useful for assessing the amount of aerobic exercise, self-report of aerobic exercise tends to only modestly predict aerobic fitness level in adolescents (Morrow and Freedson 1994). To this end, an objective method of aerobic fitness may also be necessary when studying the effects of exercise. One such method is maximal oxygen uptake (VO_2 max). VO_2 max is the highest rate of oxygen consumption by an individual during exercise, and can ultimately determine an individual's ability to perform aerobic exercise (Armstrong and van Mechelen 2008). Below, I briefly describe the physiology underlying aerobic exercise, as well as how VO_2 max is measured. A more in-depth schematic of these processes can also be found in **Figure 1**.

During exercise, engaged muscles need energy (adenosine triphosphate, ATP), and ATP stores must be replenished for exercise to be sustained. Rapid replenishment first occurs through anaerobic catabolism of glucose, or glycolysis, which results in the by-products of carbon dioxide and water in the mitochondria or is further reduced into lactate. A larger, albeit slower, mechanism of ATP replenishment occurs aerobically, and is most of the energy used during exercise that occurs for longer than 1 minute. Thus,

aerobic fitness supports aerobic replenishment of energy, and is reflective of the ability to deliver and efficiently use oxygen by the working muscles (Armstrong and van Mechelen 2008) (**Figure 1a**). Given the importance of oxygen for energy during aerobic exercise, maximal oxygen uptake can be assessed with VO_2 max testing. This type of test includes incrementally increasing exercise intensity until volitional exhaustion. Oxygen uptake will increase almost linearly during the progressive exercise test until no further increase can be seen (a VO_2 plateau). A clearly defined plateau is required by definition to assess an individual's VO_2 max value. In pediatric exercise science, not all children and adolescents demonstrate a plateau, so a more common measurement of aerobic fitness in young individuals is VO_2 peak, or the highest VO_2 value observed during testing (Armstrong and van Mechelen 2008) (**Figure 2**).

In postpubertal adolescents and adults, regular aerobic exercise has been shown to relate to better oxygen delivery and utilization by the body, and thus higher oxygen consumption (e.g. VO_2 values) (Krahenbuhl et al. 1985; Rowland 1985). However, VO_2 testing is expensive and time intensive for the participant and researcher. Furthermore, while maximal VO_2 can be influenced by aerobic exercise, it should be noted that a number of genetic factors may also influence one's VO_2 max. In a familial heritage study, maximal heritability estimate for trainability of VO_2 max was approximately 47% (Bouchard et al. 1999). Furthermore, genetic composition of muscle fibers may impact VO_2 capacity. Specifically, slow-twitch muscle fibers have more mitochondrial mass (compared to fast-twitch muscle fibers) and thus can lead to greater mitochondrial capacity and increased oxygen consumption (Robergs and Roberts 1997). Therefore, similar to subjective methods, using the objective measure of VO_2 max as a sole marker as aerobic fitness also has its limitations.

Nonetheless, VO_2 max is still thought to be the "best single measurement of

aerobic fitness" (Armstrong and van Mechelen 2008). Thus, in the exercise and cognition literature, VO_2 max (or VO_2 peak) is largely used to define groups of varying levels of fitness, or as an independent variable of aerobic fitness (Etnier et al. 2006; Erickson et al. 2009; Chaddock et al. 2010a; Chaddock et al. 2010b; Pontifex et al. 2010; Voss et al. 2010a; Chaddock et al. 2011a; Erickson et al. 2011; Voss et al. 2011; Chaddock et al. 2012a; Chaddock et al. 2012b; Chaddock et al. 2012c). In order to stay consistent with the literature, in the current study we also assess and interpret VO_2 peak as an index of aerobic fitness. However, given the limitations that exist with the current methods, it may be especially useful to use multiple techniques to assess aerobic exercise. For this reason, both subjective (self-reports of aerobic activity levels) and objective (VO_2 peak) measurements were collected in the current dissertation in attempt to more fully characterize the relationships between aerobic exercise and brain-behavior relationships in adolescents.

Aerobic exercise: physiological and psychological adaptations

Aerobic exercise results in physical changes in both the periphery and the central nervous system, and it is associated with a number psychological benefits (Physical activity and health: a report of the Surgeon General 1996).

Peripheral Adaptations

A number of physical changes that occur with aerobic exercise training can be found in **Figure 1b** (Armstrong and van Mechelen 2008). Cardiovascular adaptations include enlargement of the heart and ventricular volume, as well as increases capillarization of the heart muscle and an increase in stroke volume. As a result of greater stroke volume, a lower resting heart rate and a slower increase in heart rate during exercise is also seen following aerobic training. Additional cardiovascular changes include lower blood pressure, greater absorption of oxygen from the

bloodstream, and increased blood volume. Changes are also seen to occur to the respiratory system, including increased lung ventilation and diffusion, as well as decreased utilization of the lactic acid system during exercise. Transformations also occur in the skeletal muscle, including increases in mitochondria size and number, capillarization, energy stores (glycogen, free fatty acids, and triglycerides), myoglobin stores, as well as increases in slow-twitch muscle fibers. Together, these physical adaptations allow for better oxygen delivery and utilization by the body, and thus higher oxygen consumption as detected by VO_2 values during aerobic VO_2 max testing (Armstrong and van Mechelen 2008).

Central Nervous System Adaptations

In addition to these peripheral changes, adaptations to the central nervous system also occur with chronic aerobic exercise training. Exercise increases cerebral blood volume (Swain et al. 2003; Pereira et al. 2007), as well as blood vessel surface area in the DG of the hippocampus (van Praag et al. 2005). Increases in angiogenesis (growth of new blood vessels) are also seen following chronic training, as vascular volume fraction is larger in the cerebellum and motor cortex (Black et al. 1990; Swain et al. 2003) in rats, and in the motor cortex in non-human primates (albeit this was moderated by age)(Rhyu et al. 2010). Angiogenesis in the hippocampus, as demarcated by an increase in glucose transporter (Glut1) positive vessel density, has also been reported in mice (Van der Borght et al. 2009). These vasculature changes are thought to be mediated by increases in endothelial cells and signaling factors such as vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF-1) which are stimulated by exercise (For review see van Praag 2008).

Neurotransmitter systems and additional growth factors are also affected by aerobic exercise (Meeusen et al. 2001; van Praag 2009). General motor behavior in

rodents leads to widespread increases in acetylcholine in the hippocampus, striatum, and frontal cortex (Day et al. 1991). Exercise-induced changes in NMDA receptor subtypes are also seen with regard to the glutamatergic system, including increases in NR2A and NR2B in the hippocampus (Molteni et al. 2002; Farmer et al. 2004). Physical exercise also impacts the monoamine system (For review see Chaouloff 1989). Increases are seen in tryptophan hydroxylase in the dorsal raphe neurons originating in the brainstem following exercise. Tryptophan hydroxylase is the rate limiting enzyme of serotonin biogenesis, and serotonergic neurons from the raphe nucleus innervate a number of brain areas including the hippocampus, which expresses serotonergic receptors. Furthermore, exercise has been shown to increase extracellular serotonin in the ventral hippocampus (Wilson and Marsden 1996). In addition, catecholamines (including dopamine and norepinephrine), glutamate, and GABA have been shown to increases in the striatum with an acute bout of exercise, although basal levels were found to be diminished in trained rats (Meeusen et al. 1997). Exercise-adaptations have also been noted in dopamine receptors. Endurance training results in greater dopamine binding to striatal D2 receptors, as well as increased dopamine metabolism in both young and old runners compared to controls (MacRae et al. 1987a; MacRae et al. 1987b). Moreover, exercise-related motivation has been linked to ventral striatal D1 receptors, as blockade of these receptors have been shown to decrease voluntary wheel running in mice genetically bred to be high runners (Roberts et al. 2012). Lastly, exercise also activates additional growth factors, including brain derived neurotrophic factor (BDNF) both within and outside the hippocampus (For review see Cotman et al. 2007). BDNF is important for synaptic plasticity, new cell growth, as well as cell survival and has been shown to activate a wide array of signal cascades including mitogen-activated protein kinase (MAPK), calcium/calmodulin-dependent kinase II (CAMKII), cAMP

response element-binding (CREB), and synapsin I (Vaynman et al. 2003; Vaynman et al. 2004a).

Psychological Benefits

Physical activity and aerobic exercise have been shown to relate to increases in positive mood, as well as decreases in self-reports of stress, depression, and anxiety in both clinical and non-clinical samples of adults (For review see Byrne and Byrne 1993). Similar benefits have also been seen in healthy and institutionalized adolescents, along with decreases in hostility, behavioral problems, confusion, and fatigue with aerobic training (Brown et al. 1992; Norris et al. 1992; Kirkcaldy et al. 2002). For these reasons exercise has been recommended as a potential treatment for psychiatric mood disorders (Barbour et al. 2007). While the mechanism(s) for these changes are unclear, it has been proposed that aerobic exercise effects on serotonin, dopamine, and norepinephrine may underlie these benefits (Ransford 1982; Chaouloff 1989; Dunn and Dishman 1991).

Chronic aerobic exercise and cognition

Acute exercise effect studies utilize a single session of aerobic exercise that occurs immediately preceding the measured outcome, whereas studies of chronic effects examine how repeated, regular, long-term aerobic exercise activity relates to dependent variables of interest (Coles and Tomporowski 2008; Hillman et al. 2008; Tomporowski et al. 2008; Hillman et al. 2009b; van Praag 2009). Although interesting, the acute effects of exercise are beyond the scope of the current project, as little research has been performed on this topic and how it relates to memory (Coles and Tomporowski 2008; Hillman et al. 2008). Moreover, the interpretations of an acute exercise study design are limited, given that it is difficult to parse out if changes in cognition are due to the specific effects of exercise, or reflect general increases in

arousal that accompany aerobic exercise (Hopkins et al. 2012). Thus, the current dissertation project was specifically interested in examining how repeated, long-term aerobic exercise influences brain structure and function.

In this regard, chronic aerobic activity has been shown to enhance cognition in both adult and aging rodents and humans (van Praag et al. 1999; Hillman et al. 2008). In rodents, aerobic exercise has been shown to increase learning and retention of memory as assessed by spatial (see below) and non-spatial tasks (Hopkins et al. 2011). In humans, working memory and executive performance has been found to be better in active elderly compared to their sedentary peers (Yaffe et al. 2009). Furthermore, a number of exercise intervention studies have been performed, in which non-active individuals are asked to participate in an exercise program for an extended period of time, and data is collected both before and after the intervention. A meta-analysis of 18 of these exercise intervention studies showed that aerobic exercise improves various aspects of cognition, including visuospatial skills, executive functioning, cognitive control, and speed of processing (Colcombe and Kramer 2003).

Similar to adult and aging rodent studies, exercise has been shown to enhance cognition in adolescent and young rats (Uysal et al. 2005; Lou et al. 2006; Hopkins et al. 2011). Furthermore, exercise is thought to also benefit human children. Specifically, some of the first reports of exercise and cognition in children showed a positive relationship between activity levels and measurements of scholastic performance, such as mathematic and reading achievement (Sibley and Etnier 2003; Castelli et al. 2007). Only within the past decade has there been an impetus to further examine how exercise impacts more direct measures of cognition in children, with most studies focusing on executive measures of attention and inhibition (Sibley and Etnier 2003; Hillman et al.

2005; Buck et al. 2008; Hillman et al. 2009a; Stroth et al. 2009; Pontifex et al. 2010; Voss et al. 2011; Chaddock et al. 2012a).

Aerobic exercise, learning and memory, and the hippocampus

Rodents

In young, adult, and aged rodents, wheel running has been shown to improve performance on a variety of spatial memory tasks including the Morris Water Maze, T-maze, and Y-maze (van Praag et al. 1999; Uysal et al. 2005; van Praag et al. 2005; Van der Borght et al. 2007). Aerobic exercise has also been shown to increase neurogenesis (Kim et al. 2004; van Praag 2008). Wheel running results in a 3- to 4-fold increase in both the production and survival of new cells in the DG (van Praag 2008) and increases in new cell growth have been shown to relate to larger DG volumes (Clark et al. 2008). While the exact mechanisms are unknown, a number of the physiological changes outlined above are thought to underlie exercise-induced neurogenesis. Specifically, vascular changes, including increases in endothelial cells as well as blood surface volume and surface area in the DG, may allow for trophic factors such as BDNF and IGF-1 to reach newly proliferating cells. Exercise-induced serotonin may also contribute, as hippocampal DG cells express 5-HT 1A receptors, which have been implicated in cell proliferation in this area (Radley and Jacobs 2002). In addition to neurogenesis, regular aerobic running has also been shown to increase neuron density and dendritic morphology in a number of hippocampal regions, including the DG and in the CA1 and CA3 subregions (Vaynman et al. 2004a; Uysal et al. 2005; Redila and Christie 2006).

These exercise-induced changes in hippocampal structure, including increases in neurogenesis, have been linked with the improvements seen in performance on spatial memory tasks (van Praag et al. 1999; Uysal et al. 2005; van Praag et al. 2005; Van der Borght et al. 2007). Beyond neurogenesis, a number of the other physiological changes

are likely to contribute to the exercise-induced benefits in learning and memory. For example, blocking BDNF's receptor in the hippocampus has been shown to reduce the exercise-induced enhancements in both spatial learning and memory as assessed by the Morris Water Maze (Vaynman et al. 2004b). The alterations in neurotransmitters may also play a role. Dopaminergic and serotonergic projections innervate the hippocampus and the frontal cortex, and thus alterations in these systems with exercise may lead to better learning and memory performance. Furthermore, as previously mentioned, motivation and emotion can also facilitate learning via connections between the hippocampus and other limbic structures such as the ventral striatum (Pennartz et al. 2011). Thus, exercise-related changes in ventral striatal dopamine may also contribute to the enhancements in spatial learning and memory reported in the literature.

Humans

Similar to the rodent literature, exercise has also been associated with improvements in memory performance and hippocampal volume in the elderly, and more recently, children. Cross-sectional and intervention designs have shown aerobic fitness, as determined by VO_2 peak, to positively relate to both short-term spatial delayed (3 seconds) match-to-sample performance and hippocampal volume in elderly subjects (ages: 55-81 years) (Erickson et al. 2009; Erickson et al. 2011). Furthermore, hippocampal volumes have been found to mediate the relationship between aerobic fitness (as measured by VO_2 peak or max) and memory performance in one of these samples (Erickson et al. 2009). In addition, aerobic intervention in adults (ages: 21-45 years) resulted in increased cerebral blood volume restricted to the DG, thought to reflect an *in vivo* correlate of neurogenesis (Pereira et al. 2007). Interestingly, this *in vivo* marker of neurogenesis also correlated with improved learning seen to occur as a result of exercise training (Pereira et al. 2007).

Little research has examined how exercise relates to learning and memory during childhood and adolescence. The paucity of exploration of exercise and memory relationships in children and adolescents may have stemmed from a 2003 meta-analysis examining the impact of physical activity on cognition in children (Sibley and Etnier 2003). Specifically, the authors concluded that while fitness was associated with better overall cognitive function, it did not relate to memory ability in youth ages 4 to 18 years of age (Sibley and Etnier 2003). However, this was based on 7 studies of memory, many of which did not have adequate control groups, and were not published in peer-review journals (Sibley and Etnier 2003). Beyond this meta-analysis, only two published studies, to date, have compared memory performance between high and low-fit children (ages: 9-10 years), as defined based on their VO_2 max value falling above the 70^{th} percentile or below the 30^{th} percentile according to normalized data (Chaddock et al. 2010a; Chaddock et al. 2011a). Chaddock and colleagues (Chaddock et al. 2010a) examined memory performance for novel visual stimuli between high and low-fit children. The novel visual stimuli were presented to participants using a relational (learning and recognizing visual triplets) or item-based paradigm (learning individual stimuli and recognizing them as either old or new). The results showed that lower-fit participants displayed a trend towards poorer performance on the material encoded relationally, although no differences were seen for item-based memory performance. Moreover, hippocampal volumes were found to mediate the relationship between aerobic fitness (as measured by VO_2 max) and relational memory performance. A similar study was published by the same researchers, examining relational versus non-relational encoding and recognition memory of face and house stimuli pairs (Chaddock et al. 2011a). Again, lower-fit individuals performed worse for recognition memory of

house and face pairs encoded relationally, whereas no group differences were seen in performance on the non-relational memory condition.

Taken together, these findings suggest that aerobic exercise is associated with larger hippocampal volume in children and the elderly, which plays a mediating role in the learning and memory benefits that result from aerobic activity. Although these relationships are similar to those seen in rodents, to date, the human studies have failed to employ readily available translational learning and memory paradigms. Translational research is necessary to create links between basic science and clinical practice, which may allow for a better understanding of how exercise influences brain structure and function. Furthermore, it cannot be automatically assumed that these exercise and memory relationships seen in children manifest themselves in a similar fashion in adolescents. As previously outlined above, distinct structural and functional changes are specific to childhood versus adolescence, and the adolescent time period is thought to be an additional sensitive period of brain development (Dahl 2004; Casey et al. 2008). Despite the documented proclivity of the adolescent brain to be impacted by exogenous factors (White and Swartzwelder 2004; Uysal et al. 2005), it remains unclear if and how exercise influences learning and memory during adolescence.

The first two aims of this dissertation research were to 1) assess the relationships between aerobic fitness and learning and memory behavior, as well as 2) examine the relationship between aerobic exercise and adolescent hippocampal volume in adolescents. In Chapter 3, I combine a translational learning and memory task (a virtual Morris Water Task (vMWT)) and structural neuroimaging techniques to address how exercise relates to brain volume and learning and memory in adolescents. *I hypothesized that greater aerobic fitness would be related to larger bilateral hippocampal volumes, as well as better performance on a number of learning and*

memory tasks, including the vMWT. Given that exercise induces neurogenesis, structural morphology, and neurotrophic factors in the hippocampus (van Praag et al. 1999; Uysal et al. 2005), I hypothesized that the relationship between aerobic fitness and learning and memory would be mediated by changes in hippocampal volume.

Functional neural circuitry of learning and memory

FMRI is a safe and non-invasive neuroimaging technique that measures blood oxygen level-dependent (BOLD) changes that occur with mental activity (Buxton 2002; Huettel et al. 2009). In particular, this method is based on the fact that changes in blood flow and oxygenation are tightly coupled to neural activity (see **Figure 3**). When local neurons are activated, there is an increase in blood flow carrying oxygenated blood. This oxygen-rich blood displaces paramagnetic, deoxygenated blood, ultimately resulting in an improved MR signal. By mapping changes in the BOLD signal, we can indirectly measure neuronal activity across time, and in relation to task demands (Buxton 2002; Huettel et al. 2009). Moreover, fMRI has a reasonable temporal resolution (in seconds) as well as superior spatial resolution for measuring brain activity in subcortical structures, such as the hippocampus, when compared to other neuroimaging techniques (Huettel et al. 2009). For these reasons, fMRI is a useful tool in studying the neural correlates of learning and memory in humans.

While there are a number of ways to assess learning and memory using fMRI, one of the most commonly utilized and powerful task-related designs is the subsequent memory effect paradigm (Kim 2011). Using this paradigm, brain activity can be examined during the encoding of information that is later remembered or forgotten (See *Introduction* and *Figure 1* of Chapter 4 for details).The primarily advantage of using this task is that it has been readily used by the field of learning and memory (over 100+ studies to date), and consistent patterns of memory-related brain response have

emerged. This rich literature is invaluable when hypothesizing and interpreting how the neural circuitry of learning and memory may be affected by various factors. Previous research has shown that neural circuitry underlying successful memory encoding using this task includes not only the activation of hippocampus and prefrontal areas, but also a submissive, deactivating default mode network (DMN)(see **Figure 4**)(Kim 2011). Although memory performance and structural changes in some of these brain regions (e.g. the hippocampus) have been shown to be influenced by aerobic exercise (Erickson et al. 2009; Chaddock et al. 2010a; Erickson et al. 2011), the influence physical fitness has on the neural circuitry underlying memory encoding has yet to be elucidated.

Given that exercise leads to adaptations in blood volume, cerebral blood flow, angiogenesis, and oxygen utilization, it is feasible that exercise may influence the fMRI BOLD signal. However, if this was the case, it would be expected that increased levels of aerobic fitness would lead to global changes in task-related brain activity. Furthermore, if aerobic exercise leads to changes in the BOLD signal, the direction of task-activation should be similar in all areas involved in the task. To date a number of studies have been used to assess how aerobic exercise influences task-related BOLD signal during cognitive control tasks, as well as BOLD-related functional connectivity (Voss et al. 2010a; Voss et al. 2010b; Voss et al. 2011; Chaddock et al. 2012a). Aerobic exercise did not result in global changes in brain activity in any of these studies. Furthermore, studies on acute exercise show that exercise-induced changes in these factors that may potentially affect the BOLD signal, including cerebral blood flow and cerebral oxygen to glucose uptake, are largely transient, with the values returning to pre-exercise levels within 60 minutes or less after completion of aerobic exercise (Dalsgaard et al. 2002; Williamson et al. 2009). A study on exercise and hippocampal blood flow also revealed specific rather than global changes following aerobic training (Pereira et al.

2007). Together, these findings suggest that aerobic exercise may not lead to global changes in blood flow, but rather the relative task-related changes seen in the BOLD signal with aerobic exercise may instead reflect neuronal adaptations.

In this regard, the third aim of the current project was to fill this existing gap in the literature by examining if aerobic exercise is associated with differences in the neural processes underlying successful learning and memory performance using fMRI. *I hypothesized that aerobic exercise would enhance memory performance on a verbal associative subsequent memory encoding task. I also hypothesized that successful memory encoding would be subserved by the hippocampus and PFC, as well as deactivation of regions of the DMN as seen by previous studies. However, given that exercise increases new cell growth, as well as a number of neurotrophic factors in the hippocampus (van Praag 2009), I hypothesized that hippocampal neurons may be more efficient, resulting in less hippocampal BOLD signal as measured by fMRI, when successfully encoding new memories.* Data addressing these hypotheses are presented in Chapter 4.

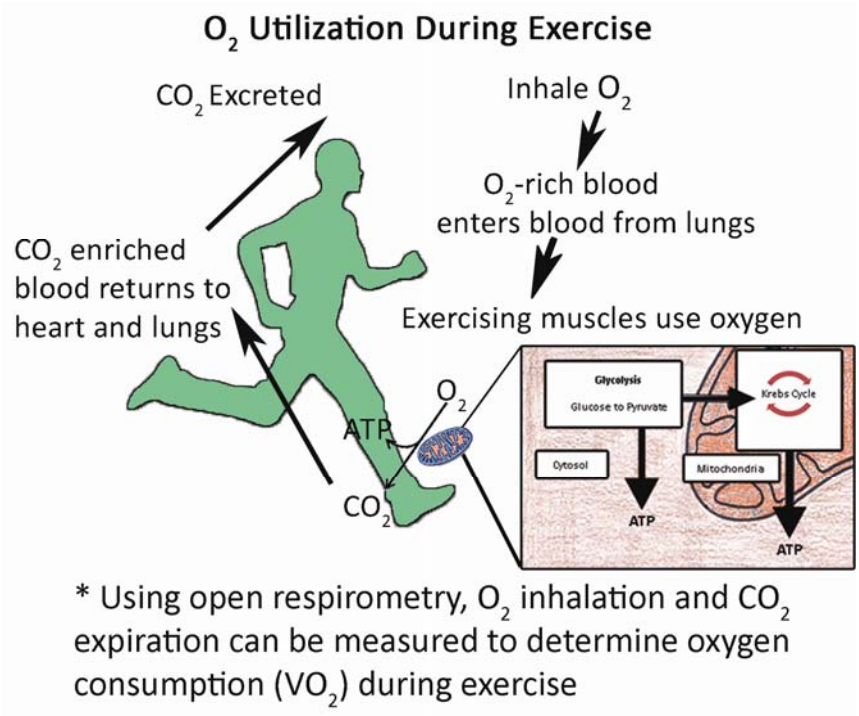
Summary

It is important to determine how exogenous and environmental factors impact the typically developing brain and behavior in order to help create better youth-targeted prevention and treatment programs for psychopathology. As established in the animal literature and reported in a handful of human studies, exercise may have beneficial effects on learning and memory, as well as affect hippocampal structure (van Praag 2009). It remains unclear if similar benefits of exercise on brain and behavior are present in human adolescents. Furthermore, beyond these changes, it is unclear if aerobic exercise may also influence the functional neural circuitry important for learning and

memory. Thus, the studies performed for this dissertation aimed to determine whether aerobic exercise relates to hippocampal structure and function in adolescents.

Figures

a)



b)

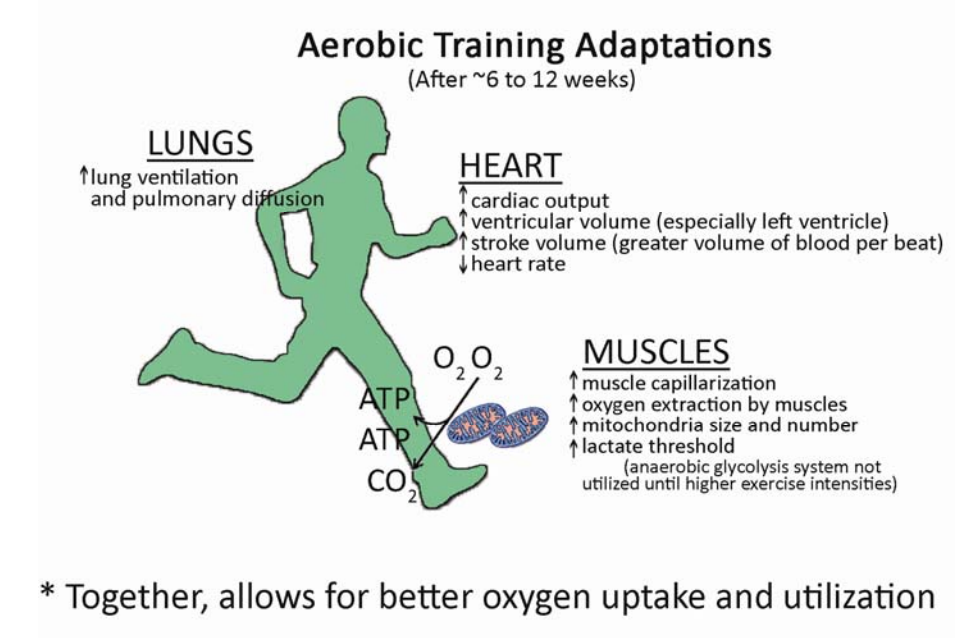


Figure 1. a) Oxygen use during an episode of aerobic exercise. b) A number of physiological adaptations seen with regular, chronic exercise, which allow for better oxygen utilization during aerobic exercise. Abbreviations: O_2 = oxygen; CO_2 = carbon dioxide; ATP = adenosine triphosphate (energy).

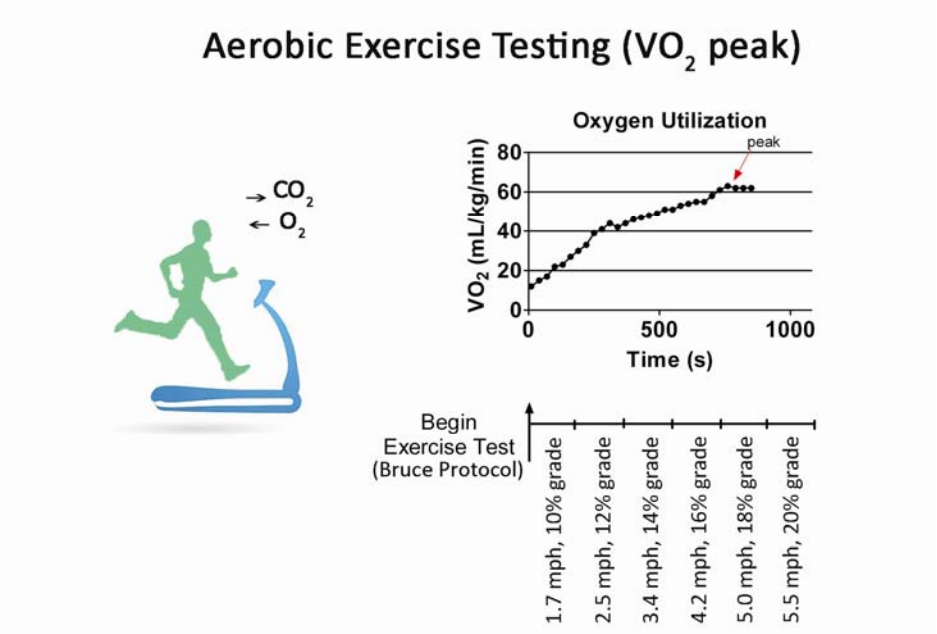


Figure 2. An example of how oxygen consumption (VO_2 peak) can be determined in the lab. Using open respirometry, oxygen inhalation and carbon dioxide expiration can be assessed while performing a progressively more intense treadmill workout (e.g. the Bruce Protocol; increase in speed and grade every 3 minutes). The highest VO_2 value obtained is known as VO_2 peak -- an objective marker of aerobic fitness in children and adolescents. Abbreviations: O_2 = oxygen; CO_2 = carbon dioxide; MPH = Miles per hour.

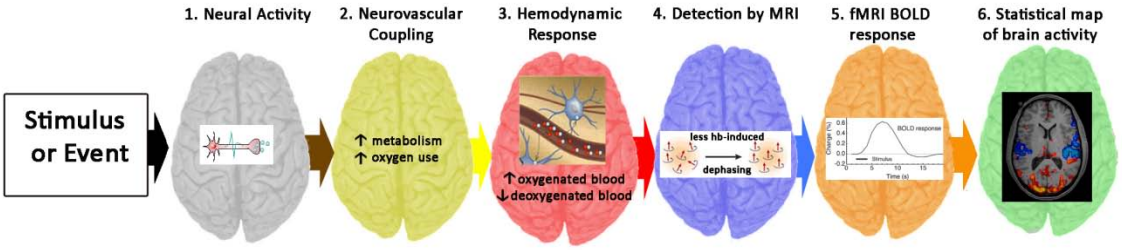


Figure 3. A schematic of how fMRI signals are used to indirectly measure brain activity.

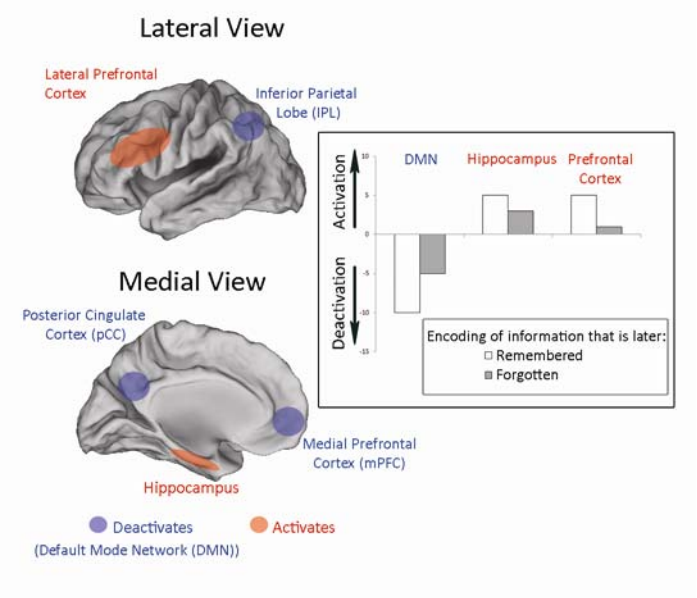


Figure 4. A simplified diagram of brain areas involved in memory encoding as seen by subsequent memory effect fMRI paradigm. Prefrontal cortex and hippocampal regions activate during encoding of new information, whereas default mode network (DMN) regions deactivate more to information later remembered than to information later forgotten.

Chapter 2. General approach

Participants

To test my hypotheses, a single group of adolescents were assessed. All participants were recruited through fliers, advertisements, and mailings distributed throughout the community and underwent comprehensive structured interviews as part of an ongoing study focused on adolescent neurodevelopment. Briefly, following written consent and assent from all youth and one of their biological parents, both youth and parent participated in separate structured telephone interviews to determine eligibility. Inclusionary criteria for youth included being male, 15 to 18 years of age, and meeting either high or low-active criteria defined below. Exclusionary criteria for youth included current diagnosed DSM-IV psychiatric disorder [Diagnostic Interview Schedule for Children Predictive Scales (DISC-PS-4.32b)(Lucas et al. 2001; Hoven et al. 2005)]; significant substance use (>10 lifetime alcoholic drinks or 2 drinks/occasion, > 5 uses of marijuana, any other drug use, or > 4 cigarettes per day) [Brief Lifetime version of the Customary Drinking and Drug Use Record (Brown et al. 1998)]; reported history of psychotic disorders in biological parents [Family History Assessment Module (FHAM)(Rice et al. 1995)]; major medical condition or significant head trauma [Structured Clinical Interview (SCI)(Brown et al. 1994)]; left-handedness [Edinburgh Handedness Inventory (Oldfield 1971)], or irremovable metal. All youth and their parent were each compensated $10 for completing the comprehensive structured interviews.

Procedure

After obtaining parental consent and youth assent, youth completed a structured telephone interview, which included assessment of aerobic activity participation. Using a modified version of the Youth Adolescent Activity Questionnaire (YAAQ; see Appendix), the number of hours of regular, organized physical activity was collected from youth for

each season (summer, fall, winter, spring) over the past year, including aerobic exercise and weight lifting. This questionnaire was chosen because seasonal format questionnaires, such as the YAAQ, have been shown to increase the accuracy of self-report of physical activity in adolescents (Rifas-Shiman et al. 2001). Youth were then determined eligible for the study if they met the criteria for being a high (HA) or low-active (LA) adolescent. HA youth were defined as those participating in an average of ≥10 hours per week of regular, organized aerobic physical activity purposely performed to allow for improvement or maintenance of aerobic fitness, including basketball, soccer, football, track, cross country, and swimming across one or more seasons (summer, fall, winter, spring) within the past year. LA youth were defined as those individuals that had participated in ≤1.5 hours of aerobic physical activity per week over the past year. These criteria were set forth, as significant increases in aerobic fitness have been seen in adolescents who participated in ≥10 hours of aerobic exercise per week (Brown et al. 1972; Weber et al. 1976; Lussier and Buskirk 1977). Furthermore, the hope was that the relatively extreme categorizations (≥10 versus ≤1.5 hours per week) would maximize the likelihood of detecting group differences. Based on these criteria, 17 youth were enrolled in the study as LA and 17 were HA. Every attempt was made to match HA and LA youth on a number of demographic variables

Once enrolled in the study, youth then completed three sessions at Oregon Health & Science University within a 15 day time window, including 1) a learning and memory assessment session, 2) aerobic fitness assessment, and 3) a MRI scanning session. HA youth were asked to participate in the study during the season in which they were most physically active based on their YAAQ self-report. Youth were compensated $100 for completing these 3 visits.

Participant Characterization

General Intelligence: Participants were administered the 2-subtest version of the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999) to estimate intellectual functioning.

Socioeconomic Status (SES): Information was gathered on SES by administering the Hollingshead Index of Social Position (ISP) to parents, as well as collecting information on total household income, as part of the structured telephone interview.

Body Mass Index: Strong associations exist between sedentary lifestyle and obesity, and differences have been noted between obese and non-obese individuals using neuroimaging techniques (Carnell et al. 2011). Thus, height and weight were obtained and the Center for Disease Control and Prevention Child and Teen Calculator was used to determine BMI (Center for Disease Control 2011).

Pubertal Status: Although more common in girls, intensive physical exercise has been reported to delay pubertal maturation (Georgopoulos et al. 2010). Thus, pubertal status was assessed using the self-rating Pubertal Development Scale (PDS) (Petersen et al. 1988).

Personal Lifestyle Questionnaire (PLQ): The Revised PLQ (Mahon et al. 2003) was used to assess general differences in lifestyle that may also explain group differences in performance or brain response. This is a 24-item questionnaire that has six subscales, including Nutrition (4 items), Safety (3 items), Relaxation (5 items), Health Promotion (4 items), Substance Use (4 items), and Exercise (3 items). The items are measured on a 4-point scale from 1 (never) to 4 (always). After reverse scoring some items, higher total score values indicate more positive health practices.

Extracurricular Activities: As another measure to assess differences in lifestyle between the groups, youth were asked the frequency in which they participated in extracurricular activities (including sports, clubs, recreational activities, etc.) on a scale of 1 (never) to 4 (at least once/week). In addition, a list of activities was obtained and the number of activities was summed for each subject. Furthermore, because physical activity has been shown to be negatively associated with time spent playing video games in adolescents (Janz and Mahoney 1997), average time spent playing video games was assessed. Subjects reported the console type used (e.g. PlayStation, etc.), the average number of hours on each console type per day (i.e. sessions), and the number of sessions per console type per week. Number of hours and number of sessions were summed across console types and multiplied together to assess the average number of hours of video games played per week.

Participant Demographics

One participant's parent (LA) chose not to disclose total household income, and one subject (HA) did not complete the PLQ. Participant characteristics, aerobic fitness, and body composition results can be found in **Table 1**. The majority of HA youth participated in an average of 10+ hours per week over the past year (n = 11), and the remaining HA that played sports more seasonally, still averaged a relatively high amount of aerobic exercise over the entire year (mean = 7.78, standard deviation = 2.4, n = 6) hours. The groups were matched on age, IQ, and were not significantly different on a number of lifestyle behaviors, including nutrition, relaxation, health promotion, safety, substance use, frequency of extracurricular activities, number of extracurricular activities, and video game habits. The groups were also matched on BMI. Although both groups came from households that made above the national income average, the HA had an overall higher SES and median household income, as reported by their parents.

Self-report of pubertal maturation was also different between the groups, with HA being less mature compared to LA. To account for these differences, SES and puberty were covaried for in all subsequent analyses. VO_2 peak testing was used to objectively measuring aerobic fitness and aerobic differences expected between the groups based on self-report of aerobic exercise participation. VO_2 peak values were significantly different between the groups, confirming that HA youth had better aerobic fitness compared to LA youth.

Analytic Strategy

The rationale for enrolling participants based on HA and LA criteria was two-fold. First, it was my hope that by examining differences between individuals with relatively large differences in aerobic exercise levels (≥10 versus ≤1.5 hours), I could maximize the likelihood of detecting a relationship between aerobic exercise and brain and behavior in adolescents.

Secondly, as previously stated, measurements of aerobic exercise in humans each have their strengths and weaknesses (See *Introduction: Aerobic Exercise*). Enrolling subjects based on self-reports allowed for a relatively easy, and more cost-effective way to evaluate physical activity (i.e. via the initial phone interview) in a large array of adolescents to find eligible participants based on the HA and LA criteria.

Although youth were recruited and consented for the study based on physical activity level, I also chose to collect an objective measurement of aerobic fitness (VO_2 peak) as part of the study design in hopes of confirming subjective reports. However, this measurement also allowed me to examine if VO_2 peak related to hippocampal structure and function across the entire sample. Therefore, separate analyses were run using group (HA vs. LA) and VO_2 peak as the independent variable of aerobic exercise in hopes of more fully characterizing the relationships between aerobic exercise and

brain and behavior in this sample of adolescents. Given that recruitment and enrollment was based on the HA and LA criteria, self-report aerobic activity levels had a bi-modal distribution (≥10 or ≤1.5) with little variance within each group [HA: mean = 11.3, standard deviation = 3.4; LA: mean = .26, standard deviation = .5], and thus, were not appropriate to use as an independent variable in the subsequent linear-based analyses.

In Chapters 3 and 4, I highlight the significant results seen using either independent variable of aerobic exercise (group, VO_2 peak); all other analyses can be found in the included supplementary material. Moreover, the difference in results seen between these two measurements of aerobic exercise (group versus VO_2 peak) is discussed in Chapter 5.

Demographics	**HA**	**LA**	
N	17	17	
Age	16.6 (.8)	16.2 (.8)	*t(32) = -1.36, p = .19*
% Caucasian	82.4	82.4	
IQ[a]	117.1 (11.8)	118.0 (8.1)	*t(26.1) = 0.26, p = .79*
SES[b]	18.3 (5.9)	26.5 (12.9)*	*t(22.6) = 2.39, p = .03*
Median Household Income[b] (Thousands)	130	90[¥]	
Puberty[c]	3.06 (.4)	3.3 (.3)*	*U = 80, z = -2.24, p = .026*
Aerobic Fitness			
Aerobic Activity (hrs/wk over past year)[d]	11.3 (3.4)	.26 (.5)	*t(16.6) = -11.33, p < .001*
Aerobic Activity (hrs/wk in season scanned)[d]	12.6 (3.8)	.23 (.5)	*t(16.5) = -13.39, p < .001*
VO_2 peak (mL/kg LBM/min)	77.7 (10.5)	67.0 (7.4)**	*t(32) = -3.41, p = .002*
Body Composition			
BMI[e]	21.6 (2.9)	22.4 (4.4)	*t(25.72) = .67, p = .51*
Lifestyle			
Nutrition [f¥]	12.0 (1.0)	12.4 (1.5)	*t(31) = 0.77, p = .45*
Relaxation [f¥]	15.2 (2.2)	15.4 (2.2)	*t(31) = 0.22, p = .83*
Health Promotion [f¥]	13.7 (1.3)	13.0 (2.3)	*U = 117, z = -.70, p = .51*
Safety [f¥]	14.2 (1.3)	15.0 (1.2)	*U = 86.5, z = -1.8, p = .07*
Substance use[f¥]	11.6 (.5)	11.3 (1.0)	*U = 122, z = -.58, p = .63*
Video Game Habits (hrs/week)	6.0 (5.9)	8.4 (9.6)	*U =127, z = -.60, p = .56*
Extracurricular Activities			
Frequency	4 (0)	3.5 (1.0)	*U =110.5, z = -2.09, p = .25*
Number	2.9 (1.3)	2.3 (1.3)	*U = 109, z = -1.28, p = .23*

[a] Wechsler Abbreviated Scale of Intelligence

[b] Hollingshead Index of Social Position

[c] Pubertal Development Scale

[d] Youth Adolescent Activity Questionnaire

[e] Body Mass Index

[f] Personal Lifestyle Questionnaire

Chapter 3. Aerobic fitness relates to learning on a virtual Morris water maze task and hippocampal volume in adolescents

(Portions of this chapter have been published in *Behavioural Brain Research*)

Introduction

In addition to its well-known physical benefits, aerobic exercise can positively impact the brain and improve learning and memory (For review see Hillman et al. 2008; van Praag 2009). These beneficial effects of exercise on brain and behavior have been extensively studied in the animal literature and have largely focused on the impact of exercise on hippocampal structure and learning and memory performance (van Praag et al. 1999; Uysal et al. 2005; van Praag et al. 2005; Van der Borght et al. 2007; Clark et al. 2008). More recently, human studies have been performed to examine how physical activity and aerobic fitness relate to cognition and brain volume. However, these studies have primarily focused on exercise's effects on executive or higher-order cognitive functions (e.g. attention, inhibition, etc.), and have largely been focused on examining these relationships at either end of the age spectrum, including the elderly and young children (Kramer et al. 1999; Colcombe and Kramer 2003; Hillman et al. 2005; Buck et al. 2008; Hillman et al. 2009a; Hillman et al. 2009b; Chaddock et al. 2011a; Chaddock et al. 2012a). Thus, the goals of the current experiment were to 1) implement a translational task in examining the relationships between aerobic fitness and hippocampal structure and subsequent memory behavior, and 2) to do so in a group of adolescents, as adolescence is an additional, and important developmental time period in which the brain may be especially sensitive to environmental influences (Andersen 2003).

Aerobic exercise has been shown to affect the hippocampus, which plays a vital role in learning and memory formation (Milner 1972; Squire 1982; Squire 1992b; Squire 2004). Wheel running results in neurogenesis in the dentate gyrus (van Praag 2008), as well as increases in neuron density in hippocampal regions outside the dentate gyrus, such as in areas CA1 and CA3 (Uysal et al. 2005). Furthermore, these exercise-induced

changes in the hippocampus are associated with improved performance on spatial memory tasks (Uysal et al. 2005; Van der Borght et al. 2007). In adolescent, adult, and aged rodents, wheel running has been shown to improve performance on a variety of spatial memory tasks including the Morris Water Maze, T-maze, and Y-maze (van Praag et al. 1999; Uysal et al. 2005; van Praag et al. 2005; Lou et al. 2006). Furthermore, inhibition of neurogenesis results in blocking exercise-induced enhancements in spatial learning and memory performance (Clark et al. 2008), suggesting that the aforementioned exercise-induced changes in hippocampal neurogenesis may directly contribute to these reported learning and memory benefits.

Although the histology underlying volumetric changes seen in the human hippocampus is less clear, human studies are in agreement with the animal literature that aerobic exercise impacts hippocampal structure and learning and memory (Etnier et al. 2006; Winter et al. 2007; Erickson et al. 2009; Chaddock et al. 2010a; Chaddock et al. 2011a; Erickson et al. 2011). However, the few studies showing associations between aerobic activity, hippocampal size, and memory performance have only focused on elderly or child populations. The influence of exercise on the brain and subsequent memory has not been examined in adolescents, and previously found associations between fitness, hippocampal volumes, and behavior may not be ubiquitous across the lifespan. For example, the aerobic effects seen in previous studies on aging adults may not be applicable to younger individuals, as normal age-related processes may interact with, and contribute to, the ultimate effect of aerobic exercise on brain and behavior that have been reported. This concept that aerobic exercise may not have unique actions at different parts of the lifecycle is also likely true for adolescence. During adolescence, the brain continues to develop both structurally (Giedd et al. 1996a) and functionally (Durston et al. 2006; Fair et al. 2009), with a number of changes seen in

hippocampal volume between childhood and adulthood (Giedd et al. 1996b; Gogtay et al. 2006). Given the dynamic properties of the teenage brain, this period may be especially sensitive to the effects of exercise. In support of this idea, the effect of exercise on changes in hippocampal structural has been shown to be age-dependent, with more robust hippocampal neurogenesis seen in adolescents and young rats compared to compared to older ones (Kim et al. 2004).

Lastly, although studies have examined the relationships shown between physical activity, hippocampal volume, and memory, no study, to date, has examined these relationships using a directly translational learning and memory task. Rather, the previous human studies have examined learning and memory tasks that cannot be used in animals, as they often require additional higher-order cognitive processes such as language or other executive functions (Winter et al. 2007; Chaddock et al. 2011a). Interestingly, virtual spatial navigation tasks that are analogous to the infamous Morris Water Maze rodent task have been successfully created and utilized to assess hippocampal-dependent spatial learning and memory (Burkitt et al. 2007; Nowak and Moffat 2011). Yet, to our knowledge, no study has used such translational approaches to examine the influence of exercise on the hippocampus and subsequent memory in humans. Given that human cross-sectional studies are correlational in nature, and therefore are limited by their inability to determine causal relationships, translational designs may help to better elucidate our understanding of how exercise affects the human brain and behavior.

In the current study, we examine the relationships between aerobic fitness level, hippocampal volume, and learning and memory in adolescents, ages 15 to 18. Furthermore, in an attempt to increase the translational aspect of this experiment, we utilized a validated and previously published virtual Morris Water Task (vMWT)(Nowak

and Moffat 2011) as one of our measures to assess the influence of aerobic fitness on learning and memory in these youth. Based on previous human and animal research (Uysal et al. 2005; Chaddock et al. 2010a; Erickson et al. 2011), we hypothesized that greater aerobic fitness would be related to larger bilateral hippocampal volumes, as well as better performance on all tasks of learning and memory, including visuospatial memory on the vMWT. Furthermore, in order to show some regional specificity of exercise's effect on brain and subsequent behavior, we also examined the relationships between total gray and white matter volume and aerobic fitness, as well as with learning and memory performance. Given that exercise induces neurogenesis, specifically in the hippocampus (van Praag et al. 1999; Uysal et al. 2005), we also hypothesized that the relationship between aerobic fitness and learning and memory would be mediated by changes in hippocampal volume.

Materials and methods

Participants

Participants included 34 eligible male youth. All participants were recruited through fliers, advertisements, and mailings distributed throughout the community and underwent comprehensive structured interviews as part of an ongoing study focused on adolescent neurodevelopment. Briefly, following written consent and assent from all youth and one of their biological parents, both youth and parent participated in separate structured telephone interviews to determine eligibility. Inclusionary criteria for youth included being male and between the ages of 15 to 18 years old. Exclusionary criteria for youth included current diagnosed DSM-IV psychiatric disorder [Diagnostic Interview Schedule for Children Predictive Scales (DISC-PS-4.32b)(Lucas et al. 2001; Hoven et al. 2005)]; significant substance use (>10 lifetime alcoholic drinks or 2 drinks/occasion, > 5 uses of marijuana, any other drug use, or > 4 cigarettes per day) [Brief Lifetime version

of the Customary Drinking and Drug Use Record (Brown et al. 1998)]; reported history of psychotic disorders in biological parents [Family History Assessment Module (FHAM)(Rice et al. 1995)]; major medical condition or significant head trauma [Structured Clinical Interview (SCI)(Brown et al. 1994)]; left-handedness [Edinburgh Handedness Inventory (Oldfield 1971)], or irremovable metal. All youth and their parent were each compensated $10 for completing the comprehensive structured interviews, and youth were further compensated $100 for completing behavioral tests and MRI scanning.

The current study limited its recruitment to male adolescents for a number of reasons. Specifically, a number of intrinsic sex differences have been reported in hippocampal volumes (Giedd et al. 1996b; Gogtay et al. 2006), aerobic capacity and physical fitness level (Krahenbuhl et al. 1985; Riddoch et al. 2004), and virtual maze task performance (Astur et al. 2004; Lovden et al. 2007; Newhouse et al. 2007). Furthermore, there is overwhelming evidence that aerobic exercise negatively impacts pubertal development in girls (Sherman and Thompson 2004), which may alternatively impact brain development and behavior (Sisk and Zehr 2005) (also see 2.4.3 *Pubertal Status below*). Due to these various gender-specific disparities between female and male adolescents, we chose to begin to address the question of how exercise impacts hippocampal structure and function in one sex. By examining these relationships in males only, we aimed to reduce variability that may otherwise arise from inherent and aerobic-induced differences between the sexes.

Demographic information

Information was gathered on age, ethnicity, grade point average (GPA), and socioeconomic status (SES) as part of the structured telephone interview to determine eligibility. SES was assessed by administering the Hollingshead Index of Social Position

(ISP) to parents. The Hollingshead ISP determines socioeconomic status based on occupation and educational attainment of each parent (Hollingshead 1975).

Procedure

Youth completed three separate sessions at Oregon Health & Science University within a 15 day time window, including 1) a learning and memory assessment session, 2) aerobic fitness and physical activity assessment, and 3) a MRI scanning session. Given that there are also acute effects of exercise that have been previously reported on learning and cognition (Coles and Tomporowski 2008; Hillman et al. 2009b), aerobic fitness testing never preceded the other two sessions on the same day.

Learning and memory assessment

Rey Auditory Verbal Learning Test (RAVLT)

The RAVLT (Rey 1941; Rey 1964) is a widely accepted neuropsychological test used to evaluate verbal learning and memory. This test was chosen because recall on this task has been shown to correlate with hippocampal volume in healthy elderly individuals (Reiman et al. 1998), and aerobic fitness in healthy adults (Pereira et al. 2007). Briefly, this test asks participants to learn a list (list A) of 15 unrelated words over 5 trials with an immediate recall after each trial. A second, novel 15 word list (list B) is then presented, again followed by a recall trial. Immediately after the second list, participants are asked to recall words from list A. In addition, participants are asked to recall the first word list after a 20 minute delay. The dependent measure for verbal learning was total number of words learned from list A over the 5 trials, normalized for age, whereas a retention score (computed by subtracting the immediate recall of trial 5 from the delay recall) was used as the primary dependent variable for verbal recall memory, as it takes into account the number of words initially encoded (baseline learning effects)(Sowell et al. 2001).

Virtual Morris Water Task (vMWT)

Spatial learning and memory was assessed using a previously published, computerized vMWT protocol (Nowak and Moffat 2011). This task was designed to mirror the rodent spatial navigation test, known as the Morris Water Maze, in order to help compare assessment of spatial learning and memory between rodents and humans. The vMWT was chosen because bilateral lesions of the hippocampus impairs probe memory performance in rats and mice (Eichenbaum et al. 1990), and hippocampal size has been shown to correlate with vMWT memory performance in humans (Driscoll et al. 2003).

The vMWT was displayed on LCD computer monitor, and participants used a joystick (Logitech Attack 3) to move through the environment. The task consisted of 1 practice trial, 6 learning trials, a 30 minute delay probe trial, and a visual platform trial.

For the practice trial, participants were immersed into a vMWT environment that consisted of a pool of water within a larger room with ceiling, floor, walls, and four objects placed around the pool. Within the pool, there was a hidden platform that the participants were instructed to find as fast as possible. When the participant “swam” over the platform, it emerged from the water and hoisted them “out of the pool” for 10 seconds. During this 10 second period, the participant was rendered immobile except for the ability to rotate 360 degrees. This practice trial allowed participants to gain experience with the environment and sensitivity of the joystick, as well as how the vMWT platform works.

After the practice trial, the participant completed 6 learning trials. These included introducing the participant to a new vMWT environment that was similar to the practice trial, except there were six objects which were all different than the practice trial, and the hidden platform was now located in a new location. Participants were instructed to locate

the hidden platform as quickly as possible during the 6 trials. They were informed that the platform would remain in the same location. Participants began each learning trial from one of six random start positions. As in the practice, they remained on the platform for 10s each time they found it and were only able to rotate 360 degrees during this time. During each trial, the computer recorded the *x,y* coordinate position approximately every .02 seconds and was used to calculate total distance traveled on each trial. The computer also recorded the total distance traveled in each quadrant of the pool, including the quadrant in which the platform was located, also known as the target quadrant. Percent distance traveled in the target quadrant was calculated for each subject across all 6 trials. The amount of learning that occurred across the 6 trials was determined for each participant by calculating delta (δ), or the change, between percent distance traveled in the quadrant between trial 1 and trial 6 (δ = trial 6 percent distance in target quadrant– trial 1 percent distance in target quadrant) and used as the dependent measures for spatial learning.

Thirty minutes following the last learning trial, a 60 second probe trial was completed. Unbeknownst to the participant, the platform was removed from the pool. All participants began the probe trial in the same start position. The percent distance traveled in the goal quadrant and the number of times the participant intercepted the platform, had it been present, were determined for each participant as the dependent variables of spatial delayed memory recall.

After the probe trial, participants were placed into the same pool used during learning, but the platform location was visible and marked by 4 red flags. Participants started from one of the six random start positions, and were instructed to navigate to the visible platform as fast as possible. Latency to travel to the visible platform was determined for each subject. This trial was used as a control trial to ensure visuomotor

ability did not relate to aerobic fitness.

Aerobic fitness and physical activity assessment

Aerobic fitness and activity levels were examined in adolescents by collecting self-reports of aerobic exercise activity levels, as well as objectively measuring their aerobic capacity.

Youth Adolescent Activity Questionnaire

A modified version of the Youth Adolescent Activity Questionnaire (YAAQ) was administered during the structured interview with the youth to assess participation in aerobic activities over the past year. The YAAQ asks detailed questions about average physical activity participation across all four seasons of the year, as well as the number of hours per week spent doing each activity (Wolf et al. 1994). The number of hours performed doing aerobic activity per week were then calculated to give the total number of hour of aerobic exercise per week for each of the four seasons, as well as the average of aerobic activity across the entire year. This questionnaire was chosen because seasonal format questionnaires, such as the YAAQ, have been shown to increase the accuracy of self-report of physical activity in adolescents (Rifas-Shiman et al. 2001). As previously mentioned, based on self-report, youth were identified as either high-active (HA) or low-active (LA).

Aerobic Capacity Testing

Aerobic fitness was measured by determining the highest rate of oxygen that could be consumed during exercise (VO_2 peak). VO_2 peak is a measure of the highest rate of an individual's body to transport and utilize oxygen during incremental exercise, and is thought to be the most valid objective measurement of aerobic physical fitness (Armstrong and Welsman 2007). The same computerized indirect calorimetry system (VMax Series, V6200 Autobox, Sensormedics, VIASYS Healthcare) was used to assess

VO_2 peak in each subject during a Bruce Protocol (Bruce et al. 1973). Specifically, the Bruce Protocol required participants to run on a motor-driven treadmill that began at a speed of 1.7 mph and 10% grade, with increases in speed and grade every 3 minutes until volitional exhaustion. In addition, heart rate was measured throughout the fitness test, and ratings of perceived exertion were assessed every 2 minutes on a scale of 0 (very easy) to 10 (very hard). VO_2 peak values were only considered valid if the participant had delivered maximal effort on the test as defined by at least one of the following the physiological criteria as outlined by Armstrong and van Mechelen (Armstrong and van Mechelen 2008): 1) oxygen consumption remained at a steady state despite an increase in workload, as evidenced by a plateau in oxygen consumption, 2) heart rate reached ≥ 200 beats per minute, 3) the respiratory exchange ratio ≥ 1.0; and/or the subjective criteria of reporting a 10 on the perceived exertion scale.

Lean body mass (LBM) was determined just prior to aerobic testing by conducting a bioelectrical impedance test on each subject using the Body Composition Analyzer, Model 310e (Biodynamics Corp, Seattle, WA). Bioelectrical impedance analysis examines the body's impedance or resistance to a generated low-level electrical current (<1 milliamp), accurately and reliably allowing for an estimate of lean and fat tissue content to determine percentage of body fat (For review see Kyle et al. 2004). Peak oxygen consumption was then expressed in mL/kg LBM/min and used as the independent measure of aerobic fitness. Scaling by lean body mass was used as it has been found to be a more accurate way of expressing fitness in relation to aerobic metabolism and reduces the possibility of body fat as a confounding variable in youth (Dencker et al. 2010).

MRI scanning session

Images were acquired on a 3.0 Tesla Siemens Magnetom Tim Trio system (Siemens Medical Solutions, Erlangen, Germany) with a twelve channel head coil at OHSU's Advanced Imaging Research Center. Whole-brain, high-resolution structural anatomical images were acquired in the sagittal plane using a T_1 weighted MPRAGE scanning sequence (TI = 900ms, Flip Angle = 10 degrees, TE = 3.58 ms, TR = 2300 ms, acquisition matrix = 256x240, resolution = 1mm x 1mm x 1.1mm).

Brain volumes

Hippocampal Volumes

Bilateral hippocampi were manually defined (traced) on each individual's high resolution anatomical image based on modification of a previously established method (Nagel et al. 2004; Nagel et al. 2005; Medina et al. 2007; Hanson et al. 2010). The tracer (MH) was blind to participant characteristics, and had established high intra and inter-rater reliability (all intraclass correlations ≥.95) on an independent sample using this previously published protocol prior to tracing. Specifically, bilateral hippocampi were traced on contiguous coronal slices, perpendicular to the anterior-posterior commissure plane using Analysis of Functional NeuroImages (AFNI) software, and were confirmed in axial and sagittal view. The anatomical boundaries used included: the anterior boundary: demarcated by the white matter of the alveus and the anterior recess of the temporal horn; the posterior boundary: demarcated by the columns of the fornix and the pulvinar of the thalamus, followed until the tail is no longer visible; the inferior boundary: white matter of the parahippocampal gyrus; the superior and lateral boundary: determined by temporal horn and alveus/fimbria; and the medial boundary: ambient cistern. Right and left hippocampal volumes were then calculated by multiplying the number of voxels by

the associated voxel dimensions (1.1 mm^3) and were expressed as a ratio to overall intracranial volume (ICV) to account for individual differences in brain size.

Gray, White, and Total Intracranial Volume

Gray matter and white matter volume, as well as ICV (the sum of gray, white, and cerebrospinal fluid) were calculated from each subject's high-resolution T_1 weighted anatomical image using the Functional Magnetic Resonance Imaging of the Brain (FMRIB)'s automated segmentation tool (FAST) from the FMRIB's Software Library, version 4.1 (Zhang et al. 2001; Smith et al. 2004). Specifically, anatomical images were first skull-stripped using a combination of a hybrid watershed and deformable surface skull-stripping semi-automated program (Segonne et al. 2004) and manual editing. Next, skull-stripped anatomical images were used as input to FAST, which uses a Markov random field model and an associated expectation-maximization algorithm to segment 3D brain images into gray matter, white matter, and cerebrospinal fluid, while also correcting for spatial intensity variations. This algorithm has been shown to be superior to other automated and semi-automated segmentation programs for segmenting tissue types (Clark et al. 2006). Gray matter, white matter, and total ICV were then calculated by multiplying the number of voxels for that tissue type by the associated voxel dimensions (1.1 mm^3). Finally, similar to the hippocampus, gray and white matter volumes were analyzed as a ratio to overall ICV to control for individual variability in brain size.

Addressing potential theoretical confounds

A number of potential theoretical confounds were assessed and used as covariates during analyses if they met both theoretical and statistical criteria for being considered a potential confound. Theoretical explanations for these confounds can be found below. These potential confounds were then included in statistical testing if they

showed a correlation ($p \leq .09$) with both the independent (aerobic fitness) and dependent variables of interest.

Age

Developmental changes are well documented in brain and behavior across adolescence (For review see Casey et al. 2000), including changes in hippocampal volume (Giedd et al. 1996b). Thus, age was examined as a potential covariate for analyses for examining relationships between variables that were not already normalized for age.

General Intelligence and Grade Point Average

Positive relationships have been reported between aerobic fitness levels and general intelligence (IQ), as well as academic achievement in children and adults (For meta-analysis review see Sibley and Etnier 2003; Etnier et al. 2006). In addition, relationships have been reported between memory performance and IQ in teenagers and adults (Wechsler 2009). Participants were administered the 2-subtest version of the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999) to provide an estimate of overall intellectual functioning, and grade point average (GPA) was collected from youth during the initial phone interview.

Pubertal Status

Although more commonly reported in girls, intensive physical exercise has been reported to indirectly delay pubertal maturation in some athletes by contributing to energy deprivation and low body fat (Georgopoulos et al. 2010). Puberty and its related sex hormones have also been shown to relate to brain structure in adolescents (Herting et al. 2011; Peper et al. 2011), including hippocampal size, in a sex dependent manner (Bramen et al. 2011). For these reasons, pubertal development was measured in male subjects and assessed as a potential confound. Specifically, pubertal maturation was

assessed using the self-rating Pubertal Development Scale (PDS) (Petersen et al. 1988). The PDS consists of a total of 5 distinct questions for boys in regards to height growth, body hair, skin changes, vocal changes, and facial hair. For each of the 5 questions, participants were asked to rate their development on a 4 point scale (1 = has not begun yet, 2 = barely begun, 3 = definitely begun, 4 = seems complete). An average was then calculated from these responses for a pubertal development score ranging from 1 (prepubertal) to 4 (postpubertal). This questionnaire was chosen as it is a relatively noninvasive assessment of pubertal development, and because self-reports on this scale have been shown to correlate significantly with other measures of pubertal status, including physician ratings (Petersen et al. 1988).

Video Games Questionnaire

Physical activity has been shown to be negatively associated with time spent playing video games in adolescents (Janz and Mahoney 1997), and relationships have been seen between brain volumes and video game abilities (Erickson et al. 2010). Furthermore, the nature of the spatial navigation task is similar to modern video games played by youth, as it requires manual navigation around a 3-dimensional virtual world. Thus, video game experience was also assessed as a potential confound. A brief video game questionnaire was utilized to determine average amount of time spent playing video games per week for each subject. Subjects reported the video game console type used (e.g. Wii, PlayStation, PC computer), the average number of hours playing video games on this console type per day (i.e. sessions), and the number of video game sessions per console type per week. Number of hours and number of sessions were summed across video game console types and multiplied together to assess the average number of hours of video games played per week.

Analyses

Exploratory data analyses were performed to determine normality, and when necessary, appropriate transformations were used to correct for violations of this parametric assumption. When transformations were not successful in obtaining normality (as seen by visual inspection and Shipiro-Wilks test ($p < .05$)), non-parametric statistics were employed. Outliers were determined as values exceeding 3 standard deviations (SD) from the mean. Spearman's correlation was used to examine the relationship between activity self-reports and fitness (VO_2 peak) test results.

Group

Percent distance traveled in the target quadrant was assessed with a 6-within (vMWT trials), 1-between (group) repeated-measures ANCOVA, controlling for SES and puberty (see Chapter 2 for details). Separate multiple regression analyses were then performed to examine spatial and verbal learning and memory between HA and LA youth while controlling for potential confounds. To examine hippocampal volumes (corrected for ICV), a 2-within (hemisphere), 1-between (group) ANCOVA was performed, while controlling for SES and puberty.

VO_2 peak

Pearson's correlations were used to assess the relationships between the 3 theoretical confounds and the independent variable of VO_2 peak and the dependent variables of interest. To examine learning across the 6 vMWT learning trials in the entire sample, a repeated-measures ANOVA was used to examine changes in percent distance in the target quadrant for all subjects. Simple regression analyses were performed to examine if aerobic fitness (as measured by VO_2 peak) predicted verbal and spatial learning and memory. Simple regression analyses were also performed to examine if aerobic fitness predicted left and right hippocampal volumes. When one of

the above variables was determined to be a potential confound variable of interest based on the criteria outlined, follow-up multiple regression analyses were performed for identified significant relationships to determine if the results held after controlling for the potentially confounding variable. Given the number of regression analyses performed, Bonferroni corrections were applied to reduce reporting Type I errors and these results are mentioned when applicable.

Results

Group analyses showed no significant differences in hippocampal volume, nor verbal or spatial learning and memory between HA and LA youth (**supplementary material**). These analyses can be found in the supplemental material for this chapter. The focus of the results presented in the body of this chapter is the relationships between VO_2 peak, learning and memory behavior and hippocampal volumes across the entire sample of 34 male adolescents.

Participant characteristics

Participant characteristics and aerobic fitness results for the entire sample of 34 subjects can be found in **Table 1**. All subjects had IQ values well-above the normal range and came from upper middle class families. Physical activity self-reports and aerobic fitness (VO_2 peak) test results were significantly related [*Spearman's rho*(34) = .57, p =.001]. Given that VO_2 peak results is the current gold standard in assessing aerobic fitness (Armstrong and Welsman 2007), VO_2 peak was selected as the independent variable used to examine the global relationships between aerobic exercise and brain structure and behavior.

Assessment of potential confounds

Statistical results for the potential theoretical confounds can be found in supplemental material (**Supplemental Table 1**). Only one of the proposed theoretical

confounds met the aforementioned criterion and was included as a statistical covariate during hypothesis testing. That is, a significant negative relationship was seen between pubertal status and VO_2 peak fitness results [$r(34) = -.37$, $p = .03$], with higher aerobic fitness values related to lower self-ratings of pubertal development. In addition, negative relationships were seen between pubertal status and hippocampal volumes [left: $r(34) = -.44$, $p = .01$; right: $r(34) = -.34$, $p = .05$]. No relationship was seen between puberty and ICV alone [$r(34) = .22$, $p = .21$]. Thus, pubertal status was used as a covariate in follow-up analyses examining aerobic fitness and hippocampal and gray matter brain volumes.

Aerobic fitness and learning and memory

Rey Auditory Verbal Learning Test (RAVLT)

Data for 1 subject were outliers on both learning and memory trials, and excluded from subsequent RAVLT analyses. Their removal had no effect on the results. Behavioral results for the group can be found in Table 1. Aerobic fitness did not significantly relate to either learning [total words learned: $R^2 = .01$, $F(1,31) = .17$, $\beta = -.07$, $p = .68$] or recall memory [retention memory score: $R^2 = .03$, $F(1,31) = 1.09$, $\beta = .18$, $p = .31$] on the RAVLT.

Virtual Morris Water Task (vMWT)

Data were not collected on all 6 learning trials for 3 participants due to technical errors, and 1 subject was an outlier on all trials, leaving N = 30 for all subsequent analyses for the vMWT. Behavioral results for the group can be found in **Table 1** and **Figure 1a**. Results showed that percent distance traveled in the target quadrant was significantly different between the 6 vMWT trials [$F(3.93, 29) = 14.46$, $p < .001$, partial $\eta^2 = .28$](**Figure 1a**). Post-hoc tests revealed a significantly larger percent of distance traveled in the target quadrant in trials 2 thru 6 when compared to trial 1 [p's < .001], as well as between trial 2 and trials 3, 4, 5, and 6 [$p's < .05$]. A trend was also seen

between trial 3 and trial 5 (p =.08). Simple regression analyses showed aerobic fitness to be a significant predictor of the amount of learning that occurred (δ) during the vMWT task [R^2 = .34, F(1,29) = 14.64, β =*.59, p* =.001](**Figure 1b**). However, aerobic fitness did not predict delayed memory recall performance on this task, as aerobic fitness did not relate to either number of platform crossings [R^2 = .05, F(1,29) = 1.36, β =*-.22, p* =.25] or percent distance traveled in the target quadrant during the probe trial [R^2 = .03, F(1,29) = .91, β =*.18, p* =.35]. Importantly, no relationship was seen between aerobic fitness and latency to travel to the platform in the visible platform control trial [R^2 = .001, F(1,29) = .04, β =*-.04, p* =.84, showing aerobic fitness was unrelated to visual or motor processes on this task.

Aerobic fitness and hippocampal volumes

Bilateral hippocampal, gray, and white matter volumes are reported in **Table 1.** Aerobic fitness showed a significant positive relationship with both the left [R^2 = .32, F(1,32) = 15.3, β =*.55, p* <.001] and the right hippocampus [R^2 = .17, F(1,32) = 6.5, β =*.41, p* =.016] (**Figure 2**). No significant relationships were seen between aerobic fitness and total gray matter volume [R^2 = .03, F(1,32) = 1.0, β =*.18, p* =.32] or white matter volume [R^2 = .01, F(1,32) = .19, β =*-.08, p* =.66], or ICV [R^2 = .05, F(1,32) = .09, β =*-.05, p* =.76]. Follow-up analyses were run to determine if aerobic fitness remained a significant predictor of hippocampal volume after covarying for pubertal status (**Supplemental Table 2**). While aerobic fitness showed positive relationships with the volume of the right and left hippocampus, negative relationships were seen between pubertal status and hippocampal volume. With both in the model, aerobic fitness remained a significant predictor of hippocampal volume for only the left hippocampus [β = .47, p = .004, semipartial correlation (sr^2) = .19](**Supplemental Table 2**).

Brain volumes and learning and memory

RAVLT

Significant positive relationships were seen between learning on the RAVLT and bilateral hippocampal volumes [left: R^2 = .12, F(1,31) = 4.14, β = .34, p =.05; right: R^2 = .15, F(1,31) = 5.26, β = .38, p =.029]. While RAVLT failed to show a significant association with total gray matter volume [R^2 = .08, F(1,31) = 2.78, β = .29, p =.11], larger total white matter volume was related to greater learning performance on the RAVLT [R^2 = .25, F(1,31) = 10.2, β = .50, p =.003]. However, only the relationship between white matter volume and learning passed Bonferroni correction (p < .013). No significant relationships were seen between verbal recall and brain volumes [left hippocampus: R^2 = .00, F(1,31) = .002, β = .01, p =.96; right hippocampus: R^2 = .01, F(1,31) = .18, β = -.08, p =.67; gray matter: R^2 = .02, F(1,31) = .50, β = -.13, p =.49; white matter: R^2 = .02, F(1,31) = .63, β = -.14, p =.44].

vMWT

Significant positive relationships were also seen between the amount of learning (δ) on the vMWT and bilateral hippocampal volumes [left: R^2 = .26, F(1,29) = 10.06, β = .51, p =.004; right: R^2 = .18, F(1,29) = 6.07, β = .42, p =.02]. On the contrary, total gray matter [R^2 = .08, F(1,29) = 2.49, β = .29, p =.13] or white matter [R^2 = .004, F(1,29) = .10, β = .06, p =.75] volume did not significantly predict spatial learning on the vMWT. After employing Bonferroni correction (p < .013), only the relationship between left hippocampal volume and spatial learning (δ) remained significant. Brain volumes did not, however, predict delayed memory performance on the vMWT probe trial [platform crossings: left hippocampus: R^2 = .002, F(1,29) = .05, β = .04, p =.82; right hippocampus: R^2 = .00, F(1,29) = .01, β = .02, p =.92; gray matter: R^2 = .01, F(1,29) = .27, β = .09, p =.61; white matter: R^2 = .001, F(1,29) = .04, β = .04, p =.85; percent

distance in target quadrant: left hippocampus: R^2 = .014, F(1,29) = .40, β = .12, p =.53; right hippocampus: R^2 = .001, F(1,29) = .04, β = -.04, p =.85; gray matter: R^2 = .01, F(1,29) = .40, β = .12, p =.53; white matter: R^2 = .02, F(1,29) = .49, β = .13, p =.49].

Given that the above results showed that aerobic exercise predicts learning on the vMWT, as well as hippocampal volume, and hippocampal volume predicts vMWT learning (δ), follow-up multiple regression analyses were performed to determine if hippocampal volume predicts vMWT learning (δ), while controlling for aerobic fitness. Both relationships between the bilateral hippocampus and vMWT learning (δ) were no longer significant [left: β = .277, p =.13, sr^2 = .05; right: β = .237, p =.16, sr^2 = .04], suggesting that the relationship seen between the hippocampus and spatial learning was driven by their individual associations with aerobic fitness. Although it was hypothesized that hippocampal volume would mediate the relationship between aerobic fitness and spatial learning, the absence of a relationship between the hippocampus and learning when controlling for aerobic fitness violates the necessary criteria for a potential mediating variable using the classic causal steps approach, as outlined by Baron and Kenny (Baron and Kenny 1986). Thus, hippocampal volume was not a mediator of the relationship between aerobic fitness and vMWT learning (δ) in the current sample.

Discussion

This is the first study of its kind to show that associations between exercise and learning on the Morris Water Maze translate to humans on a virtual Morris Water Task. In addition, this is the first study to examine the influence of aerobic exercise on brain structure and function in a human adolescent sample, a population which has been previously overlooked in the exercise literature. The results show that aerobic fitness relates to hippocampal volume and spatial learning on the vMWT. Similar results were not found for verbal memory, or for total gray or white matter volume, perhaps

suggesting some specificity in the influence of aerobic exercise on the adolescent brain and behavior or that effects may be somewhat larger in the spatial domain.

As hypothesized, aerobic fitness statistically predicted larger hippocampal volumes in adolescents. This finding is in agreement with the animal literature showing exercise-induced neurogenesis in the hippocampus. Specifically, aerobic exercise has been shown to promote cell proliferation and survival in the dentate gyrus of the hippocampus (van Praag et al. 2005), and exercise-induced increases in new cell growth have been shown to be associated with increases in the volume of the DG (Clark et al. 2008). Increases in angiogenesis (Van der Borght et al. 2009) and changes to hippocampal dendritic morphology (Redila and Christie 2006) have also been seen to occur following aerobic activity. Although MRI technology does not have the ability to detect such changes at the cellular level, increases in neurogenesis and angiogenesis may contribute to larger hippocampal volumes, which can subsequently be detected by structural MRI. Beyond neurogenesis, exercise may also lead to larger hippocampal volumes by increasing dendritic spines and arborization of pyramidal cells in the dentate gyrus and CA1 regions (Redila and Christie 2006; Stranahan et al. 2007). Regardless of the underlying neural mechanism, these findings extend previous work showing that physical activity levels and aerobic capacity relate to larger hippocampal volumes in elderly adults (Erickson et al. 2009; Erickson et al. 2011) and young children (Chaddock et al. 2010a). Taken together with the current study, these findings strongly suggest that aerobic exercise influences hippocampal size at multiple stages of life.

In addition to hippocampal volume, aerobic exercise was associated with better visuospatial learning in adolescents. The creation of virtual spatial navigation tasks that mirror commonly utilized tests to assess learning and memory in animals (Levy et al. 2005; Baker and Holroyd 2009), such as the vMWT (Burkitt et al. 2007; Nowak and

Moffat 2011) are an invaluable tool in allowing for translational research to be performed. By examining how aerobic exercise relates to performance on a vMWT, we were able to replicate in human adolescents, previous work in animals showing that exercise training enhances learning behavior in young rodents (Uysal et al. 2005; Lou et al. 2006). Although the current data are correlational, together, these findings suggest that exercise influences spatial learning across both human and rodent species. However, in terms of memory performance, aerobic exercise did not relate significantly to either spatial delayed recall memory on the vMWT, or verbal learning or delayed memory recall on the RAVLT in the current study. These findings failed to replicate previous research. For example, one animal study found exercise to increase spatial memory retention using the Morris Water Maze; although this relationship was shown in aging rodents (van Praag et al. 2005). In addition, a number of associations have been reported between physical activity levels and improved performance on a wide variety of other learning and memory tasks in children (Chaddock et al. 2010a; Chaddock et al. 2011a) and adults (Winter et al. 2007; Erickson et al. 2009; Erickson et al. 2011), including learning novel vocabulary words (Winter et al. 2007), item and relational memory for visual stimuli (Chaddock et al. 2010a; Chaddock et al. 2011a), and a visual short-term delayed-match to sample task (Erickson et al. 2009; Erickson et al. 2011).

One possibility is that these apparently divergent results in the human literature may stem from the fundamental differences between learning *versus* memory processes, as well as the specific tasks used in each study. Learning can be defined as long-term change in behavior that results from prior experience, whereas remembering is the response or recount of information that was experienced earlier (Domjan 2003). Learning and memory can also be broken down further into a number of interdependent, but unique, processes including encoding, retention (consolidation and storage), and

retrieval; all of which have distinct neural and molecular mechanisms (Abel and Lattal 2001). For example, hippocampal NMDA receptors are important for encoding associative place memory, whereas delayed memory retrieval depends on AMPA receptors (Bast et al. 2005). At a systems level, memory encoding requires the hippocampus, whereas as retrieval is subserved by a number of brain regions, including the hippocampus, but also the parietal and prefrontal cortices (Rugg et al. 2002). Therefore, the apparent discrepancies between some prior studies and the current one that shows aerobic exercise relates to spatial learning but not delayed memory recall on the vMWT, may result from aerobic exercise having different effects on the neural and/or molecular pathways underlying each component of the learning and memory process.

Beyond the differences in learning *versus* memory mechanisms, the differences seen between our study and previous research may be due to the tasks used to assess learning and/or memory. For example, unlike in the current study, previously published findings employed memory tasks that do not specifically assess episodic memory, but rather short-term memory (Erickson et al. 2009; Erickson et al. 2011) or in some cases involve memory tasks that also tap executive functions (Chaddock et al. 2011a), which rely on cortical brain regions, such as the prefrontal cortex and sensory cortices (Jonides et al. 2008). Moreover, it is also possible that aerobic fitness was not found to relate to memory performance in the current study, as both tasks utilized had a relatively short retrieval delay (≤ 30 minutes), and longer retrieval delays may be more sensitive to exercise effects. Lastly, there are differences between the current study and other work in study design, particularly regarding the length of time between physical exercise testing or intervention and cognitive assessment. In the current study, we ensured that memory performance was not directly influenced by any carryover effects of acute

exercise from aerobic fitness testing by not allowing aerobic exercise testing to proceed the MRI scan or the memory assessment sessions, whereas in the previous study showing the beneficial effect of exercise on verbal learning and memory, novel words were learned within 15 minutes of the exercise intervention (Winter et al. 2007). Other studies did not report the time between aerobic activity and cognitive assessment (Erickson et al. 2009; Chaddock et al. 2010a; Chaddock et al. 2011a; Erickson et al. 2011). However, the idea of "time-dependent benefits" of exercise is likely an important factor, as aerobic-induced benefits in learning for animals tend to peak following a delay, whereas the benefits of physical activity on memory performance are best seen immediately following exercise (Berchtold et al. 2010). Thus, more research manipulating time between physical activity and cognitive assessment is warranted.

Another factor that might contribute to the current results is insufficient statistical power. Although a number of findings did not reach statistical significance, effect sizes suggest that some of the non-significant findings did have medium effect sizes. For example, a significant differences was not seen for vMWT learning (δ) or hippocampal volumes between high and low-active youth (**supplementary material**), but both results accounted for a good proportion of the variance [δ group effect size: R^2 = .078; hippocampal volume group effect size: partial η^2 = .08]. These findings suggest that using activity level as dichotomous variable may have led to an issue of power in the current study. However, effect sizes were small for verbal learning and memory, and thus, it is feasible that aerobic fitness may vary in how it relates to different types of learning during adolescence. Future research should also focus on which types of memory, as well as what subcomponents (encoding, retention, retrieval), are specifically influenced by exercise and if these change across development.

The current results failed to confirm our hypothesis that hippocampal volume would mediate the relationship between aerobic fitness and learning on the vMWT. This was surprising, as the hippocampus has been found to mediate relationships seen between cardiovascular aerobic fitness and learning and memory in studies of children (ages 9-10, N = 41) (Chaddock et al. 2010a) and elderly participants (N = 109) (Erickson et al. 2009). Notably, the effect sizes of the hippocampus as a mediation variable were small to medium (Cohen's f^2 = .24 – .34). For the sample size used for mediation analyses in the current study (n = 30), an effect size of at least .40 would have been necessary to detect a significant hippocampal mediation effect, again, suggesting that the current study may have been somewhat underpowered for detection of mediation. There are also a number of additional mechanism(s) that occur outside the hippocampus that may help to explain the beneficial impact of aerobic exercise on spatial learning. For example, changes in neurotransmitter levels, including those important for learning and memory such as dopamine and acetylcholine, are up-regulated following exercise and may mediate exercise's induced actions on learning (For review see Meeusen et al. 2001). Furthermore, neurotrophic factors, such as brain derived neurotrophic factor (BDNF), are also thought to be important for the beneficial cognitive effects of exercise, and increases in BDNF mRNA are not limited to the hippocampus, but have been shown in the cortex following physical activity in rodents (Llorens-Martin et al. 2008). Therefore, exercise induced chances in neurotransmitter or neurotrophic factors may be important mechanisms for the relationships seen between aerobic fitness and vMWT learning in adolescents.

Beyond aerobic fitness, we found that verbal learning in adolescents was positively associated with total white matter volume, although white matter volume and verbal learning were not significantly related to aerobic fitness. These findings are similar

to previous work showing white matter volume to relate to other cognitive functions in youth (Silveri et al. 2008). Using structural MRI, increases in white matter volume have been repeatedly shown across adolescence (Giedd et al. 1996a; Giedd et al. 1999b). These increases in white matter volume are thought to reflect increases in myelination, as post-mortem-studies have found that neurons in the frontal cortices (Yakovlev and Lecours 1967) and parahippocampus (Benes et al. 1994) continue to myelinate during adolescence and into adulthood. Given that myelination increases the rate of neuronal signaling conduction, these findings might suggest that improved verbal learning is achieved through more efficient and faster neural processing. Further research is needed to determine if more specific relationships may exist between the white matter volume relationship with verbal learning detected here, including if it is driven by specific increases in myelination in brain regions that subserve memory processes such as the hippocampus and frontal lobes (Squire 2004).

Conclusion

This study employed a translational task to examine how exercise relates to hippocampal structure and learning and memory in adolescents. These results show that aerobic fitness relates to larger hippocampal volumes and better learning on a virtual Morris Water Task in adolescents. Taken together with previous studies on young children and aged individuals, these findings suggest that aerobic exercise may benefit the brain and learning at multiple stages across the lifespan. Future studies should address what additional types of memory may benefit from aerobic fitness during adolescence, and if similar relationships are seen in adolescent girls. Lastly, this study was correlational in nature, thus longitudinal exercise intervention studies are essential to confirm that aerobic exercise results in hippocampal structural changes and enhanced spatial learning in adolescents.

Table 1. Participant characteristics, task performance, and brain volumes. Means and SD are presented unless otherwise noted.

Participants	**All subjects**	**HA**	**LA**
Age	16.4 (.82)	16.6 (.8)	16.2 (.8)
% Caucasian	82.4	82.4	82.4
GPA	3.4 (.4)		
SES[a]	22.4 (10.8)	18.3 (5.9)	26.5 (12.9)
IQ[b]	117.6 (10.75)	117.1 (11.8)	118.0 (8.1)
Aerobic Exercise (hrs/week)[c]	6.2 (5.8); range: 0-18	11.3 (3.4)	.26 (.5)
VO_2 peak (mL/kg LBM/min)	72 (10.5)	77.7 (10.5)	67.0 (7.4)
RAVLT[d]			
Total words learned (z-score)	1.7 (.8)	1.7 (.6)	1.7 (1.0)
Memory Retention Score	-.4 (1.5)	-.13 (1.3)	-.65 (1.8)
vMWT[e]			
Learning (δ)	.35 (.2)	.42 (.21)	.30 (.22)
Delay Memory (Probe)			
% Distance in target quadrant	69 (20.0)	67 (25.3)	66.4 (17.8)
Platform Crossings	3.7 (2.3)	3.6 (2.2)	3.8 (2.4)
Swim Time (seconds)	4.8 (1.8)	5.0 (2.3)	4.6 (1.4)
Brain Volumes (mm^3)			
Left Hippocampus	3942.5 (445.9)	4102.8 (395.1)	3782.3 (446.6)
Right Hippocampus	4126.5 (485.9)	4243.0 (457.6)	4010.0 (498.8)
Gray Matter	707579.9 (43137.3)	715481.2 (48635.7)	699678.6 (36604.1)
White Matter	550300.4 (39717.9)	555748.8 (40630.5)	544852.04 (39238.9)

[a] Hollingshead Index of Social Position

[b] Wechsler Abbreviated Scale of Intelligence

[c] Youth Adolescent Activity Questionnaire

[d] Rey Auditory Verbal Learning Test

[e] virtual Morris Water Task

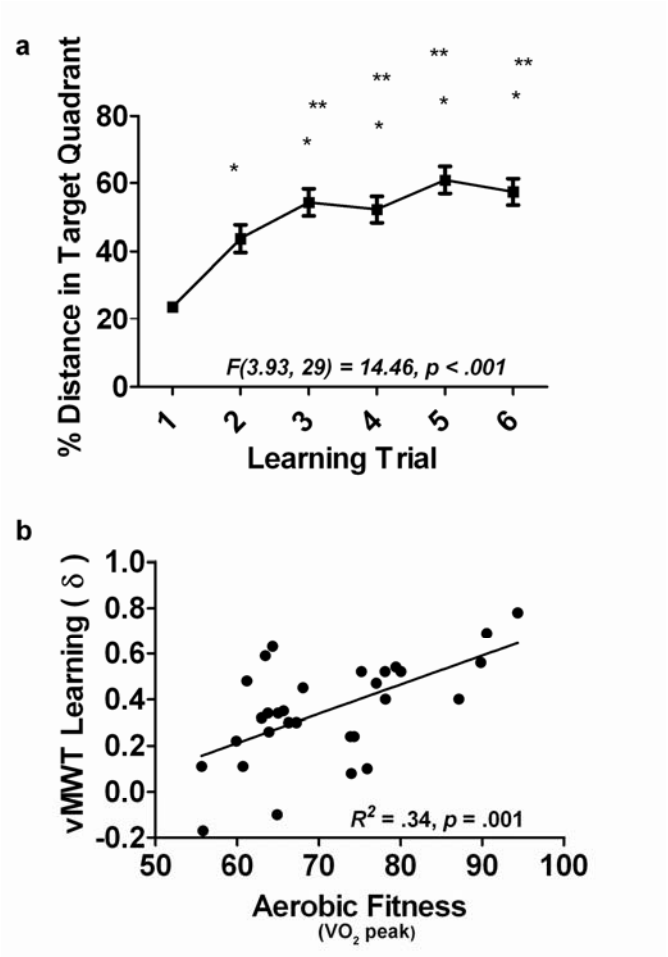


Figure 1. Virtual Morris Water Task Learning **a)** Percent distance traveled in the target quadrant across the 6 trials. * denotes post-hoc *t*-test results; significantly different from trial 1 (* *p's* < .05) and from trial 2 (** *p's* < .05). *F*-statistic of the repeated measures ANOVA and its associated *p*-value are also shown. **b)** Aerobic fitness (as measured by VO_2 peak) predicts the amount of learning that occurred (δ =percent distance in target quadrant in trial 6 –percent distance in target quadrant in trial 1) during the vMWT task. R^2 and associated *p*-value is also reported for this relationship. Higher δ reflects better learning.

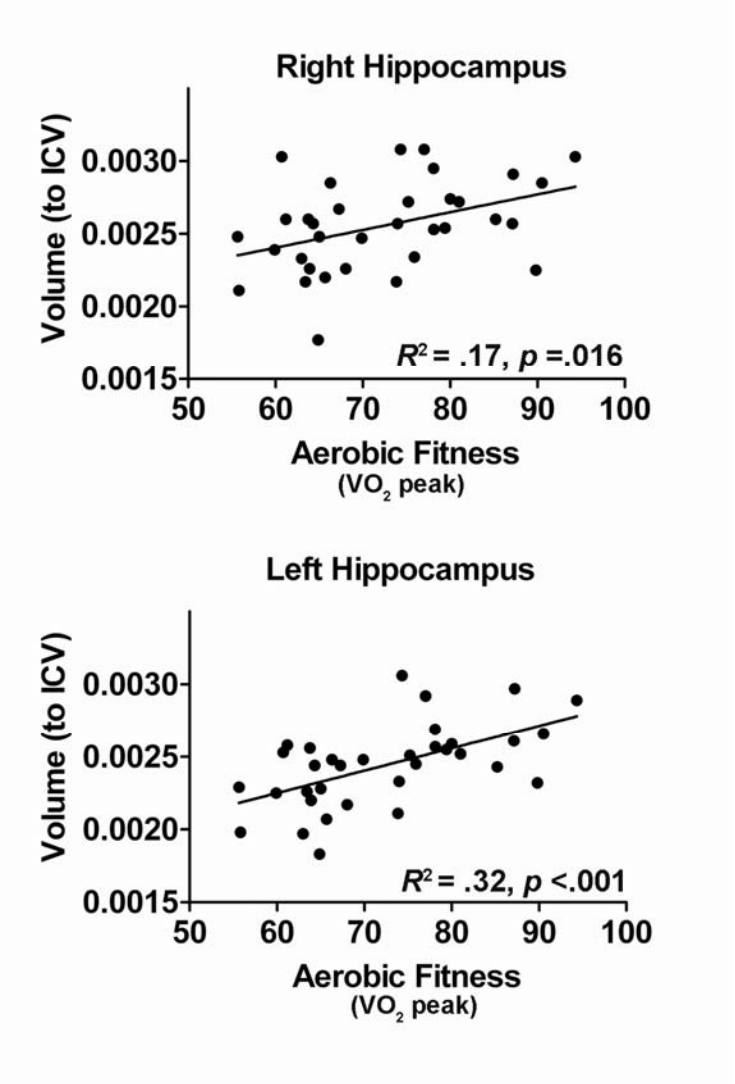


Figure 2. Aerobic fitness (as measured by VO_2 peak) predicts right and left hippocampal volumes. Volumes (in mm^3) reported controlling for Intracranial Volume (ICV). R^2 and associated *p*-values are also reported for each relationship.

Chapter 3. Supplemental Material

Additional vMWT variables of Interests

Additional variables of vMWT performance can be computed across a learning trial including: latency, or time, it takes to find the hidden platform; cumulative distance, or the distance traveled to reach the hidden platform; and percent time spent in the target quadrant that holds the hidden platform. Percent time spent in the target quadrant can also be assessed as a measurement of memory in the probe trial (Vorhees and Williams 2006). Covariance for time and distance for the entire sample, as well as each group were very high (entire sample: ≥ .895; HA: ≥ .935; LA: ≥ .941).

Correlation analyses were performed to examine the relationship between VO_2 peak and other outcome variables on the vMWT, including time to the platform, cumulative distance to platform, and time spent in the target quadrant. Spearman's rho was used to examine time, distance, and percent time for learning trials as these data were non-normal (Kolmogorov-Smirnov, p's < .05). Pearson's correlations were run for all other analyses. Due to technical problems, time was not collected for one subject during learning trial 6, and thus, was excluded from all subsequent analyses examining time for this trial.

For the learning trials, the relationship between VO_2 peak and latency to the hidden platform, cumulative distance to the hidden platform, as well as percent time in the target quadrant across the 6 learning trials were examined. A correlation matrix of this data can be found in **Supplementary Table 5**. For trial 6, VO_2 peak showed a significant negative relationship with cumulative distance [*rho*(29)=-.40, *p* =.03] and a significant positive relationship between percent time in target quadrant [*rho*(29)= .43, *p* = .02]. VO_2 peak was not significantly related to latency, cumulative distance, or percent time on any of the other vMWT learning trials [*p*'s >.56]. For the probe trial, no

relationship was seen between VO_2 peak and percent time in the target quadrant [$r(30)=.18$, $p=.35$]. No significant relationship was seen between cumulative distance to the visible platform in the control trial [$r(30)=-.04$, $p=.83$].

HA and LA group analyses and results

Rey Auditory Verbal Learning Test (RAVLT)

Separate multiple regression analyses were performed to examine verbal learning (total number of words learned over 5 trials), and verbal recall memory (retention score). Group results can be found in **Supplementary Material Figure 1**. Putting group (dummy coded) in the model as a single predictor did not predict verbal learning [$R^2 = .003$, $\beta = -.05$, $t = .28$, $p = .78$] or recall memory [$R^2 = .026$, $\beta = .17$, $t = .95$, $p = .35$]. After covarying SES and puberty in the model, group remained a non-significant predictor of verbal learning [group: $\beta = .07$, $t = .36$, $p = .73$, $sr^2 = .0036$] and verbal recall memory [group: $\beta = .39$, $t = 1.96$, $p = .06$, $sr^2 = .11$].

Virtual Morris Water Task (vMWT)

Group results for percent distance and percent time in target quadrant, as well as latency and cumulative distance to the hidden platform can be found in **Supplementary Material Figure 2**. Group differences in spatial learning were explored by examining both percent distance traveled in the target quadrant across the 6 vMWT learning trials, as well as delta (δ = trial 6 percent distance in target quadrant– trial 1 percent distance in target quadrant). Results showed a significant main effect of trial [$F(1, 3.9) = 14.41$, $p < .001$, partial $\eta^2 = .34$], but not of group [$F(1, 28) = .38$, $p = .54$, partial $\eta^2 = .01$] nor the interaction term [$F(1, 3.9) = 1.60$, $p = .18$, partial $\eta^2 = .05$] (**Supplemental Material Figure 2**). Post-hoc tests showed that percent distance traveled was significantly different in trial 1 versus all other trials (2 thru 6), and trial 2 was also significantly

different from trials 5 and 6 (all *p*'s < .05). ANCOVA, covarying for SES and puberty, showed similar results [trial: $F(1, 3.8) = 3.07$, $p = .02$, partial $\eta^2 = .11$]; group: $F(1, 25) = .08$, $p = .78$, partial $\eta^2 = .003$]; interaction: $F(1, 3.8) = 1.2$, $p = .32$, partial $\eta^2 = .04$]. Simple regression analysis showed that group was not a significant predictor of delta [$R^2 = .078$, $F(1,29) = 2.36$, $\beta = .28$, $t = 1.53$, $p = .14$] (**Supplemental Material Figure 2**); multiple regression analyses, controlling for SES and puberty, depicted similar results [group: $\beta = .33$, $t = 1.60$, $p = .12$, $sr^2 = .08$].

No significant group differences were seen for the additional outcome variables across the learning trials [percent time: group: $F(1, 27) = .08$, $p = .78$, partial $\eta^2 = .003$; latency: group: $F(1, 27) = .19$, $p = .67$, partial $\eta^2 = .007$; cumulative distance: $F(1, 28) = .008$, $p = .93$, partial $\eta^2 = .0002$] (**Supplementary Material Figure 2**). Similar results were seen using ANCOVA to covary for SES and puberty.

Group differences in delayed spatial memory recall were assessed by simple regression analysis, to determine if group predicted percent distance and percent time traveled in the target quadrant and the number of times the participant intercepted the platform during the probe trial. Results showed that group was not a significant predictor of delayed spatial recall memory [percent distance: $R^2 = .028$, $F(1,29) = .01$, $\beta = .17$, $t = .89$, $p = .38$; percent time: $R^2 = .046$, $F(1,29) = 1.36$, $\beta = .22$, $t = 1.17$, $p = .25$; platform crossings: $R^2 = .001$, $F(1,29) = .03$, $\beta = -.03$, $t = -.18$, $p = .86$](**Supplemental Material Figure 3**). Results did not change after controlling for SES and puberty [percent distance: group: $\beta = .166$, $t = .81$, $p = .42$, $sr^2 = .023$; percent time: group: $\beta = .25$, $t = 1.21$, $p = .24$, $sr^2 = .048$; platform crossings: group: $\beta = -.15$, $t = -.72$, $p = .48$, $sr^2 = .017$].

Regression analysis also showed that neither latency nor cumulative distance to the visible platform differed significantly between the groups [latency: $R^2 = .015$, $F(1,29) = .41$, $\beta = .05$, $t = .22$, $p = .83$, cumulative distance: $R^2 = .009$, $F(1,29) = .25$, $\beta = .09$, $t =$

.49, p = .62], even with controlling for SES and puberty [latency: group: β = .12, t = .64, p = .53, sr^2 = .0016; cumulative distance: group: β = .02, t = .29, p = .91, sr^2 = .0004].

Brain volumes

Bilateral hippocampal volumes are presented in **Supplemental Material Figure 4** for both groups. To examine hippocampal volumes (corrected for ICV), a 2-within (hemisphere), 1-between (group) ANOVA was performed. Results showed no significant main effect of hemisphere [F(1,31) = .15, p = .70, partial η^2 = .005], group [F(1,31) = 2.67, p = .11, partial η^2 = .08], or group-by-hemisphere interaction [F(1,31) = 1.23, p = .27, partial η^2 = .005]. ANCOVA, covarying for SES and puberty, showed identical results [hemisphere: F(1,29) = .06, p = .81, partial η^2 = .001; group: F(1,29) = 1.91, p = .18, partial η^2 = .07; interaction: F(1,29) = .48, p = .49, partial η^2 = .02]. No significant differences were seen between the groups for gray matter volume [R^2 =.029, F(1,33) = .95, β = .17, p = .34], white matter volume [R^2 =.002, F(1,33) = .07, β = .05, p = .79], or ICV [R^2 =.009, F(1,33) = .28, β = .09, p = .60]; similar results were seen after controlling for puberty and SES.

Additional comments

Given that the results for spatial learning and hippocampal volumes were in the same direction as seen in the main results of Chapter 2 (VO_2 peak correlations), the lack of significant results using activity level as dichotomous variable may be an issue of power. In fact, the effect sizes were small to medium using group. While power is one possible factor, differences in what each independent variable is able to capture about aerobic fitness may also contribute to these disparate findings. Both of these ideas are discussed further in **Chapter 5**.

Supplemental Table 1. Potential theoretical confound results. ***Bold-Italics*** indicate significant Pearson's *r* correlations and associated *p*-values ($p < .10$).

	Age	Puberty	IQ	GPA	Video Games
RAVLT (N =33)					
Total Words Learned [a]	-----	r(33) =-.03, p=.88	r(33) =.04, p=.81	r(33) =-.08, p=.64	r(33) =-.05, p=.77
Memory Retention Score	**r(33) =.37, p=.04**	r(33) =-.13, p=.41	r(33) =.05, p=.80	r(33) =.01, p=.94	r(33) =.17, p=.34
vWMT (N =30)					
Learning (δ)	r(30) =.16, p=.40	r(30) =-.23, p=.21	r(30) =.18, p=.35	r(30) =.13, p=.50	r(30) =-.21, p=.27
Probe- % distance in target quadrant	**rho(30) =-.43, p=.02**	rho(30) =-.17, p=.36	rho(30) =.03, p=.89	rho(30) =.06, p=.77	rho(30) =-.28, p=.14
Probe Crossings	r(30) =.26, p=.13	r(30) =-.29, p=.12	r(30) =.05, p=.80	**r(30) =.32, p=.08**	r(30) =.02, p=.93
Swim Time	r(30) =-.22, p=.24	r(30)=-.29, p=.12	**r(30) =-.33, p=.07**	r(30) =.04, p=.85	r(30) =.18, p=.35
VO_2 peak (N =34)					
	r(34) =.05, p=.80	**r(34) =-.37, p=.03**	r(34) =.28, p=.11	r(34) =.12, p=.50	**r(34)=-.39, p=.02**
Brain Volumes [b] (N=34)					
Left Hippocampus	r(34)=.005, p=.98	**r(34)=-.44, p=.01**	r(34)=.25, p=.16	r(34)=.12, p=.49	r(34)=-.02, p=.93
Right Hippocampus	r(34)=-.14, p=.44	**r(34)=-.34, p=.05**	r(34)=.24, p=.16	r(34)=.15, p=.40	r(34)=.08, p=.67
Gray Matter	r(34)=.05, p=.76	**r(34)=-.29, p=.09**	r(34)=-.09, p=.61	r(34)=.06, p=.76	**r(34)=-.39, p=.02**
White Matter	r(34)=-.002, p=.99	r(34)=-.19, p=.27	r(34)=-.03, p=.88	r(34)=.09, p=.61	r(34)=.002, p=.99

[a]Already normalized for age, so no age analysis needed
[b]Corrected for intracranial volume (ICV)

Supplemental Table 2. Relationships between aerobic fitness and hippocampal volumes with and without controlling for pubertal status. R^2 and *p*-value of the overall model, as well as the β, associated *p*-values, and semipartial correlation (sr^2) for each predictor variable for simple and multiple regression analyses. ***Bold-Italics*** indicate significant β and associated *p*-values ($p < .05$).

Brain Volume[a]	Model 1				Model 2							
	Predictor(s): Aerobic Fitness				Predictor(s): Aerobic Fitness & Pubertal Status							
	R^2	*p*	VO_2 β	*p*	R^2	*p*	VO_2 β	*p*	sr^2	*Puberty* β	*p*	sr^2
Left Hippocampus	.32	< .001	***.57***	***< .001***	.38	.001	***.47***	***.004***	.19	-.26	.09	.06
Right Hippocampus	.17	.016	***.41***	***.016***	.21	.025	.32	.06	.09	-.22	.21	.04

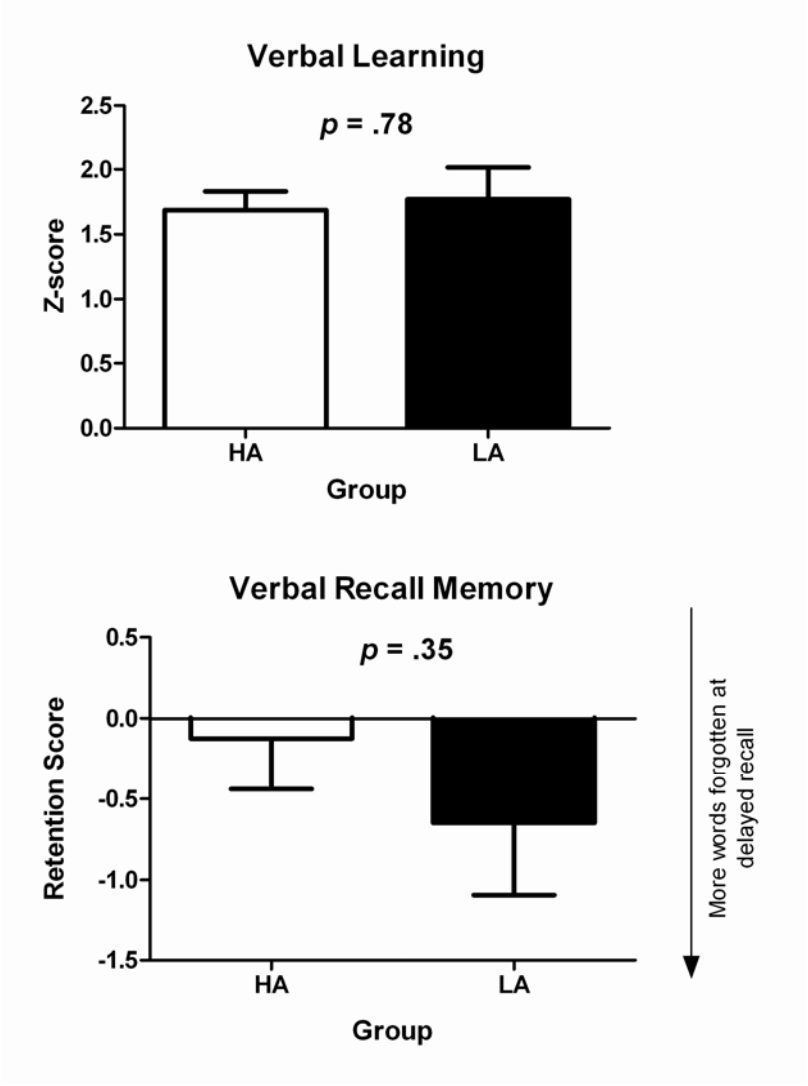
[a] Corrected for intracranial volume

Supplemental Table 3. Simple *Pearson's* correlation matrix between aerobic fitness (VO_2 peak), hippocampal volumes, and spatial and verbal learning and memory variables. **Bold** font denotes a significant correlation.

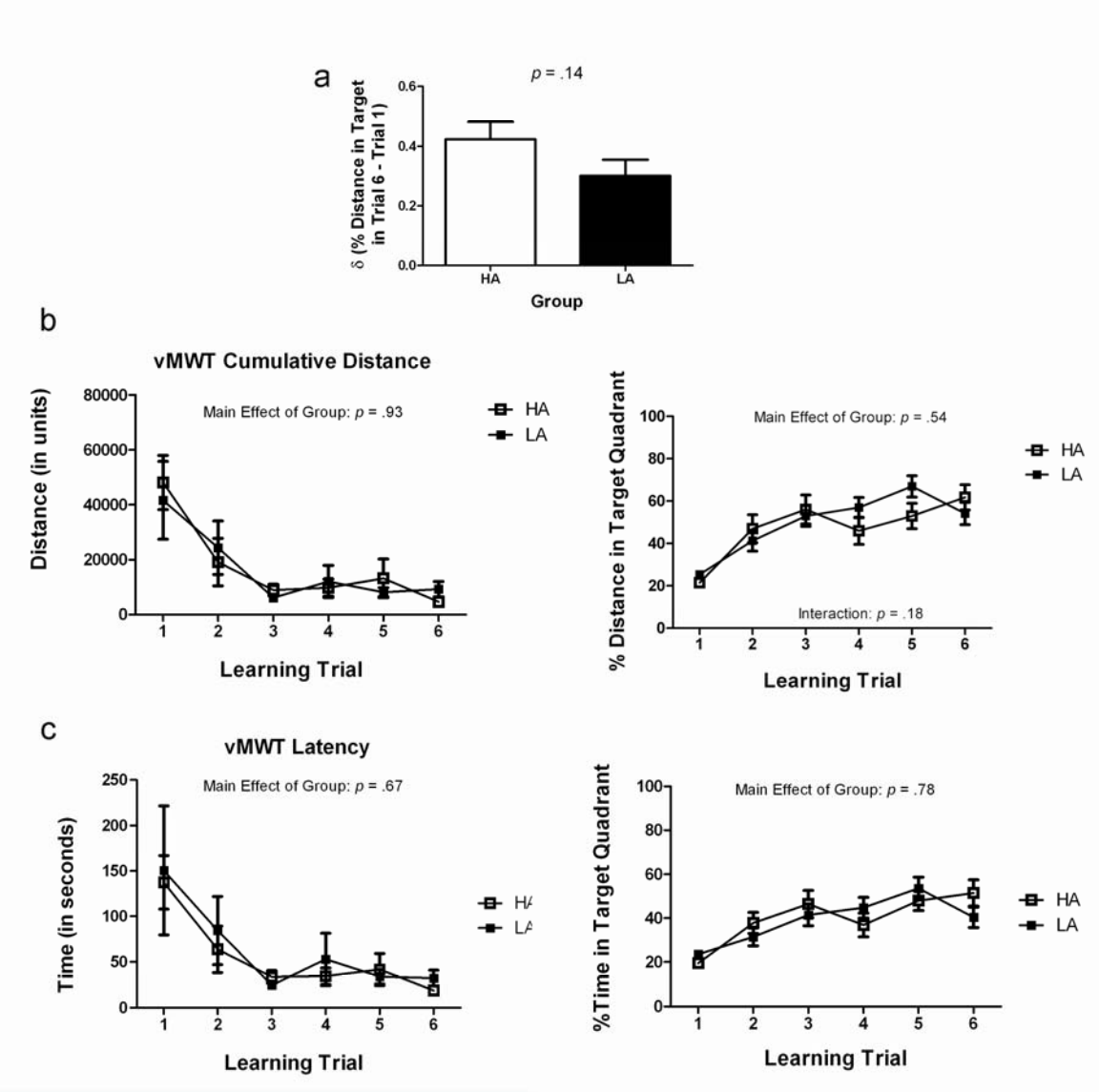
	Aerobic fitness (VO_2 peak)	Left hippocampus	Right hippocampus
Aerobic fitness (VO_2 peak)	--	--	--
Left hippocampus	***r(34) = .568*** ***p <.001***	--	--
Right hippocampus	***r(34) = .411*** ***p =.016***	--	--
Spatial learning (δ)	***r(30) = .586*** ***p <.001***	***r(30) = .514*** ***p =.004***	***r(30) = .422*** ***p =.02***
% Distance in TQ	*r(30) = .225* *p =.23*	*r(30) = .044* *p =.82*	*r(30) = -.036* *p =.85*
Platform crossings	*r(30) = -.216* *p =.25*	*r(30) = -.015* *p =.94*	*r(30) = .020* *p =.92*
Verbal learning	*r(33) = -.07* *p = .68*	***r(33) = .34*** ***p = .05***	***r(33) = .38*** ***p = .03***
Verbal memory	*r(33) = .18* *p = .31*	*r(33) = .008* *p = .96*	*r(33) = -.08* *p = .67*

Supplemental Table 4. Spearman's rank correlation matrix for aerobic fitness (VO_2 peak) and additional vMWT outcome variables for each of the six learning trials.

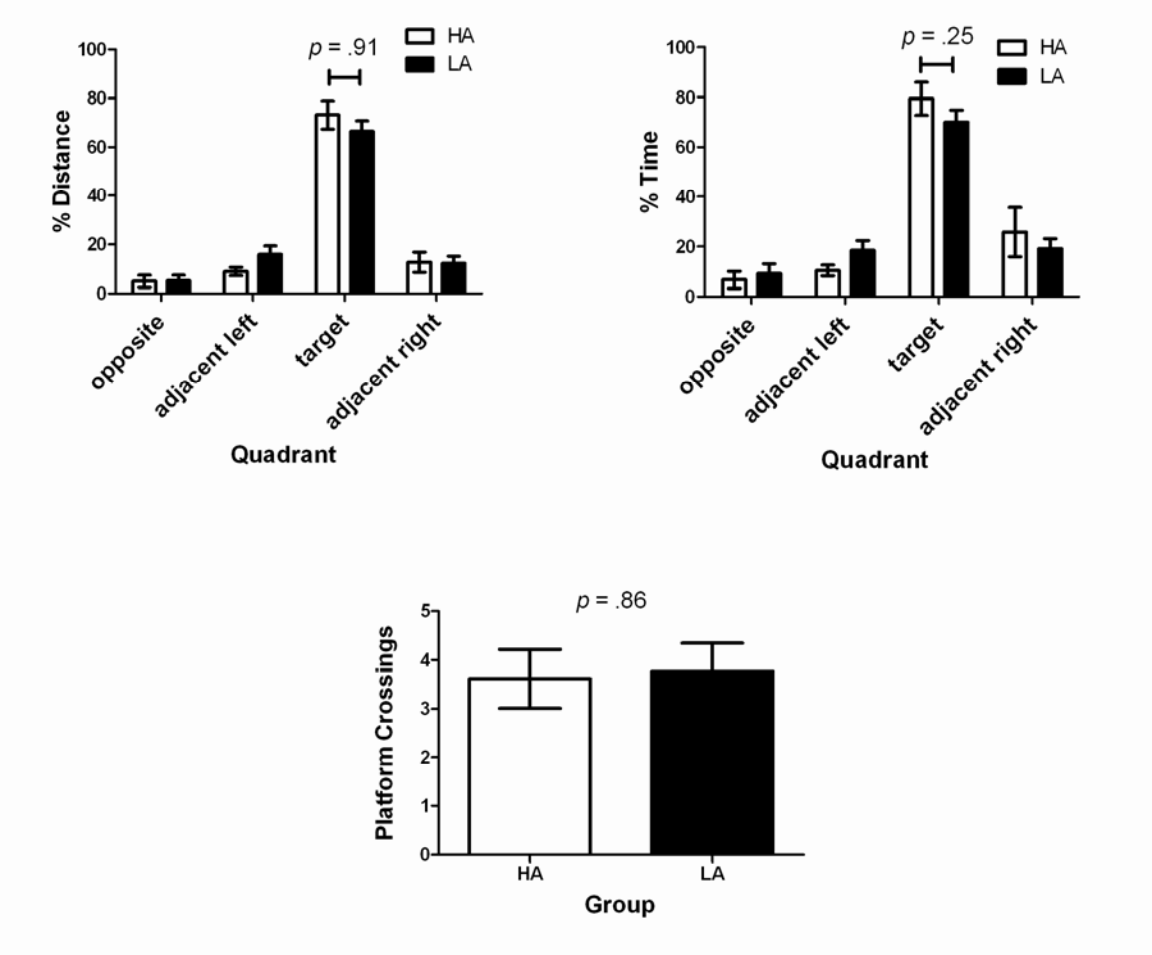
Trial	Latency & VO_2 peak	Cumulative Distance & VO_2 peak	% Time & VO_2 peak
1	*rho(30) = -.034* *p =.86*	*rho(30) = .005* *p =.98*	*rho(30) = -.273* *p =.144*
2	*rho(30) = .065* *p = .732*	*rho(30) = .119* *p = .564*	*rho(30) = -.044* *p = .818*
3	*rho(30) = .026* *p = .891*	*rho(30) = -.091* *p = .633*	*rho(30) = .087* *p = .649*
4	*rho(30) = .016* *p = .932*	*rho(30) = -.087* *p = .646*	*rho(30) = -.297* *p = .112*
5	*rho(30) = .111* *p = .561*	*rho(30) = -.036* *p = .849*	*rho(30) = .049* *p = .798*
6	*rho(29) = -.078* *p = .686*	***rho(29) = -.402*** ***p = .028***	***rho(29) = .428*** ***p = .021***



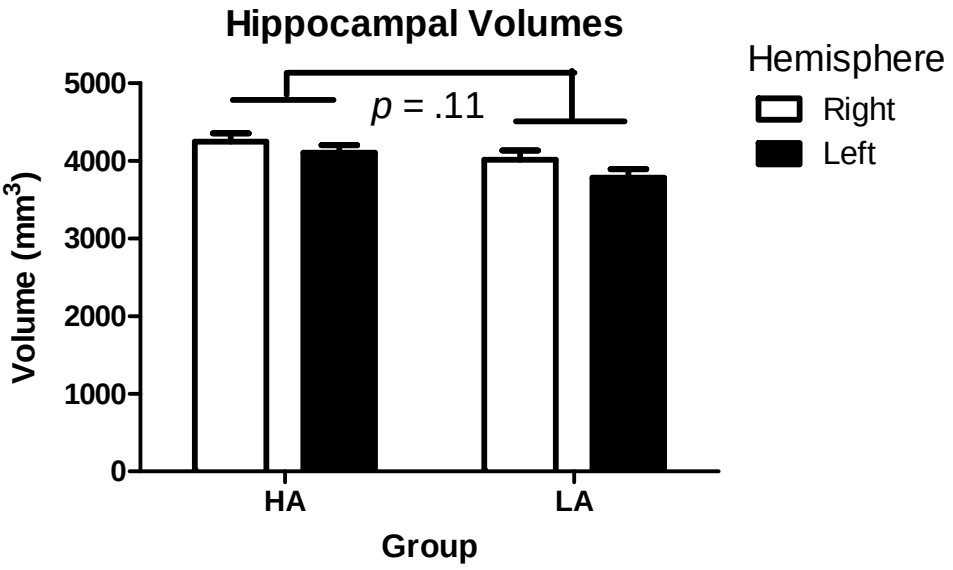
Supplemental Figure 1. RAVLT performance (mean ± SEM) by group. a) Verbal learning; better performance is reflected by higher Z-score values. b) Verbal recall memory; better performance is reflected by larger positive retention scores; retention scores reflect the total items recalled at delay minus the total items learned on trial 5. HA = high-active youth; LA = low-active youth.



Supplemental Figure 2. vMWT learning performance (mean ± SEM) by group. a) Spatial learning as measured by delta (δ), or percent (%) distance in target quadrant in trial 6 minus % distance in target quadrant in trial 1; b) distance (both cumulative as well as % distance in target quadrant); c) latency (both latency as well as % time in target quadrant). HA = high-active youth; LA = low-active youth.



Supplemental Figure 3. vMWT memory performance (probe trial) by group. % Distance and % Time spent in each of the quadrants during the probe trial is shown, as well as number of platform crossings.



Supplemental Figure 4. Hippocampal volumes for each hemisphere (mean ± SEM) by group. HA = high-active youth; LA = low-active youth.

Chapter 4: Differences in brain activity during a verbal associative memory-encoding task in high and low-active adolescents

(This work has been submitted to the *Journal of Cognitive Neuroscience*)

Introduction

Research has shown that exercise benefits learning and memory performance in adults and aged individuals (Winter et al. 2007; Erickson et al. 2009; Erickson et al. 2011). Recent studies have also found exercise to benefit memory performance in youth. Chaddock and colleagues (2010a; 2011a) showed that less aerobically-fit children (ages 9-10) displayed poorer performance on relational encoding of visual material, although aerobic fitness level did not correlate with non-associative memory performance. These findings are consistent with the possibility that physical fitness may enhance performance by changing the underlying neural circuitry of relational memory processing. However, despite the beneficial effect of exercise on memory performance, the neurobiological mechanism(s) remain unclear.

The benefits of exercise on learning and memory are thought to be mediated by exercise-induced changes in brain areas that directly subserve these processes, such as the prefrontal cortex and hippocampus. In rodents, exercise has been shown to stimulate new cell growth in the hippocampus, and this neurogenesis is associated with improved performance on spatial memory tasks (van Praag et al. 2005; Van der Borght et al. 2007; Clark et al. 2008). Similarly, human studies have found that aerobic exercise may also influence hippocampal structure. Using a cross-sectional design, aerobic fitness was related to better visuospatial short term memory, and this effect was mediated by larger hippocampal volumes in a large sample of elderly adults (Erickson et al. 2009). Similar results were seen using a longitudinal, intervention study. Individuals who engaged in aerobic exercise 3 days a week, for 1 year, had a 2% increase in the volume of the anterior hippocampus, as well as improvements in spatial memory (Erickson et al. 2011). Associations between aerobic fitness and larger hippocampal volumes and better memory performance have also been reported in children

(Chaddock et al. 2010a; Chaddock et al. 2011a) and adolescents (Herting and Nagel In Press). Beyond the hippocampus, exercise-induced increases in brain volume, including prefrontal (PFC) and temporal cortices have been described in elderly populations (Colcombe et al. 2003; Colcombe et al. 2006). Despite evidence for exercise-related structural changes in the brain, the impact of physical fitness on neural circuitry important for learning and memory has yet to be elucidated.

Functional magnetic resonance imaging (fMRI) is one technique that can be used to examine the neurobiological substrates underlying learning and memory. To date, a number of studies have implemented a subsequent memory effect paradigm to examine brain areas subserving successful encoding of new memories (Kim 2011). In this paradigm, subjects are presented with a series of stimuli to encode in the MRI scanner. After encoding, participants perform a subsequent memory task, and encoding stimuli are then sorted into trials that were later *remembered* versus (vs.) those that were later *forgotten*. FMRI blood oxygen level-dependent (BOLD) signal that is greater for remembered vs. forgotten stimuli reflects neural activity in brain regions that contribute to successful learning, whereas BOLD signal that is greater for stimuli that are later forgotten (vs. remembered stimuli) depicts neural activity in brain areas that are thought to interfere with successful learning and memory (Rugg et al. 2002; Kim 2011). Using this paradigm, encoding of successfully remembered information activates the left inferior frontal cortex, and bilateral fusiform gyrus, hippocampus, premotor cortex, and posterior parietal cortex (Kim 2011). However, the degree to which each of these brain areas is activated in the right versus left hemisphere varies by stimulus modality (verbal vs. pictorial) and the encoding process (associative vs. item). In contrast to successful encoding, areas that have been implicated in subsequent forgetting include brain regions that have previously been determined to be part of the default mode network (DMN).

The DMN is a large-scale network comprised of the posterior cingulate cortex (pCC), medial prefrontal cortex (mPFC), lateral parietal cortices, retrosplenial cortex, and angular gyrus (Raichle et al. 2001; Fox et al. 2005). These brain regions were originally identified as a potential network, as they show decreased activation during a variety of goal-directed tasks. Based on this, a hypothesis emerged suggesting that the DMN is continuously active, but is suppressed when resources are required for externally-focused cognitive processing, ultimately resulting in deactivation of the DMN and activation of the task-specific networks (Raichle et al. 2001; Fox and Raichle 2007). During learning, the DMN may need to deactivate to allocate resources to areas important for encoding, such as the PFC and hippocampus. Evidence for this idea comes from a number of studies showing that DMN regions, most notably the pCC and lateral parietal lobes, show greater deactivation during encoding of later remembered than forgotten items, suggesting that deactivation of the DMN is essential for processing information so that it can later be remembered (Daselaar et al. 2004; Daselaar et al. 2009; Kim et al. 2010; Vannini et al. 2011). Thus, neural circuitry underlying successful memory encoding includes not only task-related activation of hippocampal and prefrontal cortices, but also a deactivation of the DMN.

The goal of the current study was to fill gaps in the existing literature by examining how exercise influences memory-related brain activity in adolescence-- a period of rapid brain development. Brain activity was examined between 17 high-active (HA) and 17 low-active (LA) male adolescents during a verbal, associative subsequent memory effect fMRI paradigm. Based on previous research showing a positive association between exercise and memory performance in children and adults (Erickson et al. 2009; Chaddock et al. 2010a; Chaddock et al. 2011a; Erickson et al. 2011), we hypothesized that HA youth would have enhanced memory performance on a verbal

associative memory task compared to LA youth. Verbal memory encoding is largely subserved by the left PFC and hippocampus in both youth and adults (Maril et al. 2010; Kim 2011). Moreover, the anterior hippocampus has been shown to be specifically involved in relational memory encoding (Lepage et al. 1998; Giovanello et al. 2004; Chua et al. 2007). Therefore, we also hypothesized that successful memory encoding would be subserved by the left anterior hippocampus and PFC, as well as deactivation of regions of the DMN. Lastly, given that exercise increases new cell growth, as well as a number of neurotrophic factors in the hippocampus (for review see van Praag 2009) which may lead to greater plasticity and excitability of the neurons, it is possible that hippocampal cells may be more efficient at learning in individuals who engage in more aerobic exercise. Therefore, we hypothesized that hippocampal neurons may be more efficient, resulting in less hippocampal BOLD signal activation in HA youth compared to their LA peers, when successfully encoding new memories.

Materials and methods

Participants

Participants included 34 eligible male youth, ages 15 to 18 years. Participants were recruited from the community as part of an ongoing adolescent neurodevelopment study. Following written consent and assent, the youth and a parent participated in separate structured telephone interviews to determine eligibility. Inclusionary criteria for youth included being male, 15 to 18 years of age, and meeting either high or low-active criteria defined below. Exclusionary criteria included current DSM-IV psychiatric diagnoses [Diagnostic Interview Schedule for Children Predictive Scales (4.32b)(Lucas et al. 2001; Hoven et al. 2005)]; significant substance use (>10 lifetime alcoholic drinks or 2 drinks/occasion, > 5 uses of marijuana, any other drug use, or > 4 cigarettes per day) [Brief Lifetime Customary Drinking and Drug Use Record (Brown et al. 1998)];

history of psychotic disorders in biological parents [Family History Assessment Module (Rice et al. 1995)]; major medical condition or significant head trauma [Structured Clinical Interview (Brown et al. 1994)]; left-handedness [Edinburgh Handedness Inventory (Oldfield 1971)], or irremovable metal. Youth and parents were each compensated $10 for completing the interviews, and youth were compensated $100 for completing behavioral tests and MRI scanning.

Activity classification

A modified version of the Youth Adolescent Activity Questionnaire (YAAQ) was administered to youth to assess participation in aerobic exercise over the past year. The YAAQ asks detailed questions about physical activity participation across all four seasons of the year, as well as the number of hours per week spent doing each activity (Wolf et al. 1994). Seasonal format questionnaires, such as the YAAQ, have been shown to increase the accuracy of self-report of physical activity in adolescents (Rifas-Shiman et al. 2001). Based on hours of aerobic activity reported by the youth on the YAAQ, HA youth were defined as those participating in an average of ≥10 hours per week of regular, organized aerobic physical activity purposely performed to allow for improvement or maintenance of aerobic fitness, including basketball, soccer, football, track, cross country, and swimming across one or more seasons (summer, fall, winter, spring) within the past year. LA youth were defined as those individuals that had participated in ≤1.5 hours of aerobic physical activity per week over the past year. The majority of HA youth participated in an average of 10+ hours per week over the past year (n = 11), and the remaining HA that played sports more seasonally, still averaged a relatively high amount of aerobic exercise over the entire year (mean = 7.78, standard deviation = 2.4, n = 6) hours. HA youth were asked to participate in the study during the season in which they were most physically active based on their YAAQ self-report. LA

youth were defined as those individuals that had participated in ≤1.5 hours of aerobic physical activity per week over the past year. These criteria were set forth, as significant increases in aerobic fitness have been seen in adolescents who participated in ≥10 hours of aerobic exercise per week (Brown et al. 1972; Weber et al. 1976; Lussier and Buskirk 1977), and relatively extreme categorizations (≥10 versus ≤1.5 hours per week) maximize the likelihood of detecting group differences. Based on these criteria, 17 youth were considered LA and 17 were HA youth.

Participant characterization

General Intelligence: Participants were administered the 2-subtest version of the Wechsler Abbreviated Scale of Intelligence (Wechsler 1999) to estimate intellectual functioning.

Socioeconomic Status (SES): Information was gathered on SES by administering the Hollingshead Index of Social Position (ISP) to parents, as well as collecting information on total household income as part of the structured telephone interview.

Body Mass Index: Strong associations exist between sedentary lifestyle and obesity, and differences have been noted between obese and non-obese individuals using neuroimaging techniques (Carnell et al. 2011). Thus, height and weight were obtained and the Center for Disease Control and Prevention Child and Teen Calculator was used to determine BMI (Center for Disease Control 2011).

Pubertal Status: Although more common in girls, intensive physical exercise has been reported to delay pubertal maturation (Georgopoulos et al. 2010). Thus, pubertal status was assessed using the self-rating Pubertal Development Scale (PDS) (Petersen et al. 1988).

Personal Lifestyle Questionnaire (PLQ): The Revised PLQ (Mahon et al. 2003) was used to assess general differences in lifestyle that may also explain group differences in performance or brain response. For example, individuals who exercise more may be more conscientious about health in general, such as eating healthier and abstaining from unhealthy behaviors, substance use. Since diet (Molteni et al. 2004; van Praag 2009) and substance abuse (Moss et al. 1994; Crews et al. 2000; De Bellis et al. 2000; Tapert et al. 2004; Nagel et al. 2005; Crews et al. 2006; Medina et al. 2007) have been shown to impact the brain and behavior, it was especially important to assess each subject's lifestyle habits. This is a 24-item questionnaire that has six subscales, including Nutrition (4 items), Safety (3 items), Relaxation (5 items), Health Promotion (4 items), Substance Use (4 items), and Exercise (3 items). The items are measured on a 4-point scale from 1 (never) to 4 (always). After reverse scoring some items, higher total score values indicate more positive health practices.

Extracurricular Activities: As another measure to assess differences in lifestyle between the groups, youth were asked the frequency in which they participated in extracurricular activities (including sports, clubs, recreational activities, etc.) on a scale of 1 (never) to 4 (at least once/week). In addition, a list of activities was obtained and the number of activities was summed for each subject. Furthermore, because physical activity has been shown to be negatively associated with time spent playing video games in adolescents (Janz and Mahoney 1997), average time spent playing video games was assessed. Subjects reported the console type used (e.g. PlayStation, etc.), the average number of hours on each console type per day (i.e. sessions), and the number of sessions per console type per week. Number of hours and number of sessions were summed across console types and multiplied together to assess the average number of hours of video games played per week.

Aerobic fitness assessment

In addition to self-report, aerobic fitness was objectively measured by peak aerobic uptake (VO_2 peak) for each participant. VO_2 peak is a measure of maximum capacity of an individual's body to transport and utilize oxygen during exercise, and is thought to be the most valid objective measurement of physical fitness (Armstrong and Welsman 2007). VO_2 peak was measured using the same computerized indirect calorimetry system (VMax Series, V6200 Autobox) during a Bruce Protocol (Bruce et al. 1973). VO_2 peak values were only considered valid if the participant delivered maximal effort on the test, as defined by at least one of the following the physiological criteria (Armstrong and van Mechelen 2008): 1) oxygen consumption remained at a steady state despite an increase in workload, as evidenced by a plateau in oxygen consumption, 2) heart rate reached ≥ 200 beats per minute, 3) the respiratory exchange ratio ≥ 1.0; and/or the subjective criteria of reporting a 10 on the perceived exertion scale. Lean body mass (LBM) was determined just prior to aerobic testing by conducting a bioelectrical impedance test on each subject using the Body Composition Analyzer (Model 310e; Biodynamics Corp), allowing for peak oxygen consumption to be expressed in mL/kg LBM/min.

Image acquisition

Images were acquired on a 3.0 Tesla Siemens Magnetom Tim Trio system (Siemens Medical Solutions, Erlangen, Germany) with a twelve-channel head coil. Whole-brain, high-resolution structural anatomical images were acquired in the sagittal plane using a T_1 weighted MPRAGE scanning sequence (TI = 900ms, Flip Angle = 10 degrees, TE = 3.58 ms, TR = 2300 ms, acquisition matrix = 256x240, resolution = $1mm^3$). Whole-brain functional images were collected in the axial plane, oblique to the anterior-posterior commissure (AC-PC), using a T2*-weighted echo planar blood oxygen

level dependent (BOLD) interleaved sequence (TR = 2000 ms, TE = 30 ms, FOV= 240 mm, flip-angle = 90°, 33 slices no gap, resolution = 3.75 mm^3). Stimuli were viewed through a mirror mounted on the head coil and responses made through a button box.

Verbal associative memory encoding task

A subsequent memory paradigm was used and consisted of 2 phases: encoding in the MRI scanner and a subsequent memory recognition test outside the scanner (**Figure 1**). For encoding, a rapid event-related design subsequent memory word-pair fMRI task was used to assess intentional verbal associative memory encoding. Stimuli consisted of two unrelated words presented horizontally in white font against a black background. A total of 231 word-pairs were presented for 2.75 seconds each, followed by .25 seconds of visual fixation across 3 runs using E-prime software (Psychology Software Tools, Pittsburgh, PA). Words were implemented from a published subsequent memory paradigm by Kuhl et al. (2010), and to stay consistent with the published paradigm, were randomly combined to construct the novel pairs. Fixation stimuli consisted of a white crosshair presented against a black background. Each word-pair was then followed by an additional period of fixation varying from 0 to 10 seconds(s), using an optimized (created by optseq2 from Freesurfer; surfer.nmr.mgh.harvard.edu/optseq) jittered event-related design (Dale 1999). Participants were instructed to “learn which word pairs are presented together, as you will be tested on them later.” In addition to learning word-pairs, they were told to make a purely subjective decision about whether or not the words “fit” together with a button press for “yes, the words fit together,” or “no, the words do not fit together”. These instructions were chosen to ensure the subjects attended to both words and processed them associatively. Both groups showed a high percentage of “no fit” responses, supporting the purposely chosen unrelated nature of the word-pairs used as stimuli [HA

= 84 ± .029%; LA = 82.8 ± .034%; *U = 136.5, z = .28, p = .77*]. Groups also did not differ on reaction time to make responses during encoding [HA = 1586 ± 44.9 milliseconds (ms); LA = 1590 ± 52.8 ms; *U = 127, z = .60, p = .56*].

For the subsequent memory test, participants completed a self-paced association recognition memory test outside the scanner approximately 20-minute post-encoding. The association recognition memory test included 231 trials presented on a computer via E-prime software (Psychology Software Tools, Pittsburgh, PA), where participants were given the first word presented from each of the 231 word-pairs and two words presented underneath it, and were instructed to "choose which word was correctly paired with the presented word in the scanner". Out of the two word choices presented for each trial, one was always the correctly paired word, and the other was a foil with equal familiarity (i.e. another word that presented as part of a different word-pair in the scanner). After every trial, participants were asked if they had high or low confidence in their decision. All participants received the same order of word-pairs for encoding and the subsequent memory tests. By using a subsequent association recognition memory test, we were able to classify neural response of trials during encoding that were later remembered (correctly identified association on subsequent memory test) versus those that were later forgotten (incorrect identified association on subsequent memory test). Furthermore, by assessing subjective confidence on recognition performance we were able to further classify correct responses into high confidence correct and low confidence correct. The assumptions for these classifications are that high confidence correct associations reflect those word-pairs that the individual had a strong memory of, whereas the low confidence correct associations may reflect a sense of either familiarity or correct guessing. Therefore, because the root of low confident correct associations may be more ambiguous, group differences in associative memory were examined between

encoding trials that were later remembered correctly with high confidence ("*remembered*") versus those associations that were later incorrectly identified on subsequent memory test ("*forgotten*") (**Figure 1**). In addition to measuring recognition memory performance (accuracy and reaction time), a type 2 signal discriminability (*d'*) was calculated p(high confidence | correct) – p(high confident | incorrect) for each subject to assess above chance performance (Clarke et al. 1959).

Image preprocessing

Data were processed and analyzed using Analysis of Functional NeuroImages (AFNI) (Cox 1996). The first 3 TRs of each scan were excluded to allow for steady state magnetization. Preprocessing included slice timing correction, motion correction, co-registration of functional to anatomical images, and spatial smoothing using a Gaussian filter (full width at half maximum = 6 mm kernel). To further reduce movement related confounds, root mean square (RMS) of within-run motion across the 6 motion parameters were calculated for each subject, and TRs showing greater than 2.5 mm or 2.5° in any of the 6 parameters were removed from the subsequent analyses. Importantly, RMS values were low (< 1) for all participants, and RMS values between groups was not statistically different [$U = 134$, $z = .36$, $p = .73$]. Next, functional masks were created to mask out non-brain areas, and then time series data were normalized to the time series mean.

To determine if aerobic activity in adolescents has a global impact on brain activity, or does so with some degree of regional specificity, we examined the amplitude and shape of the BOLD signal in a hypothetical control region, the primary visual cortex. To this end, an exploratory analysis was performed where the BOLD time course for the task was modeled with 9 individual tent functions that were evenly spaced from onset of the stimulus (0s) to 12s post-stimulus, without assuming a particular shape (Cox 1996).

Bilateral primary visual cortex regions of interests (ROIs) were created (4mm diameter spheres centered at Talairach coordinates ±17, -95, 8), and time courses of the HRF for later remembered and forgotten word-pairs were extracted and plotted for each group. The results showed no significant differences in the peak amplitude or the time course for the HRF (*p*'s ≥ .13, Cohen's *d* = .21)(**Supplemental Figure 1**). Given these results, a gamma-variate HRF was assumed for examining group-related differences in BOLD response during memory encoding. Using a deconvolution process, regressors representing high confident correct (*remembered*), low-confident correct, and incorrect (*forgotten*) word-pair stimuli were modeled with stimulus times corresponding to the onset of stimuli presentation, with the duration of the event coded as the length of each stimuli convolved with a gamma-variate HRF (Cohen 1997). Brain response during encoding of low confident correct stimuli was included in the model but was not analyzed. The estimated baseline in this model was comprised of the BOLD signal from the entire time course, linear drift, unmodeled fixation periods, and regressors of no interest (e.g., 6 motion parameters)(Cox 1996). Contrasted images for average percent signal change of *remember vs. baseline*, *forgotten vs. baseline*, and *remember vs. forgotten* were created. Functional data sets were resampled into 3 mm^3 voxels and transformed into standard Talairach coordinates (Talairach and Tournoux 1988) prior to group-level comparisons.

Hippocampal regions of interests (ROIs)

Bilateral hippocampal ROIs were manually traced on each individual's high resolution anatomical image based on a previously established method (Herting and Nagel In Press). The tracer (MH) was blind to participant characteristics, and established high intra and inter-rater reliability (all intraclass correlations ≥.95) on an independent sample using this previously published protocol prior to tracing. Bilateral hippocampi

were traced on contiguous coronal slices, perpendicular to the AC-PC plane using AFNI, and were confirmed in axial and sagittal view. ROIs were resampled to match the functional data (3 mm^3 voxels).

Susceptibility artifact assessment

Regions of the medial temporal lobe are especially susceptible to signal loss due to its relative location near the petrous bone (Ojemann et al. 1997). To assess hippocampal signal-to-noise ratios (SNR), hippocampal ROIs were used to extract signal from the original T2* weighted echo planar images. SNR were then estimated by dividing each voxel's mean intensity by its standard deviation across time, and extracted from the hippocampal ROIs for each subject. All subjects had adequate hippocampal SNR (mean = 99.18 ± 28.5) to detect an expected 1 to 2% change in the MR signal based on the fMRI paradigm design (Parrish et al. 2000). Groups did not differ in estimated SNR values [$t(32) = .98$, $p = .33$].

Analyses

All statistical analyses were carried out using PASW 18 (Chicago, Illinois). Normality was verified on all variables, and transformations were used when appropriate. When data continued to violate normality (as seen by visual inspection and Shapiro-Wilks test ($p < .05$)), nonparametric tests were employed. Independent *t*-tests were used to examine participant demographics, aerobic fitness, BMI, as well as lifestyle differences between the two groups. Group differences in demographic data (p's ≤ .09) were used as covariates when exploring memory behavior and brain activity between the groups. To determine if memory behavior was different from chance, one-sample t-tests were used to examine *d'* from zero for each group. Furthermore, to examine memory recognition performance (accuracy and reaction time), separate 2-within

(recognition (remembered vs. forgotten), confidence (high vs. low)), 1-between (group) ANCOVA were used. Follow-up regression analyses were also used to determine if VO_2 peak values were related to the dependent variables of interest, while controlling for puberty and SES.

Brain response was examined using AFNI and included both whole-brain and ROI approaches. To assess overall task-related activity, individual whole-brain one-sample t-tests were used to examine the *remembered vs. forgotten* contrast for each group. Results were voxelwise (p <.01) and cluster-corrected (# voxels ≥ 60) for multiple comparisons, and mapped onto surface using Caret software (Van Essen et al. 2001). Whole-brain ANCOVA analysis was used to examine group differences in subsequent memory (*remembered vs. forgotten*) brain activity. These results were also corrected for multiple comparisons at both the voxelwise (p < .01) and cluster level (# voxels ≥ 48). To further assess group differences, the mean percent signal change in each of the simple contrasts (*remembered vs. baseline* and *forgotten vs. baseline*) was extracted for each participant from significant clusters, and exported into PASW for further post-hoc analyses.

A priori analyses were performed to examine group differences in brain response in the bilateral anterior hippocampus. A bilateral anterior group-level hippocampal mask was created by summing the hand-drawn ROIs across all participants and extracting the anterior one-half. ANCOVA was then used to examine group differences in memory encoding (*remembered vs. forgotten*) brain activity bilaterally in the anterior hippocampus. For this *a priori* ROI analysis, p < .05, with a cluster correction of ≥ 15 voxels was considered significant. Post-hoc regression analyses were also performed to examine how VO_2 peak related to subsequent memory brain activity for the simple

contrasts in both whole-brain and ROI-based clusters (*remembered vs. baseline* and *forgotten vs. baseline*), while controlling for puberty and SES.

Results

One participant's parent (LA) chose not to disclose total household income, and one subject (HA) did not complete the PLQ, resulting in missing data for these measures. Participant characteristics, aerobic fitness, and body composition results can be found in **Table 1**. The groups were matched on age, IQ, and were not significantly different on a number of lifestyle behaviors, including nutrition, relaxation, health promotion, safety, substance use, frequency and number of extracurricular activities, and video game habits. The groups were also matched on BMI. Although both groups came from households that made above the national income average, the HA had an overall higher SES and median household income, as reported by their parents. Self-report of pubertal maturation was also different between the groups, with HA being less mature compared to LA. To account for these differences, SES and puberty were covaried for in all subsequent analyses. VO_2 peak testing was used to objectively measure aerobic fitness and aerobic differences expected between the groups based on self-report of aerobic exercise participation. VO_2 peak values were significantly different between the groups, confirming that HA youth had better aerobic fitness compared to LA youth.

Task performance

On the recognition task, the HA and LA youth showed similar memory performance (**Table 2**). Both groups showed *d'* that was significantly above chance [HA: $t(16) = 10.8$, $p < .001$, Cohen's $d = 5.4$; LA: $t(16) = 12.4$, $p < .001$, Cohen's $d = 6.2$]; although *d'* was not significant different between the groups [$t(32) = .94$, $p = .36$, Cohen's $d = .32$]. ANCOVA results showed no main effect for accuracy performance [recognition:

$F(1,30) = 2.13$, $p = .16$, partial $\eta^2 = .066$; confidence: $F(1,30) = 1.83$, $p = .19$, partial $\eta^2 = .057$]. Furthermore, the effect of group was not significant for accuracy performance [$F(1,30) = .63$, $p = .43$, partial $\eta^2 = .02$], nor were there any significant two-way interactions [recognition: $F(1,30) = .15$, $p = .69$, partial $\eta^2 = .005$; confidence: $F(1,30) = .15$, $p = .70$, partial $\eta^2 = .005$] or a three-way interaction with group status [$F(1,30) = .45$, $p = .51$, partial $\eta^2 = .015$]. The main effects of recognition [$F(1,30) = .953$, $p = .34$, partial $\eta^2 = .03$], confidence [$F(1,30) = 2.13$, $p = .16$, partial $\eta^2 = .07$], and group [$F(1,30) = .19$, $p = .67$, partial $\eta^2 = .006$] on reaction times were not significant. In addition, the two-way interactions [recognition: $F(1,30) = 1.77$, $p = .19$, partial $\eta^2 = .056$]; confidence: $F(1,30) = .01$, $p = .91$, partial $\eta^2 = .000$] and three-way interaction [$F(1,30) = 1.69$, $p = .20$, partial $\eta^2 = .053$] with group were not significant. No significant relationship was seen between VO_2 peak and task performance (p's >.05).

Verbal associative encoding brain activity

Whole Brain: The groups showed similar overall patterns of brain activity in the *remembered* vs. *forgotten* contrast (**Figure 2**, **Table 3**). Both groups showed greater activation during the encoding of word-pairs later remembered compared to those later forgotten in a number of brain regions important for verbal memory encoding, including the left inferior, middle, and superior frontal gyri, as well as in left temporal lobe, including the parahippocampal gyrus and hippocampus. LA adolescents also showed significant activation to *remembered* vs. *forgotten* word-pairs in additional regions, including the right hippocampus, bilateral basal ganglia, and right cerebellum. For both groups, the pattern of activity during unsuccessful encoding of word-pairs was seen in key default mode regions. Both groups showed greater activity in the right inferior and superior parietal lobules, middle frontal gyrus, posterior cingulate cortex, and supramarginal gyrus during encoding of forgotten word-pairs (**Table 3**).

Despite these similarities, between-group analyses of the *remembered* vs. *forgotten* contrast revealed significant group differences in BOLD response (**Figure 3**, **Table 3**). LA youth showed greater BOLD activity in the right superior and middle frontal gyri, as well as in several DMN regions, including left superior temporal gyrus, left ventral mPFC, bilateral pCC, and right inferior parietal lobule/precuneus (IPL), when compared to HA youth. To further understand these differences, mean percent signal change in the *remembered* vs. *baseline* and the *forgotten* vs. *baseline* contrast was extracted. Results revealed two distinct patterns driving these group differences. In the right superior and middle frontal gyri, LA adolescents had significantly higher activation during encoding of remembered word-pairs compared to HA youths (e.g. **Figure 3a**, green subplot), whereas groups did not significantly differ in activation in this region when encoding later forgotten word-pairs. The remaining 4 clusters showed a different pattern (**Figure 3b**). Consistent with the fact that these 4 clusters overlapped with typical DMN areas (Raichle et al. 2001; Fox et al. 2005), HA youth showed strong deactivation in these regions during successful encoding, and less deactivation when encoding word-pairs that were subsequently forgotten. LA youth, however, had less deactivation in these regions compared to HA youth during successful encoding, but had similar, or even greater, deactivation compared to HA youth during encoding later forgotten stimuli (e.g. **Figure 3b**, blue subplot). Effect size calculations showed that these differences were large in magnitude between the groups (Cohen's *d's* ≥ 1.24), and that the effect size of group difference in these clusters was significantly larger than the effect size in the visual control region (*z*-statistics ≥ 2.02, *p*'s < .05). No significant results were found when using VO_2 peak to predict brain activity in the *remembered vs. baseline* or *forgotten vs. baseline* contrasts across all subjects (*p*'s > .05). Exploratory follow-up analyses were performed to determine if VO_2 peak explained the relationship between

group and BOLD signal in these regions. All five clusters remained significant between the groups after controlling for VO_2 peak.

*Hippocampal ROIs***:** Between-group analyses revealed bilateral increased BOLD response for the *remembered* vs. *forgotten* contrast in the LA youth compared to HA youth [Left cluster: 19 voxels; *x = -32, y= 17, z = -19,* Cohen's *d* = 1.02; Right cluster: 15 voxels; *x = 29, y = 17, z = -16,* Cohen's *d* = .88] (**Figure 3c**). Simple contrast analyses showed that HA adolescents significantly activated the left hippocampus during encoding of later *remembered* word-pairs (vs. *baseline*), whereas the LA group showed bilateral increases in hippocampal BOLD signal during encoding of remembered word-pairs (vs. *baseline*). LA youth also showed significantly greater activation in both the right and left hippocampus during the encoding of later *remembered* vs. *forgotten* stimuli. HA youth, however, did not show a difference in BOLD response in the left or right hippocampus when encoding of later *remembered* vs. *forgotten* word-pairs (**Figure 3c**, pink subplot). Group effect sizes were larger for both of the hippocampal ROIs compared to the visual control regions, albeit these did not reach statistical significance (*z*-statistics: left: *z* = 1.60, *p* = .11; right: *z* = 1.34, *p* = .18). Follow-up analyses examining the relationship between VO_2 peak and hippocampal ROI BOLD signal were not significant (*p*'s ≥ .05).

Exploratory Context-Dependent Correlation Analyses: Given that the hippocampus has been shown to be inversely correlated with DMN activity during successful memory encoding (Huijbers et al. 2011; Vannini et al. 2011), the reduced DMN deactivation reported above may imply that segregation between the hippocampus and DMN may be impaired in low-active youth during successful memory encoding. In hopes of better understanding the extent in which DMN deactivation relates to hippocampal activation in

each of the groups, context-dependent changes in functional correlations were examined using AFNI (Chen 2011). This analysis was used to determine whether group differences were seen between hippocampal functional connectivity (correlated activity) with DMN regions during trials of successful memory encoding (*remembered*) relative to the non-encoding context of the *baseline* condition. For each participant, a physiological regressor was made by removing the linear trend, extracting the average time series data from the seed regions (left and right hippocampal ROIs), and deconvolving it with a gamma-variate HRF. The encoding condition (*remembered* vs. *baseline*) was used as the psychological regressor. The psychophysiological interaction regressor was then created by multiplying the deconvolved HRF time series (physiological regressor) with a vector coded for the contextual contrast of interest (psychological regressor). Separate deconvolution regression analyses were then performed for the left and right hippocampal seed regions, using a model that included the original regressors of interest and no interest, as well as the newly created deconvolved hippocampal time course and the psychophysiological interaction regressor. ANCOVA was then used to assess the fit of the interaction regressor between the two groups within the DMN regions previously shown to be significantly different between the groups (pCC, mPFC, IPL). Multiple comparison correction for these analyses required a voxelwise threshold of $p < .005$ and cluster threshold of ≥ 8 voxels.

Results were similar for the left and right hippocampus and showed that correlation between the BOLD response in the hippocampus and the mPFC, as well as the hippocampus and right IPL, differed between the groups during successful encoding of new memories (**Table 4, Supplemental Figure 2**). In other words, functional connectivity between the hippocampus and mPFC and IPL during *remembered* vs. *baseline* conditions differed between HA and LA youth. To further understand these

findings, functional connectivity during *remembered* vs. *baseline* were investigated for each group separately. HA youth showed a strong negative correlation in BOLD response between the hippocampus and both DMN brain regions during successful memory encoding (vs. *baseline*), whereas LA youth showed positive coupling (**Table 4, Supplemental Figure 2**).

Discussion

The current study examined how aerobic fitness affects brain activity during a learning and memory task using fMRI. HA and LA adolescents displayed similar recognition memory performance on the verbal associative subsequent memory task. However, LA youth showed increased BOLD response in the right superior frontal gyrus and bilateral hippocampi during encoding of subsequently *remembered* vs. *forgotten* word-pairs compared to HA youth. Furthermore, LA youth show less deactivation in DMN regions, and had reduced negative functional connectivity between bilateral hippocampus and DMN regions, including the mPFC and IPL during successful memory encoding.

A meta-analysis of 74 adult studies found that successful verbal associative encoding requires activation of the left inferior frontal gyrus and left hippocampus (Kim 2011). Recent research also attests to the importance of DMN deactivation for successful encoding, as unsuccessful encoding has been demarcated by less deactivation in key default regions, like the pCC (Daselaar et al. 2004; Daselaar et al. 2009; Kim 2011; Vannini et al. 2011). Consistent with these findings, the current study showed that both HA and LA adolescents show greater activation of the left inferior frontal gyrus and the left hippocampus during encoding of later *remembered* (vs. *forgotten*) word-pairs, whereas encoding of later *forgotten* word-pairs was hallmarked by increased task-related BOLD response in DMN areas (as opposed to deactivation),

including the mPFC, pCC and lateral parietal cortices. These similarities to previous studies (Kim 2011) confirm that the current subsequent memory task activated the expected associative encoding neural circuitry, allowing us to proceed in examining how aerobic exercise influences memory-related processes.

Of the 231 word-pairs shown, participants *remembered* approximately 60% on the post-scan recognition memory test. Somewhat surprisingly, however, there were no group differences in performance. This did not confirm our hypothesis, and failed to echo previous work finding greater associative memory performance in aerobically higher-fit compared to lower-fit individuals (Erickson et al. 2009; Chaddock et al. 2010a; Chaddock et al. 2011a; Erickson et al. 2011). The dissimilarities between our study and previous research may be due to the relatively small sample size of the given study, which may have had insufficient power to detect a difference in memory performance between the groups. Alternatively, it is possible that the apparent disparities in the literature may be a function of the tasks used to assess learning and memory. Whereas the current study examined verbal associative encoding, differences in performance between HA and LA individuals were previously seen for verbal item memory (Winter et al. 2007), visual item and relational memory (Chaddock et al. 2010a; Chaddock et al. 2011a), and visual short-term delayed-match to sample (Erickson et al. 2009; Erickson et al. 2011). Furthermore, previous work from our lab has shown that aerobic exercise in adolescents significantly predicted spatial learning on a virtual Morris water task, however, no significant relationships were seen between aerobic fitness and verbal learning and memory performance (Rey Auditory Verbal Learning Test) (Herting and Nagel In Press). Therefore, it is possible that aerobic exercise may not have a ubiquitous effect on different types of memory. Research is

needed to determine whether different types of memory are influenced to significantly different degrees by exercise, and if these relationships change with age.

Despite the groups not differing in performance, a number of differences were seen in memory-encoding neural circuitry between HA and LA youth. First, LA youth showed greater bilateral anterior hippocampal BOLD response to encoding of later *remembered* vs. *forgotten* word-pairs compared to HA youth. To our knowledge this is the first study of its kind to examine how exercise relates to hippocampal function. While fMRI does not allow us to determine the molecular and cellular mechanisms that might underlie these findings, differences in hippocampal function may result from exercise-induced changes in neurogenesis and growth factors that have been well-documented in the animal literature (For review see van Praag 2008). In particular, aerobic exercise may lead to increases in hippocampal neurogenesis and synaptic plasticity, which may ultimately result in more efficient hippocampal neurons, as depicted by less BOLD activity during successful memory encoding in HA than LA youth. This hypothesis may help to explain why LA youth showed greater hippocampal activation during encoding of *remembered* vs. *forgotten* word-pairs compared to their HA peers. Although more research is needed to understand the underlying mechanism(s), to our knowledge, the present study is the first to extend the documented effects of exercise on hippocampal structure to hippocampal function.

In terms of patterns of activation, hemispheric differences were also seen. While HA youth showed expected unilateral activation in left hippocampus and superior and middle frontal gyrus (consistent with previous reports (Maril et al. 2010; Kim 2011)), LA youth displayed bilateral BOLD response in these areas during successful versus unsuccessful encoding. Recruitment of an adjacent hemispheric homologue has been repeatedly interpreted as a compensatory mechanism to allow groups to obtain similar

performance (e.g. children vs. adults (Gaillard et al. 2000; Moses et al. 2002), patients vs. controls (Thiel et al. 2006; Jensen et al. 2011), young adults vs. aging (Dolcos et al. 2002)). Bilateral hippocampal activation has also been shown to occur as demands increase during associative memory encoding, suggesting that the contralateral hippocampus may be recruited to prevent poorer memory (Ulrich et al. 2010). Taken together, engagement of the right hippocampus and PFC during successful memory encoding may suggest LA youth need to recruit additional brain regions to perform similarly to HA youth. Future work should examine brain activity in HA and LA youth, while varying task demands, to confirm if bilateral activation is indeed compensatory.

In addition to the PFC and hippocampus, group differences were also found in deactivation patterns within DMN regions. HA youth showed greater deactivation than LA youth in key DMN regions, such as the mPFC and pCC, for *remembered* vs. *forgotten* word-pairs. This difference was primarily driven by less deactivation in the *remembered* (vs. *baseline*) condition in LA youth. Exploratory context-dependent functional connectivity further showed that HA youth showed strong negative correlations between bilateral hippocampus and mPFC and right IPL, suggesting the hippocampus is inversely cooperating with the DMN during successful memory encoding. These findings converge with existing literature suggesting that hippocampal activation and strong deactivation of the DMN co-occur to allow for successful memory encoding (Daselaar et al. 2004; Daselaar et al. 2009; Huijbers et al. 2011; Kim 2011; Vannini et al. 2011). Interestingly, LA youth did not show this strong negative correlation in BOLD response between the hippocampus and mPFC and the right IPL. This result may reflect poorer cooperation between the hippocampus and DMN during encoding of memories in LA youth. However, it is important to note that neither directionality nor cause and effect with regard to brain and behavior can be resolved

from this type of analysis. Context-dependent correlation analyses could either reflect that encoding new memories influences functional connectivity between the hippocampus and DMN, or alternatively, that the hippocampus influences the effect that the psychological state of memory encoding has on DMN activity. Nonetheless, together, these findings suggest that the hippocampus and DMN regions show altered connectivity in LA youth when encoding later remembered word-pairs when compared to their HA peers.

Given that DMN deactivation is thought to be essential for successful encoding and is not seen in populations with or at-risk for impaired memory performance (Miller et al. 2008; Pihlajamaki et al. 2009), it is somewhat surprising that LA youth display this pattern of deactivation during the successful encoding of new memories, and also are able to obtain similar task performance to their HA peers. There are a number of possible explanations that might help to explain this finding. First, perhaps the aforementioned compensatory activation of the right hippocampus and PFC allow LA youth to successfully encode new memories despite poor DMN deactivation. Specifically, the engagement of the right hemisphere in LA youth may occur as a result of poor inverse cooperation between the hippocampal and DMN, ultimately helping LA youth perform comparably to their HA peers. Another possibility is that atypical DMN deactivation does not lead to poor memory performance in LA youth because additional compensatory processes occur during the retrieval process. That is, the current study focused on how aerobic exercise affects brain response during the encoding process of successful memory creation. However, a successful memory requires not only encoding of new information, but also storage, consolidation, and retrieval. LA youth may, therefore, show atypical brain response during encoding that may be compensated for at later stages of memory processing, which ultimately results in adequate task

performance. Lastly, although deactivation was of a smaller magnitude than seen in HA youth, LA adolescents might have sufficient DMN deactivation to encode an adequate amount of information to perform well on a relatively short-term memory recognition task. However, differences in DMN deactivation during learning of later remembered word-pairs may have resulted in a different level of encoding between HA and LA youth that may have not been assessed with the current paradigm. For example, it is possible that differences in memory performance would be seen using a longer memory retrieval delay, or a more difficult type of memory test (e.g. free recall). More research is warranted to examine how exercise influences the neural mechanisms underlying other stages of memory, such as retrieval, as well as various types of memory performance (free recall, recognition, etc).

Beyond these speculative functional explanations, the finding that LA youth show atypical patterns of DMN deactivation is in agreement with previous research showing that aerobic exercise influences DMN activity in the elderly. Voss and colleagues (2010) recently reported that decrements in functional connectivity between DMN regions, which occur with normal aging, are mitigated following 12 months of aerobic training. This study also showed exercise to restore functional differentiation between the DMN and other large-scale brain networks in elderly individuals (Voss et al. 2010b). Although similar research on DMN functional connectivity and exercise has not been published in younger populations, our results implicate that aerobic exercise may also affect the integrity of the DMN, as well as the functional distinction between DMN and other task-positive networks in younger developing populations. Future research in our lab aims to determine if the intrinsic organization and functional connectivity of the DMN is influenced by aerobic exercise in adolescents.

Overall, this study provides an important extension of previous research examining how aerobic exercise impacts learning and memory across the lifespan. We have shown several distinct differences in memory-related neural circuitry between LA and HA adolescents. In studies of this nature, there is often a concern that other non-exercise related factors may account for group differences seen, such as nutrition, lifestyle factors, or environmental enrichment that coexist with exercise. In the current study, we assessed these variables, and to the best of our ability, matched the groups. Because both groups reported similar lifestyle behaviors, including their participation in extracurricular activities, the functional neural differences reported appear most parsimoniously explained as due to aerobic exercise. However, it will be important to replicate these findings, using a longitudinal aerobic exercise intervention study, to confirm the influence of aerobic exercise on memory encoding in youth.

Lastly, while a number of additional studies have used fMRI technology to study exercise-induced changes in cognitive function (Voss et al. 2010a; Voss et al. 2010b; Voss et al. 2011; Chaddock et al. 2012a), animal studies have shown that exercise can influence mitochondria (Steiner et al. 2011) and angiogenesis (Van der Borght et al. 2009), which could presumably change the shape and amplitude of the BOLD signal. Interestingly, however, previous work examining the effects of acute exercise on factors that could influence the BOLD signal (i.e. cerebral blood flow, and cerebral oxygen to glucose uptake) suggest that these effects are largely transient, with the values returning to pre-exercise levels within 60 minutes or less after completion of aerobic exercise (Dalsgaard et al. 2002; Williamson et al. 2009). Thus, in the current study youth were not allowed to exercise for 1 hour before fMRI scanning. Furthermore, a study examining 12 weeks of aerobic training (1 hr/4 times per week) on human hippocampal blood flow revealed specific, rather than global effects, which mapped onto

changes seen in neural plasticity in a rodent model (Pereira et al. 2007). These findings suggest that aerobic exercise does not lead to global changes in blood flow, a potential confound we addressed by examining group differences in the HRF in a control region. Furthermore, the effect sizes for group were significantly larger for the memory-related brain activity in the five cortical regions (*d*'s ≥ 2.02) when compared to the visual cortex (*d* = .21). In addition, the effect sizes were larger in the hippocampus (*d*'s = .88 and 1.02) compared to visual cortex; albeit these did not reach statistical significance. Together, these findings argues against the idea that exercise-induced global differences in fMRI signal is the driving factor for the differences in memory-related brain activity reported between the groups in the current study.

In summary, LA youth show a number of distinct differences in brain activity in the PFC, hippocampus, and DMN when encoding new memories, compared to their HA peers. We show that in addition to previous research on exercise-induced volumetric changes in regions important for learning and memory, aerobic fitness levels influence memory-related functional neural circuitry in youth. Moving forward it will be important to clarify the functional implications for these atypical patterns of activation in LA adolescents during memory encoding, as well as replicate these preliminary findings using an exercise intervention design. Given previous work showing exercise to benefit memory in children, adults, and elderly, future research is also needed to extend these findings to different populations, to determine if aerobic exercise can influence learning and memory neural processes across the lifespan.

Table 1. Participant characteristics. Means and standard deviations unless otherwise noted. [¥] n =16 due to missing data; * denotes $p < .05$; ** denotes $p < .01$; hrs/wk = hours per week.

Demographics	**HA**	**LA**	
N	17	17	
Age	16.6 (.8)	16.2 (.8)	*t(32) = 1.36, p = .19*
% Caucasian	82.4	82.4	
IQ[a]	117.1 (11.8)	118.0 (8.1)	*t(26.1) = .26, p = .79*
SES[b]	18.3 (5.9)	26.5 (12.9)*	*t(22.6) = 2.39, p = .03*
Median Household Income[b] (Thousands)	130	90[¥]	
Puberty[c]	3.06 (.4)	3.3 (.3)*	*U = 80, z = 2.24, p = .026*
Aerobic Fitness			
Aerobic Activity (hrs/wk over past year)[d]	11.3 (3.4)	.26 (.5)	*t(16.6) = 11.33, p < .001*
Aerobic Activity (hrs/wk in season scanned)[d]	12.6 (3.8)	.23 (.5)	*t(16.5) = 13.39, p < .001*
VO_2 peak (mL/kg LBM/min)	77.7 (10.5)	67.0 (7.4)**	*t(32) = 3.41, p = .002*
Body Composition			
BMI[e]	21.6 (2.9)	22.4 (4.4)	*t(25.72) = .67, p = .51*
Lifestyle			
Nutrition [f¥]	12.0 (1.0)	12.4 (1.5)	*t(31) = .77, p = .45*
Relaxation [f¥]	15.2 (2.2)	15.4 (2.2)	*t(31) = .22, p = .83*
Health Promotion [f¥]	13.7 (1.3)	13.0 (2.3)	*U = 117, z = .70, p = .51*
Safety [f¥]	14.2 (1.3)	15.0 (1.2)	*U = 86.5, z = 1.8, p = .07*
Substance use[f¥]	11.6 (.5)	11.3 (1.0)	*U = 122, z = .58, p = .63*
Video Game Habits (hrs/week)	6.0 (5.9)	8.4 (9.6)	*U =127, z = .60, p = .56*
Extracurricular Activities			
Frequency	4 (0)	3.5 (1.0)	*U =110.5, z = 2.09, p = .25*
Number	2.9 (1.3)	2.3 (1.3)	*U = 109, z = 1.28, p = .23*

[a] Wechsler Abbreviated Scale of Intelligence

[b] Hollingshead Index of Social Position

[c] Pubertal Development Scale

[d] Youth Adolescent Activity Questionnaire

[e] Body Mass Index

[f] Personal Lifestyle Questionnaire

Table 2. Subsequent memory task performance for each group. Means and standard deviations; RT = reaction time. All group comparisons were not significant (*p's* > *.05*).

	HA	**LA**
Overall accuracy	78.12 (9.7)	80.18 (10.03)
% High confident correct	56.67 (15.4)	62.31 (17.1)
% Low confident correct	22.04 (8.8)	17.86 (10.5)
% High confident incorrect	7.13 (7.6)	6.78 (6.5)
% Low confident incorrect	14.14 (6.9)	13.05 (8.4)
d'	49.5 (18.9)	55.5 (18.5)
High confident correct RT	2819.7 (635.7)	2624.6 (759.2)
Low confident correct RT	4279.4 (1928.9)	3698.7 (1368.2)
High confident incorrect RT	3873.8 (1590.0)	3215.7 (901.1)
Low confident incorrect RT	4457.6 (1969.0)	3793.5 (1688.0)

Table 3. Within and between-group results for *remembered vs. forgotten* contrast. Peak location, regions included, voxel number, and peak Talairach Coordinates are provided for each cluster. R = right; L = left; BA = Brodmann Area.

Peak Anatomic Location	Regions Included	# Voxels	*x*	*y*	*z*
***Remembered* vs. *Forgotten* Within Groups**					
HA					
Remembered > Forgotten					
L inferior frontal gyrus	L middle frontal gyrus; L BA 9, 11, 45, 46, 47	941	-50	29	-7
L superior frontal gyrus	L medial frontal gyrus; L BA 6, 8	96	-5	20	66
L middle temporal gyrus	L fusiform gyrus, parahippocampus; L BA 37	67	-50	-47	-10
Forgotten > Remembered					
R superior parietal lobule	L superior parietal lobule, bilateral precuneus, cuneus, posterior and mid-cingulate cortex; Bilateral BA 7, 31	1942	5	-68	57
R medial frontal gyrus	R superior, middle, and medial frontal gyrus; R BA 8, 10; Bilateral anterior cingulate gyrus; Bilateral BA 32	1006	2	59	12
R inferior temporal gyrus	R middle temporal gyrus; R BA 21	135	65	-20	-19
L inferior parietal lobule	L supramarginal gyrus; L BA 40	83	-56	-50	42
LA					
Remembered > Forgotten					
L inferior frontal gyrus	L middle frontal gyrus; L BA 11, 44, 45, 46, 47	1439	-53	23	-1
L fusiform gyrus	L superior and middle temporal gyrus, middle occipital gyrus, hippocampus, parahippocampus; L BA 21, 22	663	-59	-56	-16
L superior frontal gyrus	L medial frontal gyrus; L BA 6, 8, 9, 10	598	-8	17	66
L posterior cingulate gyrus	L parahippocampus, cuneus; L BA 30	160	-2	-56	9
L caudate	L putamen	140	-11	5	9
R cerebellum (declive)	R pyramis, R Uvula	116	11	-80	-13
L middle frontal gyrus	L BA 21	74	-59	-2	-10
R caudate	R putamen	70	14	5	15
R hippocampus	R parahippocampus, amygdala	66	20	-17	-13
L precuneus	L superior parietal lobule; L BA 7	62	-26	-71	51
Forgotten > Remembered					
R inferior parietal lobule	R supramarginal gyrus; R BA 40	461	47	-56	48
R postcentral gyrus	R BA 31; Bilateral precuneus, posterior cingulate gyrus; Bilateral BA 7	267	5	-53	72
R middle frontal gyrus	R BA 8, 9	82	35	32	48
***Remembered* vs. *Forgotten* Between Groups**					
HA < LA					
L superior temporal gyrus	L middle temporal gyrus; L BA 38	98	-29	14	-25
L ventral medial prefrontal cortex	L BA 10	74	-5	62	12
R posterior cingulate cortex	Bilateral posterior cingulate cortex/posterior midline	62	2	-50	21
R inferior parietal lobule	R precuneus; R BA 7	55	35	-44	60
R superior frontal gyrus	R middle frontal gyrus; R BA 6	54	8	26	57

Table 4. Left and right hippocampal context-dependent correlation analyses between-group results. Peak location, voxel number, and peak Talairach Coordinates are provided for each cluster. Mean psychophysiological interaction fit coefficients (± standard deviation) for each group are also reported; negative fit coefficient = negative coupling of BOLD response with hippocampus, positive fit coefficient = positive coupling in BOLD response with hippocampus; R = right; L = left.

Location	**# Voxels**	***x***	***y***	***z***	***Interaction Fit Coefficient***	
					HA	***LA***
Regions showing group differences in left hippocampal connectivity during *remembered* vs. *baseline* conditions						
Right inferior parietal lobule (IPL)	23	35	-44	60	-4.3 ± 11.0	2.3 ± 9.1
Left medial prefrontal cortex (mPFC)	12	-5	56	15	-7.7 ± 10.3	1.5 ± 15.2
Regions showing group differences in right hippocampal connectivity during *remembered* vs. *baseline* conditions						
Left medial prefrontal cortex (mPFC)	17	-5	53	18	-5.6 ± 9.3	8.2 ± 15.4
Right inferior parietal lobule (IPL)	15	35	-44	54	-3.7 ± 8.4	6.0 ± 11.7

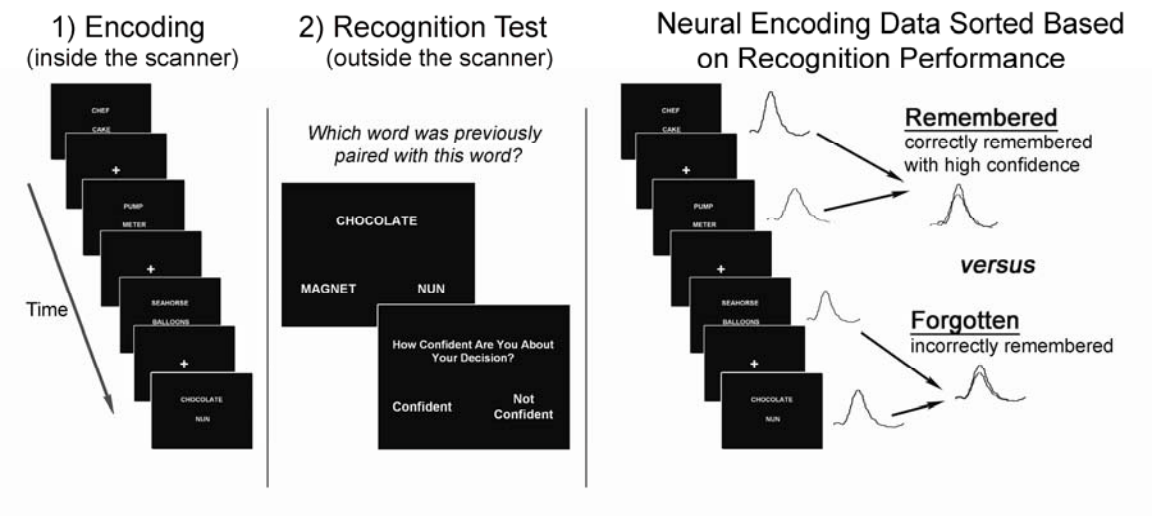


Figure 1. Verbal associative subsequent memory fMRI task. 1) Neural activity is recorded during encoding of word-pairs in the MRI scanner. 2) ~20 minutes following encoding, participants complete a recognition memory task outside of the MRI. Neural responses from encoding are then sorted according to whether the item was later *remembered* or *forgotten*, and then used to examine memory-related neural circuitry.

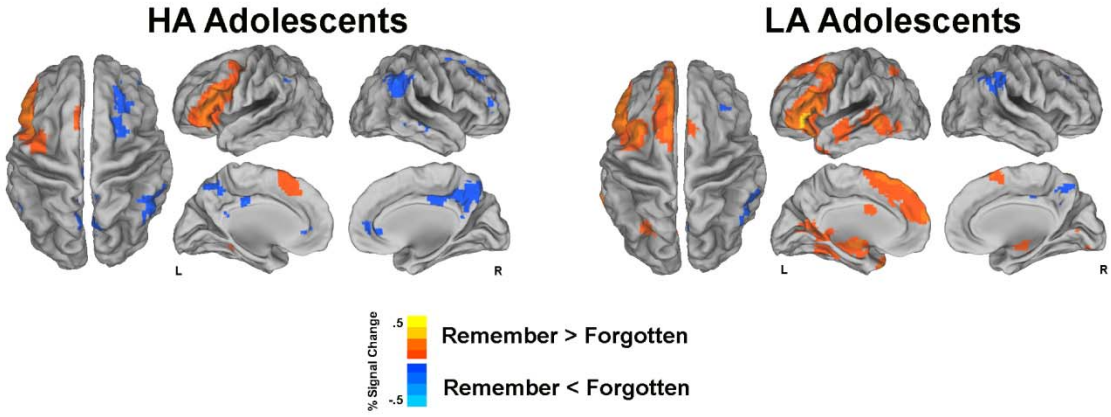


Figure 2. Individual whole brain voxelwise t-maps for *remembered* vs. *forgotten* for each group ($p < .01$, corrected for multiple comparisons).

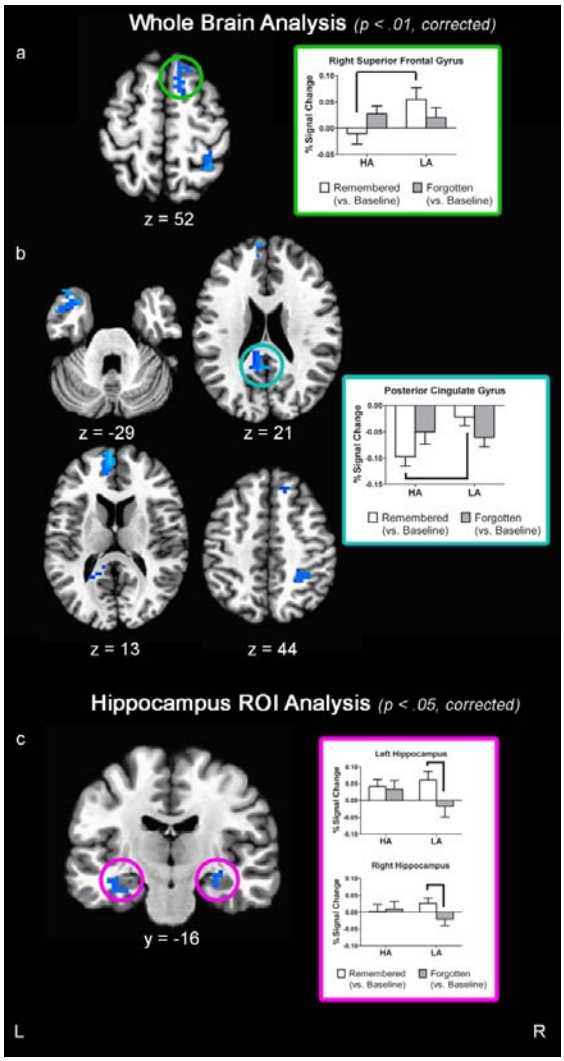
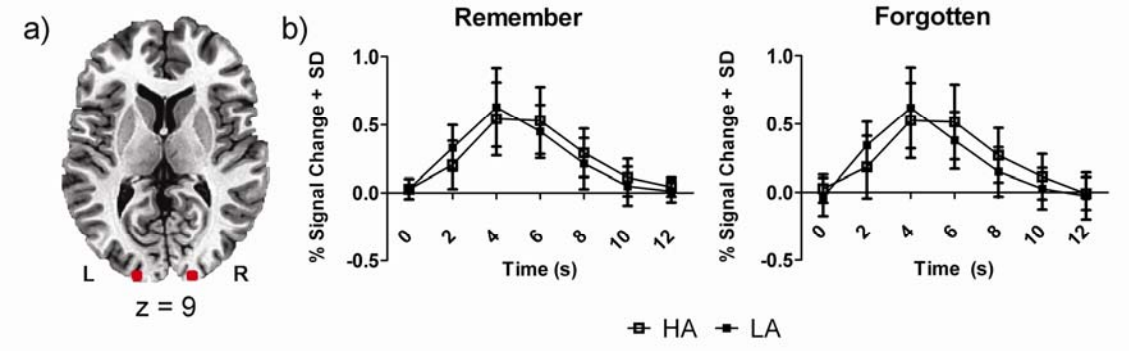
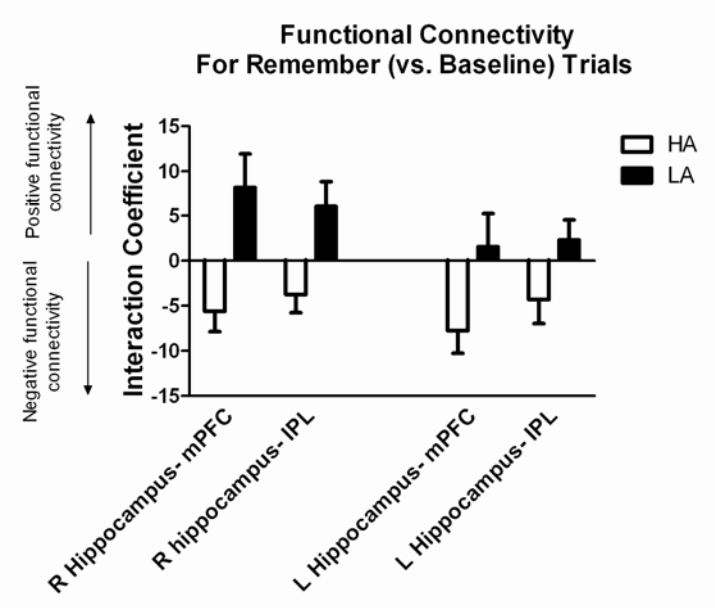


Figure 3. Between-group t-maps of areas where LA adolescents show greater brain activity than HA adolescents for *remembered* vs. *forgotten* contrast. Whole brain analysis results are driven by both a) greater activation for *remembered* (vs. *baseline*) (green subplot), and b) reduced deactivation for *remembered* (vs. *baseline*) in LA compared to HA youth. c) Hippocampus ROI analyses show greater bilateral activation for *remembered* vs. *forgotten* contrast in LA compared to HA adolescents, which is largely driven by greater activation during *remembered* (vs. *baseline*) and greater deactivation during *forgotten* (vs*. baseline*) encoding (pink subplot).



Supplemental Figure 1. a) Bilateral regions of interests (ROIs) overlaid on a Talairach template and b) the time courses of the HRF for later *remembered* and *forgotten* word-pairs for the primary visual cortex. R = right; L = left.



Supplemental Figure 2. Left and right hippocampal context-dependent correlation analyses for each group. Mean psychophysiological interaction fit coefficients (± SEM) for each group are shown below; negative fit coefficient = negative functional connectivity of BOLD response with hippocampus, positive fit coefficient = positive functional connectivity BOLD response with hippocampus; R = right; L = left.

Chapter 5. Discussion

Summary of goals and results

The overall goals of this dissertation project were to: 1) assess the relationships between aerobic fitness and learning and memory behavior in adolescents, 2) examine the relationship between aerobic exercise and adolescent hippocampal volume, and 3) determine if aerobic exercise is associated with differences in learning and memory functional neural circuitry. My overarching hypothesis was that aerobic fitness would be associated with larger hippocampal structure and greater learning-related neural functioning, and this would in turn relate to better subsequent learning and memory performance.

Chapter 3 and Chapter 4 accomplished the first two goals by examining the influence of aerobic fitness on learning and memory, as well as hippocampal volume in adolescents. I found that aerobic fitness, as measured by VO_2 peak, was positively related to greater learning on a vMWT in adolescents. However, verbal learning and memory performance, as measured by the RAVLT and the verbal associative subsequent memory task, did not relate to aerobic exercise using either VO_2 peak or group as the independent variable. Furthermore, VO_2 peak was positively related to larger left hippocampal volumes, although hippocampal volume did not meet statistical criteria for mediating the relationship between aerobic fitness and vMWT learning. Furthermore, group-based analyses (HA vs. LA youth) did not reach statistical significance.

The goal of Chapter 4 was to determine if aerobic exercise influences learning and memory neural circuitry in adolescents. This was accomplished by studying brain activity during encoding of new verbal associative memories in HA and LA adolescents. No significant differences were seen in memory performance between HA and LA youth.

However, compared to HA youth, LA teens showed less deactivation in DMN and recruited the right superior frontal gyrus less and had significant lower bilateral hippocampal activation when encoding new word-pairs. Furthermore, context-dependent analyses also showed that LA youth showed atypical functional connectivity between the hippocampus and DMN regions compared to HA youth. However, VO_2 peak did not significantly predict memory encoding-related brain activity.

Contributions to the aerobic exercise and learning and memory literature

The current findings have added to and expanded upon our knowledge of how aerobic exercise relates to learning and memory. The animal literature provides strong experimental evidence for enhancements in spatial learning and memory with aerobic exercise training (wheel running) (Uysal et al. 2005; van Praag et al. 2005; Lou et al. 2006; Clark et al. 2008; Berchtold et al. 2010). Chapter 3 presents the much-needed translational counterpart to this work, as it is the first study to examine exercise and spatial learning and memory using a human version of the classic Morris Water Maze task. Taken together with the previous literature, these findings show that exercise is related to Morris Water spatial learning across both human and rodent species.

In addition to the use of a translational task, the current set of experiments was the first to examine the associations between aerobic exercise and brain structure and function in an adolescent sample. Given that adolescence is thought of as a unique sensitive period for brain development, there has been a large push to clarify if previous brain and behavior findings in children and adult populations are also apparent in adolescents (Casey et al. 2008; Blakemore 2012). In particular, the need to more closely examine adolescent development stems from the fact that brain and behavior developmental trajectories have various onsets and are not linear processes, highlighting that it cannot be assumed that relationships during adolescence will be the

same as those seen in children or adults (Casey et al. 2008). To this end, in Chapter 3 we provide evidence that aerobic fitness is related to better spatial learning and larger hippocampal volumes in a sample of male adolescents. These findings are in agreement with results shown in elderly adults (Erickson et al. 2009; Erickson et al. 2011) and young children (Chaddock et al. 2010a). Interestingly, however, the coefficients of determination, r^2, reported for aerobic fitness as a predictor of hippocampal volume and learning and memory behavior in other age populations were somewhat smaller in magnitude from the current study (**Table 1**). While extreme caution is needed in comparing effect sizes between these studies (because methods were not synonymous and populations were not directly compared to one another), the variability may warrant future research to determine if the impact of aerobic exercise on learning and memory, as well as hippocampal size, may be non-linear over development. This is especially of interest for future research, as animal research has shown that exercise's effect varies depending on the animal's age, with more robust hippocampal neurogenesis seen in adolescents compared to older adult rats (Kim et al. 2004). Moreover, beyond learning and memory, meta-analysis has shown age to moderate the effect sizes seen between aerobic exercise and cognition, with larger effect sizes for high school and college aged samples compared to studies in children and the elderly (Etnier et al. 1997).

However, if in fact effect sizes turn out to be larger in adolescents, it will be important to consider possible additive effects of exercise that may arise from childhood, as well as those related to aerobic exercise training during adolescence. For example, participants in the current sample were also briefly asked about their level of physical activity during elementary school (see Appendix, page 139), and HA youth reported being more physically active also during this time compared to LA youth (**Table 2**). Because similar relationships have been seen in children (ages 9 to 10)(Chaddock et al.

2010a; Chaddock et al. 2011a), it cannot be ruled out that the current results may reflect persistent effects of aerobic exercise on brain and behavior stemming from earlier in childhood. This idea has yet to be fully examined, but recent research has found that aerobic fitness level and larger hippocampal volumes in 9 and 10 year olds predicts spatial short-term memory performance (3s delayed match-to-sample) one year later (Chaddock et al. 2011b); suggesting aerobic fitness may have lasting effects on the brain and behavior. If this is true, exercise performed during childhood may contribute to the positive relationships seen in the current sample of adolescents. Thus, future research is needed to directly test age-related changes seen with aerobic exercise using intervention studies during each developmental period, as well as longitudinal studies to capture if these effects endure across the lifespan. Nonetheless, taken together with previous research, the current findings suggest that aerobic exercise relates to hippocampal size and learning and memory performance during multiple stages of life.

Research on the influence of aerobic fitness on learning and memory neural circuitry has largely focused on examining volumetric changes in key memory-related brain regions. To my knowledge, Chapter 4 presents the first report of how aerobic fitness affects brain activity during a learning and memory task using fMRI. The interpretations of these findings were made in the context of the extensive published research that exists on typical fMRI BOLD response for encoding of new memories (Lepage et al. 1998; Rugg et al. 2002; Daselaar et al. 2004; Giovanello et al. 2004; Chua et al. 2007; Daselaar et al. 2009; Kim et al. 2010; Maril et al. 2010; Kim 2011; Vannini et al. 2011). Again, it is of great importance to determine if similar patterns are seen in different aged populations, and to determine if aerobic exercise during one point in development may influence brain and behavior relationships in subsequent stages of life. For example, although no performance impairments accompanied these differences

in the current sample of LA youth, it is feasible that these differences in DMN deactivation may reflect an early pattern of vulnerability for impairments in memory that may occur later in life. That is, retrospective studies have shown that low physical activity levels during childhood and adolescence are associated with adult depression (Jacka et al. 2011), and low levels of exercise habits have been linked with cognitive processing speed in old age (Dik et al. 2003) as well as increased risk for dementia (Andel et al. 2008). Moreover, both altered DMN activity and memory impairments have been previously noted in adult individuals affected by these disorders (Jones et al. 2011; MacQueen and Frodl 2011; Zhu et al. 2012). Therefore, although altered brain function was not significantly linked with behavioral differences in LA adolescents, it remains to be elucidated if reduced DMN function and poor hippocampal-DMN decoupling in LA youth may in fact lead to subsequent pathology later in life.

Overall, the studies presented in this dissertation have contributed to the scientific community's shared understanding that aerobic exercise influences learning and memory processes, as well as hippocampal structure in humans, and expands these findings to the neurodevelopmental period of adolescence.

VO_2 peak and aerobic exercise levels as predictors

In both Chapters 3 and 4, the pattern of results seen using VO_2 peak as a continuous independent variable were not synonymous to those seen when splitting the sample into two groups (based on self-report of aerobic exercise participation). Given the current limitations in accurately quantifying aerobic fitness in humans (Etnier et al. 2006; Armstrong and van Mechelen 2008), as well as the recommendation in the field of exercise and cognition to consider both the physiological and psychological benefits of exercise on cognitive processes (Etnier et al. 2006), the purpose of using both group (HA vs. LA) and VO_2 peak as the independent variable was to help clarify and better

interpret how exercise influences learning and memory in adolescents. In Chapter 3, VO_2 peak was a significant predictor of spatial learning on the vMWT and larger hippocampus volumes. While no significant differences were seen in learning and memory or hippocampus volumes between HA and LA youth, the results were in the same direction as seen with VO_2. A post-hoc power analyses showed that power estimates were larger for VO_2 versus group (power $(1 - \beta)$: VO_2 = .75; group = .34); suggesting that a lack of power may account for these differences. In Chapter 4, significant group differences were seen in relational encoding BOLD response in DMN deactivation, as well as right superior frontal gyrus and bilateral hippocampus activation; however, VO_2 peak did not significantly relate to BOLD signal during this task.

Although insufficient power may help to explain some of these results, it is also important to consider how VO_2 peak and aerobic exercise self-report measures are different in how they index aerobic exercise. Although VO_2 measurements are the "gold-standard" and "the best single measurement of aerobic fitness" (Armstrong and van Mechelen 2008), they are also influenced by genetic factors and thus do not purely reflect aerobic training (Robergs and Roberts 1997). On the other hand, the dichotomy of LA and HA groups were based on a participants' history of aerobic training not their VO_2 peak. Self-reports of aerobic training, though, can be biased by perception, and therefore using only a subjective measure of aerobic exercise may not be ideal (Armstrong and van Mechelen 2008). Furthermore, these measurements may reflect different characteristics of an individual's aerobic exercise training experience. An important factor in exercise training is the intensity with which the activity is performed. In fact, while oxygen utilization of the body can be improved with regular aerobic training, high intensity training that is at, or near, one's VO_2 peak is required to see an increase in oxygen utilization (VO_2)(Midgley et al. 2006). In this regard, aerobic quantity does not

necessarily reflect aerobic intensity. Thus, self-reports may be a good indicator of aerobic exercise quantity, whereas VO_2 peak is more likely to be influenced by aerobic intensity. Together, these reasons may account for the fact that aerobic fitness and the amount of aerobic activity do not show a 1-to-1 correspondence in adolescents [Correlation between measures from Chapter 3: *Spearman's rho*(34) = .57, *p* =.001], or anyone (Morrow and Freedson 1994). Thus, differences in results seen between VO_2 peak and aerobic training groups may stem from the differences in what each independent variable is able to capture about aerobic exercise training experience.

Beside differences inherent to each method's ability to assess aerobic fitness, the discrepancies in results may also reflect different underlying physical and psychological processes that each measurement might illustrate, and how each of these in turn may relate to brain and behavior relationships. The cellular and molecular changes that occur with aerobic exercise have been well-documented and include: increases in neurogenesis (van Praag et al. 1999; van Praag et al. 2005; Van der Borght et al. 2007), angiogenesis (Ekstrand et al. 2008; Van der Borght et al. 2009), neurotrophic factors including BDNF and insulin growth-like factor 1 (IGF-1)(Vaynman et al. 2004b; Vaynman et al. 2004a; Llorens-Martin et al. 2008), changes in glutamate and monoamine neurotransmission (For review see Meeusen et al. 2001), and mitchochondria biogenesis (Steiner et al. 2011). However, it remains unclear what aspects of aerobic exercise are responsible for these changes. Specifically, it has been suggested that both physiological and psychological explanations should be considered when thinking about, and studying, the positive relationships seen between exercise and cognition (Etnier et al. 1997; Etnier et al. 2006). Etnier et al. (2006) suggested that potential benefits of aerobic exercise on cognition are likely to not only stem from physiological mechanisms related to aerobic fitness, but also non-aerobic physiological

and psychological mechanisms that co-occur with exercise. Thus, the methods used in the current study may each illustrate distinct benefits of aerobic exercise within each of these domains.

VO_2 measures have been used and interpreted in the human literature as an index of the aerobic physiological mechanisms that change with aerobic exercise (i.e. a measure of aerobic fitness). Therefore, variables that were shown to be predicted by VO_2 peak in the current studies may be primarily driven by the aerobic benefits of exercise that are achieved with high intensity training, whereas other variables that did not relate to VO_2 peak, like those that were seen between HA and LA groups, may be better predicted by additional non-aerobic physiological and psychological changes that accompany the amount of exercise training. For example, the field has largely attributed the positive association between VO_2 and hippocampal volumes to aerobically-induced changes in neurogenesis. True experimental-designs in animals have shown wheel running to facilitate production and survival of new cells in the hippocampus, and this result is contingent upon running, as it is not seen to occur following exposure to complex environmental enrichment that does not include wheel access (Kobilo et al. 2011a). Although primarily thought to be due to the aerobic component of exercise, more recently it has been found that non-aerobic benefits of exercise may also help to explain the relationships seen between aerobic fitness and hippocampus volume, as well as increases in spatial learning. That is, with aerobic exercise, changes also occur to muscle, agility, and balance. These non-aerobic physiological processes may exert their own influences on the brain and behavior. In fact, recently, resistance training and endurance factors have been shown to have similar benefits on spatial memory and neurogenesis as aerobic exercise, but are thought to do so through distinct physiological pathways (Kobilo et al. 2011b; Cassilhas et al. 2012). Specifically, pharmacological

activation of muscle transcriptional factors has been shown to increase neurogenesis and spatial memory in sedentary animals, although these results were more modest compared to what is seen with aerobic training (Kobilo et al. 2011b). Cassilhas et al. (2012) also found that aerobic and strength training in rats lead to increases in learning and memory, but were linked to distinct exercise-induced changes in BDNF and IGF1.

Although the current study did not objectively measure balance, muscle strength, or flexibility, the number of hours spent per week lifting weights was assessed over the past year using the YAAQ. In addition to being more aerobically active, HA also reported a greater number of hours lifting weights compared to LA youth [HA: mean = 1.9, standard deviation = 1.3); LA: mean = .22, standard deviation =.62]. However, follow-up analyses showed that weight lifting did not relate to any dependent variable of interest in the current study (*p*'s > .3). Moreover, the findings in Chapter 3 and 4 remained unchanged after statistically controlling for each subject's average number of hours spent weight lifting per week across the past year, suggesting that strength training does not account for the current significant results between aerobic exercise and brain-behavior relationships. However, objective measures of non-aerobic physical changes were not obtained in the current sample. Therefore, it is feasible that both aerobic and non-aerobic processes may contribute to the significant relationships seen between VO_2 peak, spatial learning, and hippocampal volume reported above.

Unlike hippocampal volume and spatial learning, aerobic activity level group was a better predictor of memory encoding-related brain activity than VO_2 peak. This suggests that non-aerobic mechanisms, such as the non-aerobic physical changes mentioned above, including balance and flexibility, and psychological processes that co-occur with aerobic exercise, may account for these findings. A recent exercise intervention study showed that exercise-induced changes in DMN functional connectivity

patterns occurred not only in a group of elderly subjects that engaged in aerobic exercise, but also in their peers who were part of the non-aerobic flexibility, toning, and balancing “control” group (Voss et al. 2010b). Again, although these measures were not collected in the current study, it is plausible that non-aerobic physical changes that simultaneously occur with aerobic exercise may contribute to the changes in brain activity seen between HA and LA youth.

Furthermore, it is possible that exercise involves cognitive and psychological processes that may exert their own experience-dependent changes on brain function. For example, psychological changes that may occur with aerobic exercise have been described by McMorris et al. (2009). Depending on the type and mode of exercise, this includes greater experience in tracking objects, coordinating movements in space, and maintaining rules in memory. Greater use of these mental operations that are inherent in organized physical activity may exert their own distinct changes on the adolescent brain, which may contribute to the differences seen between individuals who differ in the amount of aerobic exercise they participate in (e.g. altered neurofunctional activity between HA and LA groups in the current set of experiments). It is worth noting, however, that every effort was made to reduce the likelihood of additional psychological processes to account for the current results. That is, aerobic exercise that is performed in groups (such as being on a sports team) also involves social and other environmental factors that are not specific to aerobic exercise, but rather extracurricular participation in general. In this regard, we recruited LA individuals that were equally involved in extracurricular activities (based on number and frequency of involvement); many of which include similar social and group dynamics when compared to being part of a sports team (e.g. band, science club, math team). Furthermore, animal studies have shown that increases in neurogenesis and increases in neurotrophic factors, such as

BDNF, are unique to the aerobic exercise experience and are not dependent on other factors often included in enriched environment studies (social interaction, novel stimuli, etc) (Kobilo et al. 2011a). Thus, as mentioned above, it is currently assumed that the psychological changes that may contribute to the between group data are likely to be specific to aerobic exercise training; and not related to the social context that often surrounds aerobic exercise during adolescence.

In summary, the discrepancies seen between VO_2 peak and group results may illustrate both insufficient power, the capability of each method to capture aerobic fitness experience, as well as the degree to which each measurement reflects aerobic physiological, non-aerobic physiological, and psychological benefits of aerobic exercise on brain structure and function in adolescents. Developments in the fields of exercise physiology and exercise science in quantifying aerobic training and fitness, as well as disentangling what factors may be best assessed by different methodologies will help clarify the mechanisms underlying exercise's benefits on cognition. In the meantime, the recommendation of examining multiple indices of aerobic exercise, as well as considering both physiological and psychological explanations when interpreting results in the field of exercise and cognition, is supported by the findings presented in this dissertation.

Caveats and future directions

Some limitations should be noted. First, the current set of studies evaluated the relationships between aerobic exercise and learning and memory using a variety of tasks. Further research examining aerobic exercise and additional learning and memory processes (e.g. retrieval) is necessary to fully characterize how aerobic exercise influences learning and memory in adolescents. Furthermore, although the vMWT used in the current study has been previously published as a valid assessment of spatial

learning in humans (Nowak and Moffat 2011), the set-up of this task could be improved for future research. Specifically, the current practice trial may not be ideal. The practice trial occurs in a completely different room and pool environment compared to the learning trials, including a larger-sized platform to allow for a relatively quick acclimation to finding a hidden platform and being lifted out of the pool once finding it. These differences between the practice and learning trials were chosen by the creators to help participants learn the task without experiencing the cues and environment that are necessary for spatial learning on this task. However, using the current task design, it is impossible to determine if some spatial information is encoded during this one-time practice trial that may then confound performance on the subsequent learning trials. For example, some individuals may try to initially apply the previous knowledge from the practice trial to the first learning trial until they realize the room is different, which could be construed as a type of reversal learning. Although the participants are placed in a new room to reduce any carry-over of spatial learning in the current task design, future researchers may choose to modify this task to account for this possible confound.

Secondly, the sample used in the current analyses was male, predominantly Caucasian, of a high SES background, and high-functioning (IQs well-above average). While the homogeneity of this sample reduced potential confounds, caution is warranted in the generalizability of these findings to other populations. As previously mentioned, because sex differences have been reported in a number of the outcome variables (Krahenbuhl et al. 1985; Giedd et al. 1996b; Astur et al. 2004; Riddoch et al. 2004; Gogtay et al. 2006; Lovden et al. 2007; Newhouse et al. 2007), I had chosen to examine one sex to reduce potential sex-related variability which would be hard to adequately assess within the relatively small study sample. In fact, given the present results, I would expect both similarities and differences in these relationships for female

adolescents. For example, males have been shown to have better virtual spatial navigation performance, including reduced latency and distance to reach the hidden platform, compared to age matched females (Astur et al. 1998; Astur et al. 2004; Nowak and Moffat 2011). Thus, I might expect similar relationships between exercise and spatial learning to exist in female adolescents, with higher aerobically active females showing comparable performance to non-aerobically active males. In contrast, I would expect the relationship between aerobic exercise and hippocampal volume to be more complicated in adolescent girls compared to their male peers. Long-term potentiation (LTP) is a cellular model of learning and memory (For review see Bliss and Collingridge 1993). Recently, LTP was found to increase in the DG of adolescent male rats following voluntary exercise (Titterness et al. 2011). However, despite exercising a comparable amount to males, voluntary wheel running did not enhance LTP in the DG of female rats. One interpretation of these findings is that the adolescent male brain may be more sensitive to the exercise-induced changes in hippocampal neural plasticity. However, the rats in these experiments were postnatal day 35, a time when estrogen levels are only beginning to rise (Titterness et al. 2011). This may have influenced these results, as exercise-induced up-regulation of BDNF (which may impact hippocampal volume by increasing neural growth and plasticity) varies as a function of estrogen levels in female rats (Berchtold et al. 2001). Thus, I would expect aerobic exercise to also have a positive relationship with hippocampal volumes in girls, but for this association to be moderated by pubertal development. Specifically, I would expect the relationship to be weaker in pre-pubescent girls, but to increase in magnitude as a function of pubertal maturation. Future research is needed to test these hypotheses in females, as well as to examine aerobic exercise and brain structure and function relationships in samples including greater SES and ethnic heterogeneity.

In addition to studying these relationships in a more heterogeneous group of adolescents, the current study examined these relationships in a relatively small sample size (N = 34). Therefore, the current study may have had insufficient power to detect significant relationships between some of the variables of interest. To help determine which dependent variables may be related to aerobic exercise, effect sizes were calculated. By examining effect sizes in the current analyses, I was able to clarify the magnitude of the relationships seen, rather than just relying on null-hypothesis significance testing which can be biased by small sample sizes (Cohen 1990). Effect sizes were small to medium for aerobic fitness (VO_2 peak or group) to predict vMWT learning (δ) and hippocampal volumes, but very small effect sizes were seen for verbal learning, as well as memory performance on all three of the memory tasks utilized. Taken together these findings suggest that aerobic exercise may have moderate effects that are limited to distinct processes within the larger cognitive construct of learning and memory.

Fourth, while these are the first studies to examine how aerobic exercise influences the adolescent brain and behavior, the findings are limited due to the cross-sectional nature of the data. Longitudinal or experimental intervention studies are needed to determine more direct causal effects of aerobic exercise on hippocampal structure and function in adolescents. The fact that a number of intervention studies have replicated some of the cross-sectional results, including increases in hippocampal volume following aerobic training (Erickson et al. 2011), is promising for this line of investigation. Furthermore, while previous correlational and cross-sectional studies results' have been hard to interpret due to inadequate assessment of other potential confounds (e.g. nutrition, lifestyle factors, non-aerobic activity participation) (Etnier et al. 2006), these issues were examined in the current studies detailed here, and when

necessary controlled for. The fact that significant results were seen even after controlling for these variables is also encouraging.

Fifth, more research is needed to determine the dose-response relationships between aerobic fitness (e.g. frequency, duration, mode/type) and brain structure and function. As previously mentioned, research is also needed to determine if these relationships are consistent and/or additive across the lifespan, and if exercise during childhood and adolescence has long lasting, and possibly protective effects, at later stages in life.

Despite these caveats, the current findings have potential implications for policy and future research on prevention and treatment programs for psychopathology. If these findings are confirmed, these data may be important to consider in regard to physical education and school policy. There is a continual debate over whether or not to make physical education mandatory (Hardman and Marshall 2000). Reduction or elimination of school-based physical activity has been considered to allow students to receive more time in the classroom in hopes of increasing standard-based scores. In contrast, the requirement of physical activity in the schools may help teach individuals how to live an active lifestyle, help to reduce the childhood obesity epidemic (Ebbeling et al. 2002), and from the current study, may also facilitate learning. Taken together with previous research (For review see Sibley and Etnier 2003; Chaddock et al. 2011b), the current results support that physical exercise during development relates to enhancements in distinct cognitive skills, which may ultimately contribute to better learning of topics in the classroom - effects which may be persistent into adulthood. Thus, given that schools provide a unique avenue to reach children and adolescents, helping youth implement a physically active lifestyle via physical education remains a favorable option.

Lastly, the impetus for this research project was to contribute to the scientific field's aims towards determining how exercise influences brain structure and function, and this in turn would help serve as a foundation for examining if exercise may benefit youth affected by psychiatric symptoms and disorders. Overall, in the current set of studies, exercise was seen to have a small, yet significant impact on hippocampal structure and function in a low-risk sample (as youth had well-above normal IQs, were of high SES backgrounds, and had to have met very stringent exclusionary criteria). Although the effects were on the smaller side, it is feasible that aerobic fitness may be even more beneficial for individuals with deficits in learning and memory. For example, depression, substance abuse, and schizophrenia are associated with learning and memory deficits and smaller hippocampal volumes (Campbell et al. 2004; MacMaster and Kusumakar 2004; Nagel et al. 2005; de Castro-Manglano et al. 2011). Based on the positive relationships seen in the current study, I would hypothesize that aerobic exercise training may help to mitigate these impairments, leading to learning and memory performance and hippocampal volumes more comparable to non-affected youth. Support for this idea comes from a meta-analysis on physical activity in children, which reported qualitatively larger effect sizes for mentally impaired children (mean =.43) compared to healthy youth (mean =.31); albeit statistical testing did not reach significance (Sibley and Etnier 2003). Nonetheless, aerobic exercise seems to have a small positive influence on learning and memory function in healthy adolescents, and more research is warranted to determine how exercise may benefit affected youth.

Conclusion

Overall, this work shows that aerobic exercise relates to learning performance and neural substrates of memory encoding in adolescents. While verbal learning and memory performance was not associated with aerobic fitness (either using activity group

or VO_2 peak as a predictor), spatial learning was correlated with greater aerobic fitness (VO_2 peak) on the vMWT. Higher VO_2 peak values were also shown to positively predict greater hippocampal volumes. Beyond VO_2 relationships, higher and lower aerobically active youth showed differences in brain activity when encoding new verbal memories. Interpreted in light of the vast amount of normative data on DMN deactivation and modality-specific asymmetry seen during memory encoding, LA youth show altered DMN activity and recruit compensatory regions including the right PFC and hippocampus. Taken together, these findings suggest that aerobic exercise should not be disregarded as an important environmental factor for adolescent neurodevelopment, and should be further studied as a possible treatment and prevention variable in relation to psychopathology that emerges during the teenage years.

Tables

Table 1. Coefficients of determination (r^2) for the current study, as well as similar cross-sectional aerobic fitness, hippocampal volume, and learning and memory studies in elderly adults and children.

Study	**Population**	**Coefficients of Determination**			
		Relationship between VO_2 and hippocampal volumes		**Relationship between VO_2 and learning & memory behavior**	
		Side	r^2	Learning & Memory Task	r^2
Erickson *et al.*, 2009	Elderly adults Ages 59 – 81 N = 109	Right Left	.08 .12	Accuracy on 3s delayed match to sample task	.06
Chaddock *et al.*, 2011	Children Ages 9 & 10 N = 49	Bilateral	.12	Accuracy on visual relational recognition task	.08
				d-prime on visual relational recognition task	.13
Chapter 3 Results	Adolescents Ages 15 – 18 N = 34	Right Left	.17 .32	Visuospatial learning (δ) on vMWT	.34

Table 2. Elementary school physical activity levels as assessed by youth self-report.

	HA	LA
Exercised ≥1 hour[a]	3.88 ± 1.1 (range: 2 – 5)	2.29 ± .99 (range: 1 – 4)
Participation in sports[b]	3.41 ± 1.1 (range: 2 – 5)	2.0 ± .94 (range: 1-4)

[a] Higher scores reflect more exercise days per week on average during elementary school: 1 = ≤1 day, 2 = A couple of days, 3 = About half the days, 4 = Most days, 5 = Almost every day.

[b] Higher scores reflect greater number of months of sport participation during the elementary school years: 1= Never (no sport participation), 2 = 1-3 months per year, 3 = 3 to 6 months per year, 4 = 6 to 9 months per year, 5 = 9 to 12 months per year.

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Appendix

Modified Youth Adolescent Activity Questionnaire (YAAQ)

(To be read to participant during structured telephone interview)

Subject ID: _____

NOT including phys ed (gym), **what <u>ORGANIZED ACTIVITIES</u> have you done in the past year?** **(Circle Yes or No) If yes**, please fill out how much you did that activity EACH season. Mark *"None/Zero"* for any season you did not do that activity.

Baseball or Softball

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Basketball

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Biking

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Dancing or Aerobics

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Football

Did you do this activity over the past year?

No Yes

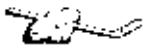
How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											

Page 1

Subject ID: _____

Spring											
Summer											



Ice, Field, Street Hockey or Lacrosse

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Jogging or Running

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Swimming

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Rollerblading, Roller skating, or Ice skating

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Skateboarding

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											

Subject ID: _____

Spring											
Summer											

Soccer

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Tennis or Other Raquet Sports

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Walking (for exercise)

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Playing Outdoor Sports (jump rope, kickball, dodgeball)

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Gymnastics or Cheerleading

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											

Subject ID: _____

Winter											
Spring											
Summer											

Strength Training Exercises (push-ups, weight-lifting)

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Volleyball

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Martial Arts, Karate, Wrestling

Did you do this activity over the past year?

No Yes

How much did you do it EACH season?

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Did you do any other sports or activities that we haven't listed?

(Please specify)

Fall _____

Winter _____

Spring _____

Summer _____

	None/ Zero	1 hr./wk.	2 hr./wk.	3 hr./wk.	4 hr./wk.	5 hr./wk.	6 hr./wk.	7 hr./wk.	8 hr./wk.	9 hr./wk.	10+ hr./wk.
Fall											
Winter											
Spring											
Summer											

Elementary School Physical Activity Questions:

(To be read to participant during structured telephone interview)

Not counting PE classes, on average, how many days a week did you exercise so that your heart rate went up and you were breathing hard for at least an hour a day during elementary?

One day or less
A couple of days
About half the days
Most days
Almost every day

How many months of the year did you participate in team sports that practiced regularly (like swimming, basketball, soccer, football, track) during the 1st to 5th grade?

Never
1-3 months of the year
3-6 months of the year
8-9 months of the year
9-12 months of the year

The above questions were then scored separately by giving a numerical value ranging from 1 to 5 for each question, with higher values reflecting higher levels of physical activity during elementary school years (based on days of exercise for question 1 and involvement in sports for question 2).

Rey Auditory Verbal Learning Test (RAVLT) Instructions:

(To be read to participant)

For trial 1: *"I am going to read a list of words. Listen carefully, for when I stop you are to repeat back as many words as you can remember. It doesn't matter in what order you repeat them. Just try to remember as many as you can."*

Word list A is read out loud with a 1-second interval between each of the 15 words. No feedback is given to the participant.

For trials 2 thru 5: *"Now I am going to read the same words again, and once again when I stop I want you to tell me as many words as you can remember, including words you said the first time. It doesn't matter in what order you say them. Just say as many words as you can remember whether or not you said them before."*

Word list A is read out loud with a 1-second interval between each of the 15 words. No feedback is given to the participant between trials.

After trial 5: *"Now I'm going to read a second list of words. Listen carefully, for when I stop you are to repeat back as many words as you can remember. It doesn't matter in what order you repeat them. Just try to remember as many as you can."*

Word list B is read out loud with a 1-second interval between each of the 15 words.

Immediately after list B trial: *"Now tell me all the words that you can remember from the first list."*

After 20 minute delay period: *"A while ago, I read a list of words to you several times, and you had to repeat back the words. Tell me the words from that list."*

Virtual Morris Water Task (vMWT) Instructions:

(To be read to participant)

Introduction to task/practice trial: *"You will be placed into the pool repeatedly and your goal on every trial is to try and find the hidden platform as quickly as you can. The platform will always be in the same place so you should try to remember it. Let's try this one for practice."*

For learning trials 1 thru 6: *"Just like in practice, you will be placed into a pool of water which is inside a larger room. Hidden underneath the surface of the water is a platform and you should try to find that platform as quickly as you can. When you find the location of the hidden platform, it will appear underneath you and raise you out of the water. When you find the platform you will have to wait for about 10 seconds before it begins the next trial.*

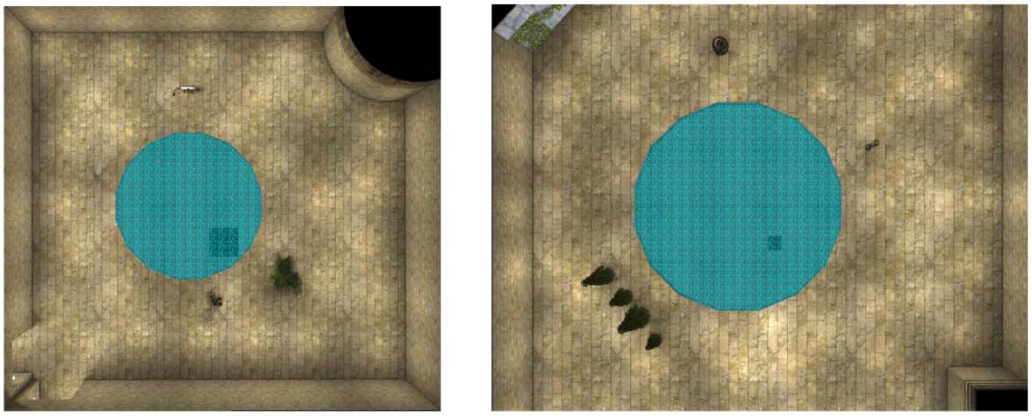
You will be placed in the pool repeatedly and your goal on every trial is to find the hidden platform as quickly as you can each time. The platform will always be in the same place. Do you understand?"

For delayed memory (probe) trial: (after 30 minute delay) "*Okay, we are going to do [the task] one more time. The environment is exactly the same as the previous trial, and the platform is in the same place. Are you ready?"*

For the control "swim" trial: "*Okay, this time the location of the platform will be designated by the placement of 1 flag at each corner. I want you to go toward it as quickly as you can and get on top of the platform."*

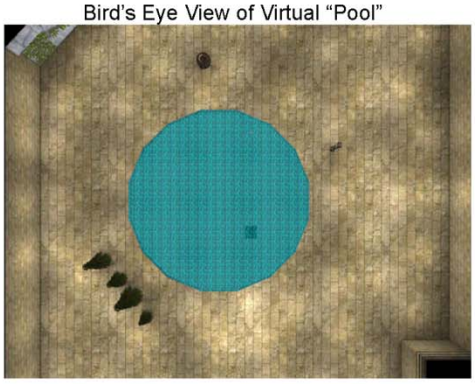
Images of virtual Morris Water Task (vMWT)

a) The different Virtual "pool rooms" used for the practice trial versus the learning trials

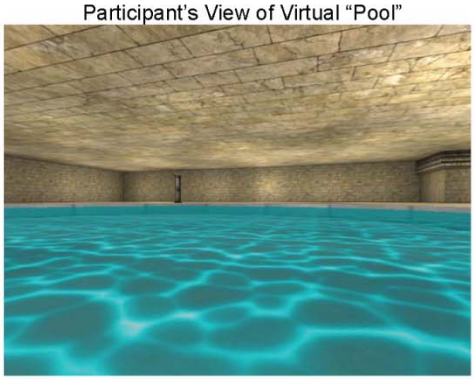


b) Pool used for learning trials and probe trial (albeit platform removed)

Bird's Eye View of Virtual "Pool"

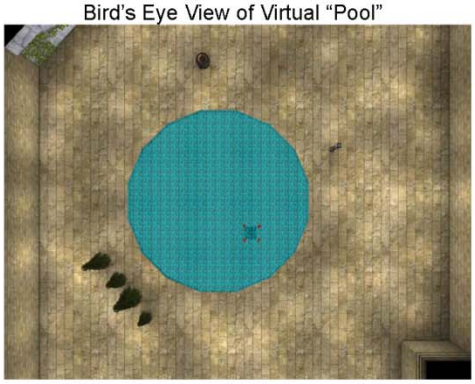


Participant's View of Virtual "Pool"

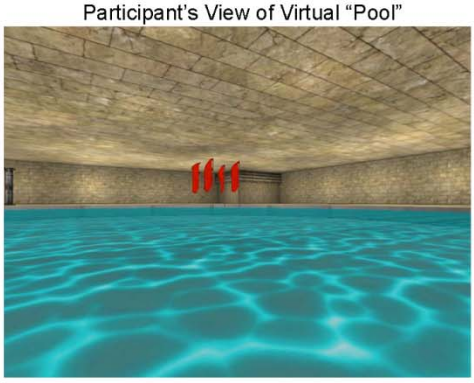


c) Pool used for control "swim" trial

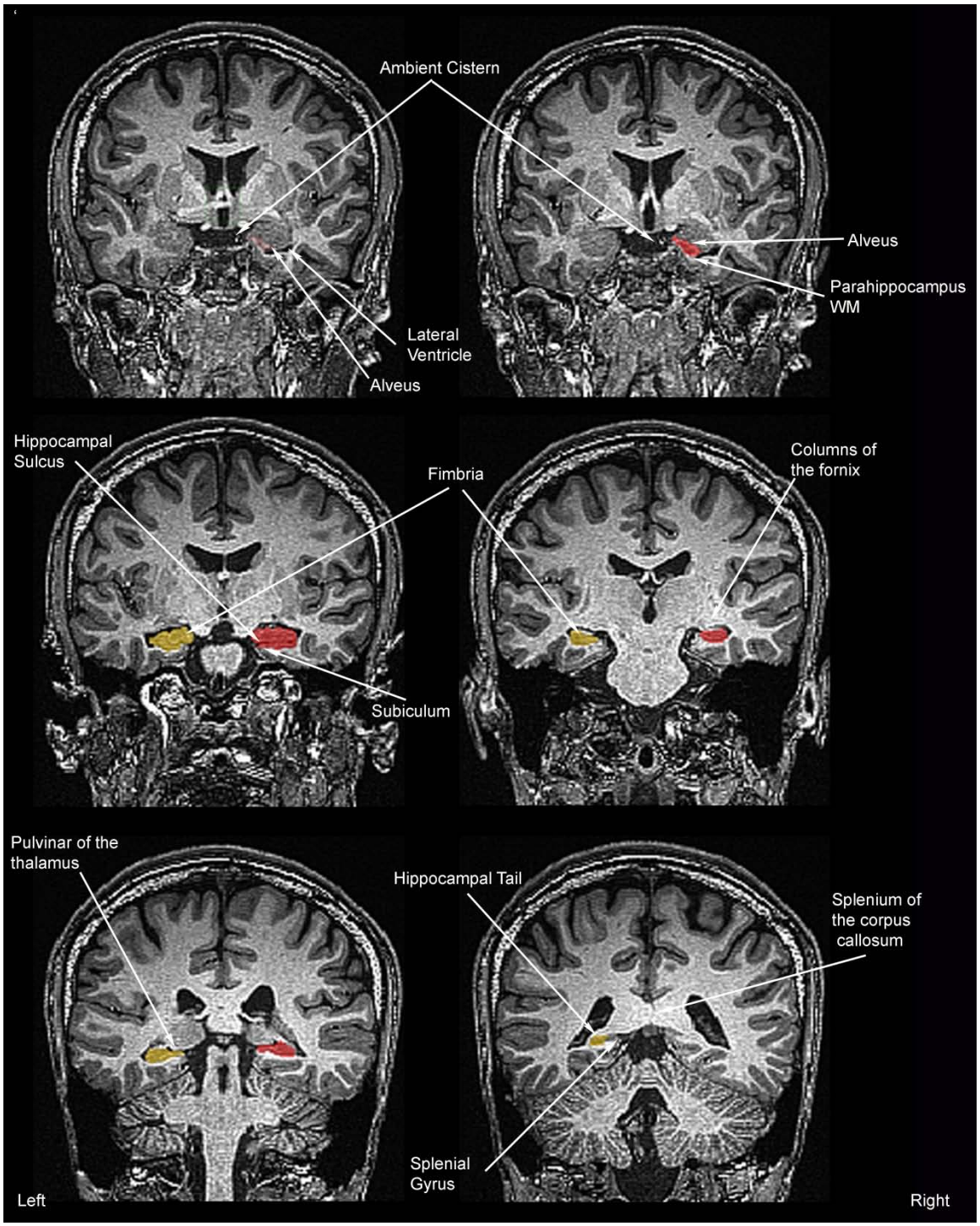
Bird's Eye View of Virtual "Pool"



Participant's View of Virtual "Pool"



Representative Hippocampal Hand-drawn Tracing



fMRI Verbal Associative Encoding Instructions:

(To be read to participant)

Practice instructions (before scan): *"You will see pairs of words on the screen that go together. Your job is to learn these pairs, as you will be tested on them later. We also want to make sure that you are reading each of the pairs carefully, so you will have to make a decision about each pair. You will have to decide if the two words "fit" together. If you think they fit together, press "1". If you don't think they fit together, press "2". However the most important thing is for you to learn the pairs so you can remember them later."*

In scanner instructions: *"This is like the task you practiced in the other room where we show you word pairs and your job is to learn which words go together. You need to decide for each pair if the words "fit" together. Press "1" if they fit and press "2" if they don't fit. However, the most important thing, though, is for you to learn all the pairs so you can remember them later as you will be tested on them.*

You will also occasionally see a screen with a plus sign in the middle of it. When you see this, simply look at the plus sign. Please remember to keep your head still through the entire test."

Recognition instructions (outside the scanner): "*Now we want to see how well you have learned the pairs of words that you just saw. For each word pairs shown to you, we will show you the first word of the word pair on the screen, as well as two words underneath that word. You're job is to decide which word was previously paired with the first word. If it is the word on the left, press "1". If it is the word on the right, press "2".*

Then you will be asked how confident you are in your decision. If confident, press "1". If not confident, press "2"."

Abbreviations

AC-PC – Anterior Commissure - Posterior Commissure
AFNI – Analysis of Functional NeuroImages
AMPA – 2-Amino-3-(5-Methyl-3-oxo-1,2- oxazol-4-yl) Propanoic Acid
ANCOVA – Analysis of Covariance
ANOVA – Analysis of Variance
ATP – Adenosine Triphosphate
BA – Brodmann Area
BDNF– Brain Derived Neurotrophic Factor
BMI – Body Mass Index
BOLD – Blood Oxygen Level-Dependent
PFC – Prefrontal Cortex
CA – Cornu Ammonis (subfield of the hippocampus
CO_2 – Carbon Dioxide
DG – Dentate Gyrus (subfield of the hippocampus)
DMN – Default Mode Network
DSM-IV – Diagnostic Statistical Manual, Version 4
EC – Entorhinal Cortex
FAST – Functional Magnetic Resonance Imaging of the Brain's Automated Segmentation Tool
FMRI – Functional Magnetic Resonance Imaging
FOV – Field of View
FWHM – Full Width at Half Maximum
GPA – Grade Point Average
HA – High-Active
HRF – Hemodynamic Response Function
ICV – Intracranial Volume
IGF-1 – Insulin-Like Growth Factor 1
IPL – Inferior Parietal Lobule
IQ – General Intelligence
IRB – Institutional Review Board
LA – Low-Active
LBM – Lean Body Mass
LTP – Long-Term Potentiation
mPFC – medial Prefrontal Cortex
MPH – Miles per Hour
MRI – Magnetic Resonance Imaging
NMDA – N-Methyl-D-Aspartic Acid
O_2 – Oxygen
OHSU – Oregon Health & Science University
PASW – Statistical Package for the Social Sciences
pCC – posterior Cingulate Cortex
PDS – Pubertal Development Scale
PLQ – Personal Lifestyle Questionnaire
RAVLT – Rey Auditory Verbal Learning Test
RMS – Root Mean Squared
ROI – Region of Interest
SES – Socioeconomic Status

SNR – Signal-to-Noise Ratio
TE – Echo Time
TI – Inversion Time
TR – Repetition Time
vMWT – virtual Morris Water Maze
MPRAGE – Magnetization Prepared Rapid Acquisition Gradient Echo
VEGF – Vascular Endothelial Growth Factor
VO_2 – Oxygen Consumption
WASI – Wechsler Abbreviated Scale of Intelligence
YAAQ – Youth Adolescent Activity Questionnaire