PIEPMEIER, AARON T., Ph.D. A Closer Look at the Role of BDNF as a Causal Link in the Physical Activity Cognition Relationship: A Dose-Response Study. (2015)
Directed by Dr. Jennifer L. Etnier. 135pp.

There is strong evidence in support of a beneficial relationship between acute physical activity (PA) and cognitive performance. However, the biological mechanisms through which this relationship operates are currently unknown. Although findings from both rodent and human research suggest brain-derived neurotrophic factor (BDNF), a protein vital to the form and function of the brain, as a potential mechanism, results from studies exploring the acute PA-BDNF-cognitive performance relationship have been mixed. The focus of the present study was to elucidate this relationship by assessing cognitive performance (i.e., memory) and acute PA-induced changes in BDNF concentrations. An experimental design utilizing a randomized control trial was used to allow for inferences of causality within the acute PA-BDNF-cognitive performance relationship. Results showed that acute light intensity PA significantly improved memory performance compared to acute vigorous intensity PA or a control (non-PA) condition. Additionally, acute PA was shown to have a non-significant effect on BDNF concentrations. Findings from this research do not support BDNF as a biological mechanism for the acute PA-cognitive performance relationship.

A CLOSER LOOK AT THE ROLE OF BDNF AS A CAUSAL LINK IN THE PHYSICAL ACTIVITY COGNITION RELATIONSHIP: A DOSE-RESPONSE STUDY

by

Aaron T. Piepmeier

A Dissertation Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Greensboro
2015

Approved by

_____
Committee Chair

To my family...

This study would not have been possible without their

love, support, and inspiration.

APPROVAL PAGE

This dissertation has been approved by the following committee of the Faculty of The Graduate School at The University of North Carolina at Greensboro.

Committee Chair _____

Committee Members _____

_____

_____

_____

_____
Date of Acceptance by Committee

_____
Date of Final Oral Examination

ACKNOWLEDGEMENTS

I would like to express my gratitude to my mentor, Dr. Jennifer Etnier for her tireless work at training me to become a scientist. I would also like to thank my committee members for their direction and assistance on this study.
Finally, thank you to my fellow students. You have made my time at UNCG an experience that I will never forget.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

There is strong evidence in support of a beneficial relationship between acute physical activity (PA) and measures of cognitive performance. Experimental studies have observed the beneficial effects of acute PA in children (Etnier, Labban, Piepmeier, Davis, & Henning, 2014; Pesce, Crova, Cereatti, Casella, & Bellucci, 2009; Piepmeier et al., *in preparation*), young adults (Chang, Tsai, et al., 2011; Etnier et al., 2014; Ferris, Williams, & Shen, 2007; Griffin et al., 2011; Labban & Etnier, 2011; Winter et al., 2007), and older adults (Chang, Chu, Chen, & Wang, 2011; Chang, Ku, Tomporowski, Chen, & Huang, 2012; Kamijo et al., 2009). Indicative of the multifaceted nature of the acute PA-cognitive performance relationship, studies exploring the effects of acute PA on measures of cognitive performance have utilized various PA modalities (e.g., cycling, treadmill, elliptical), intensities, and durations as well as varied assessments of cognitive performance (e.g., visual memory, auditory memory, problem solving, inhibition). This complicates the task of comparing results from multiple studies. However, meta-analytic techniques provide a method of synthesizing standardized effects (i.e., effect sizes) from multiple studies in order to

synthesize disparate findings and produce an estimation of the effect of acute PA on cogitive performance (Impellizzeri & Bizzini, 2012).

Meta-analytic studies have not only confirmed that there is a robust beneficial effect of acute PA on cognitve performance, but they also allow for the determination of certain aspects of the experimental designs that influence the size of the beneficial effect (i.e., moderators). Chang, Labban, Gapin, and Etnier (2012) (2012) provided information pertaining to the effect size of moderators of the acute PA-cognitive performance relationship. Findings from this review suggest that the biggest positive effects of acute PA on cognitive performance are realized when the PA intensity is “very hard”; the PA duration is more than 11 minutes; cognitive performance consists of measures of reaction time, and executive function; and cognitive performance is assessed within 15 minutes of stopping the PA. Results from the meta-analysis performed by Lambourne and Tomporowski (2010) agree with those by Chang et al. (2012) in that the largest positive effects were observed within a 15-minute post PA period. Additionally, Lambourne and Tomporowski assessed PA modality, and results suggested that the largest effects were observed in studies utilizing cycles compared to those using treadmills. Interestingly, when assessing the effect of acute PA on a specific cognitive domain (i.e., memory), results from previous meta-analyses have shown the overall effect of acute PA on memory to be small (ES=.10) (Etnier et al., 1997) or non-significant (Chang et al., 2012). However, memory is an umbrella term used to describe an entire domain of cognition. Cognitive

domains are comprised of multiple categories (e.g., memory: short-term, long-term, declarative, relational, working) making it useful to clearly identify the category of interest in order to facilitate assessment and interpretation of statistical analyses. For example, when specific categories of memory were assessed separately, Chang et al. (2012) observed significant effects. However, of the seven memory categories assessed, two were found to have positive effects (i.e., free-recall, visual short-term memory) and two were found to have negative effects (i.e., sequential memory, auditory verbal memory), and the remaining (i.e., verbal working memory, digit span [forward], figural learning test) were found to have non-signficant effects. The meta-analysis performed by Roig, Nordbrandt, Geertsen, and Nielsen (2013) provided a more detailed understanding of the effect of acute PA on memory by focusing their investigation on the differential effects of acute PA on short-term and long-term memory categories. Results indicated that, for short-term memory, the largest positive effects were observed in studies utilizing PA durations shorter than 20 minutes, light intensities, and a walking modality, and for long-term memory, the largest positive effects were observed in studies utilizing PA durations of 20-40 minutes, light intensities, and a cycling modality. Findings from the meta-analyses performed by Chang et al. (2012), Lambourne and Tomporowski (2010), and Roig et al (2013) are in agreement that positive effects may be oberved following acute PA that is at a light or very hard intensity, of a duration of 11-40 minutes, and that utilizes a cycling modality. The findings from past research indicate that

the largest PA-induced benefit to memory will be observed within 15 minutes following an acute bout of PA greater than 20 minutes.

The previously mentioned studies provide strong support for the beneficial effect of acute PA on cognitive performance. Even though the biological mechanism for this effect has not yet been determined, BDNF has been identified as a candidate. As a member of the family of neurotrophic factors, BDNF is involved in brain health and function. The vital role of BDNF in learning and memory has been established in non-human animal studies (Cirulli, Berry, Chiarotti, & Alleva, 2004; Mu, Li, Yao, & Zhou, 1999), and observations from human studies have shown its negative association with age-related cognitive decline, reductions in brain volume, and Alzheimer's disease (Erickson et al., 2010; Erickson, Miller, & Roecklein, 2011). The observed connections of BDNF to behavioral measures of learning and memory, as well as its link to memory-related neurodegenerative diseases such as dementia, is logical when one considers that the hippocampus, a region of the brain involved in the cognitive domain of memory, is a major area of BDNF expression.

The molecular properties of the BDNF protein allow it to cross the blood-brain-barrier (Pan, Banks, Fasold, Bluth, & Kastin, 1998; Poduslo & Curran, 1996), and studies have observed correlations between peripheral and central concentrations of BDNF (Klein et al., 2011). By assessing BDNF through the periphery (BDNF, obtained from blood draws from veins in the arm), studies have

shown that increases in BDNF concentrations are observed following an acute bout of PA (Etnier et al., in preparation; Ferris, Williams, & Shen, 2007; Griffin et al., 2011; Knaepen, Goekint, Heyman, & Meeusen, 2010; Rasmussen et al., 2009; Winter et al., 2007). These findings provide evidence that acute PA is a behavioral intervention by which humans may increase concentrations of BDNF.

The extant literature exploring the role of BDNF in the acute PA-cognitive performance relationship is still in its infancy. The relationship between PA-induced concentrations of BDNF and cognitive performance has been assessed with mixed findings (Etnier et al., in preparation; Ferris et al., 2007; Griffin et al., 2011; Lee et al., 2014; Skriver et al., 2014; Tonoli et al., 2014; Tsai et al., 2014; Winter et al., 2007). These inconsistent findings may be partially due to the assessment of different domains of cognitive performance between studies. For example, Etnier et al. (in preparation), Lee et al., (2014), Skriver et al. (2014), and Winter et al. (2007) observed significant relationships between PA-induced BDNF concentrations and measures of memory, while Ferris et al. (2007) and Tsai et al. (2014) observed a non-significant relationship between PA-induced BDNF concentration and measures of executive function and reaction time, respectively. Given the vital role of the hippocampus for memory performance as well as in the expression of BDNF, these findings suggest that BDNF may be involved in the relationship between acute PA and the cognitive domain of memory. However, differences in the molecular form (isoform) of the BDNF protein may be another important factor to consider when assessing its role in

the acute PA-cognitive performance relationship. Disparate findings from the aforementioned experimental studies exploring the role of BDNF in the relationship between acute PA and cognitive performance may also be partially due to the use of assessment tools (i.e., ELISA kits) that assessed either the mature isoform of the BDNF protein with a specified amount of cross-reactivity with the immature isoform, or a non-specified proportion of immature and mature protein.

As with all neurotrophins, BDNF is first expressed in an immature (pro) isoform before it is transformed into its mature (m) isoform though enzymatic modification (proteolytic cleavage). This post-translational modification changes the polarity of BDNF's effect on the form and function of the brain. BDNF goes from being an immature protein (proBDNF) involved in cell death and long-term depression (i.e., a neural process by which a postsynaptic response to a presynaptic stimulus is reduced) to a mature protein (mBDNF) involved in cell growth (e.g., neurogenesis) and long-term potentiation (i.e., a neural process by which a postsynaptic response to a presynaptic stimulus is enhanced). Depending on its molecular structure, the BDNF protein functions in a dichotomous manner and would be anticipated to influence cognitive performance differently.

To date, only a single study has been performed to assess the effect of acute PA on BDNF isoforms. Brunelli et al.(2012) assessed the effect of maximal and

submaximal PA intensities on BDNF isoform proportions in peripheral blood mononuclear cells. Maximal PA was shown to increase mBDNF levels immediately post PA, to decrease mBDNF 60 minutes post PA to levels below baseline, and to increase levels of proBDNF 30 minutes and 60 minutes post PA. Submaximal PA was shown to increase mBDNF levels 30 and 60 minutes post PA, and increase proBDNF levels 60 minutes post PA. These findings suggest that acute PA affects BDNF isoform proportions in an intensity-dependent manner. More research is needed to examine the relationship between PA-induced modifications in BDNF isoform proportions. This type of examination would benefit from further investigation into the effect of PA intensity on BDNF isoform proportions. Research is also needed to begin testing the relationship between PA, BDNF isoform proportions, and cognitive performance. Additionally, since three of the four studies that have observed an association between PA-induced changes in BDNF concentration and cognitive performance have assesse BDNF from blood serum (Etnier et al., in preparation.; Lee et al., 2014; Winter et al., 2007), future research should explore the effect of acute PA on BDNF isoform proportions in blood serum. As well as allowing for direct comparison to research exploring the PA-BDNF-cognitive performance relationship, this would also extend findings from Brunelli et al. (2012) by allowing for direct comparison between serum BDNF concentration and serum BDNF isoform proportion.

The objective of this study was to explore the relationship between acute PA intensity, BDNF (i.e., concentration, isoform proportions), and cognition prior to (pre), immediately after (post), 30 minutes after (post-30), and 60 minutes after (post-60) a vigorous intensity (vigPA), low intensity (lowPA), or control (noPA) condition using a randomized control trial (See Figure 3). Cognitive performance was assessed at the post and post-30 time points as well as 24 hours post PA. The central hypothesis was that there would be a positive intensity-dependent relationship between acute PA, systemic BDNF levels in the blood, and cognitive performance. Specifically, it was hypothesized that acute PA would have a positive direct effect (i.e., benefit) on memory. Additionally, it was hypothesized that the positive relationship between acute PA and memory would be mediated by BDNF. Specifically, PA would induce a change in systemic BDNF concentrations and isoform proportions (mBDNF÷[mBDNF + proBDNF]) and improve cognitive performance compared to controls in an intensity-dependent manner.

The rationale for examining the relationship between PA, BDNF, and cognition was that completion of the proposed research would help elucidate the role of BDNF as a mediator of the relationship between acute PA and cognition. Additionally, gaining knowledge on the ability of PA to affect BDNF isoform proportions would provide information that may aid in the determination of specific neural processes that are supported or inhibited by acute PA. These findings will be important, because understanding the mechanisms of the acute

PA-cognition relationship is critical to moving towards the prescription of PA to benefit cognitive performance and brain health.

CHAPTER II

LITERATURE REVIEW

Memory

Different methods have been used to assess and analyze “memory”, including physiological assessments of cellular activity and behavioral assessments. While investigations into memory have lead researchers to identify multiple domains of memory storage (e.g., iconic, short-term, intermediate-term, long-term, permanent), this review will only address short-term and long-term memory. Furthermore, neuropsychological tools have been designed to assess, specific subtypes of memory (e.g., relational memory, working memory). Due to their reliance on the activation of specific regions of the brain (e.g., relational memory - hippocampus, working memory – frontal lobes), distinctions pertaining to memory subtypes may be important to studies investigating mechanism of memory. Because the current study explored various memory types an overview is provided to summarize biological and psychological models of memory.

Cellular Model of Memory

Long-term potentiation (LTP) and long-term depression (LTD) are descriptors used in cellular models of memory. These are the processes by which presynaptic and postsynaptic neurons develop a dynamic relationship involving both neurons being changed by the others' activity. When an active presynaptic neuron signals a postsynaptic neuron at low levels, this results in the creation of a postsynaptic potential. When the postsynaptic potential is large enough, it will signal an action potential. However, in many areas of the brain (e.g., hippocampal regions) when a presynaptic neuron signals a postsynaptic neuron at high levels, long-term changes take place above and beyond the depolarization of the postsynaptic neuron. The presynaptic neuron increases the strength of its signaling power (i.e., releases more neurotransmitter) and postsynaptic neurons change their physiological structure to become more sensitive to the neurotransmitter produced by the presynaptic neuron (i.e., LTP). Once LTP takes place, a small stimulation by the presynaptic neuron, that originally would only elicit a small response in the postsynaptic neuron, will elicit a larger response. The coupling of an increase in presynaptic neurotransmitter release and postsynaptic sensitivity has been described as a molecular mechanism of memory. Long-term depression works in an inverse manner to long-term potentiation (i.e., a high-level stimulation no longer elicits an action potential), but is still considered a mechanism for memory. For example, certain neurons may be needed to strengthen a memory while others may interfere with

the memory. In this example, LTP would increase the sensitivity of neurons needed to strengthen a memory and LTD would decrease the sensitivity of neurons that interfere with a memory. In this way, LTP and LTD have a synergistic relationship in the creation of memories.

Memory Storage

Short-term memories are those memories that are only accessible for a few minutes. For example, one's short-term memory is utilized when remembering a newly heard phone number long enough to dial it into a phone. Therefore, assessments of short-term memory must take place within seconds to a few minutes of exposure to memory stimuli. In the context of a widely used list-learning task (i.e., Auditory Verbal Learning Task), short-term memory is assessed immediately upon hearing a list of 15 words by having the participants recall as many words as they can remember. This task is performed multiple times, with the result that some of the memory stimuli are incorporated into long-term memory. The duration of long-term memories may last from several minutes to years. Therefore, in order to infer that an assessment tool is measuring long-term memory, it must be administered long enough after exposure to the memory stimuli that short-term memories have degraded (e.g., at least 30 minutes). Investigations into the Auditory Verbal Learning Task have shown that long-term memory of certain word lists may last as long as one year post exposure. Participants' retention of previously learned material is an example of the caution that must be used in research designs exploring memory. Studies that utilize

repeated exposures to a memory stimulus should consider the effect of previous learning on the performance on subsequent assessments.

Memory Subtypes

Declarative memories are typically explained as relating to facts or specific events. The previously mentioned Auditory Verbal Learning Tasks is a good example of declarative memory. Participants are exposed to memory stimuli (i.e., word lists) and are asked to recall those memories at certain time points. However, there are other types of declarative memory that require the memory stimuli to be related to other stimuli (relational memory). A spatial learning task is an example of relational memory where one is required not only to remember the memory stimulus, but also the location of the stimulus. Relational memories are sometimes referred to as hippocampus-dependent memories, as the hippocampus has been found to be vital for performance on these types of memory tasks (Chaddock et al., 2010). Working memory is a memory subtype that is often discussed as a type of executive function or a frontal lobe-dependent memory (described below). Working memory is utilized when information must be held in one's memory and manipulated in order to complete a task. An example of a task that utilizes working memory is the "backwards" condition of the digit span task. This task assesses working memory by measuring the number of digits that participants can recall after a brief exposure. To perform this task, participants are presented with a sequence of digits, presented one digit at a time, and are asked to recall the digits in reverse order. This task

requires participants to hold the digit sequence in their mental space for the entirety of the recall. It is the act of “holding” the information in their mental space that makes this a working memory task. In comparison, the “forward” condition to the digit span task requires participants to simply recall the digit sequence. This does not require participants to hold the digit information in their mental space prior to recall. They will simply recall the digits in order.

The multiple aspects of memory require that any exploration into this construct clearly identify the subtype of memory and the specific domain of memory storage being assessed. Additionally, memory has been shown to be dependent on distinct neural regions as well as seemingly opposing cellular processes. Therefore, theoretical justifications should be provided as to the reasoning behind explorations into memory performance.

Acute Physical Activity and Cognition

Acute physical activity (PA) has been shown to enhance behavioral measures of cognition(Chang et al., 2012; Chang, Chu, et al., 2011; Chang et al., 2012; Ferris, Williams, & Shen, 2007; Labban & Etnier, 2011; Lambourne & Tomporowski, 2010; Winter et al., 2007). This beneficial relationship extends beyond more simple measures, such as reaction time, and into measures of higher order cognition, such as memory and executive functioning (e.g., planning, inhibition) (Chang et al., 2012; Lambourne & Tomporowski, 2010). Improved performance on measures of higher order cognition suggests that factors other than increased levels of neural activation generated by performing

PA (i.e., PA-induced neural activation) may be responsible for this relationship. Additionally, the intensity level of the PA seems to play a role in the relationship, with performance on cognitive tasks differing across intensity levels. Potential mechanisms of this relationship have been identified, but have yet to be tested using statistical techniques to assess mediation. Therefore, additional research is required in order to obtain a deeper, more thorough understanding of the acute PA-cognition relationship in humans. There is considerable interest in the PA-cognition relationship, as previously discussed in the literature review and the knowledgebase will benefit from further exploring the role of BDNF as a potential mechanism. The current study will extend upon past research to aid in elucidating this relationship. A greater level of understanding, once obtained, will allow for the emergence of PA prescriptions aimed at improving cognition.

Memory

Studies with non-human animals have provided robust support for the positive effect of PA on cognition (Anderson et al., 2000; Carro, Nuñez, Busiguina, & Torres-Aleman, 2000; van Praag, Shubert, Zhao, & Gage, 2005; Vaynman, Ying, & Gomez-Pinilla, 2004). Importantly, these studies often assess cognition through the use of memory tasks. For example, the Morris water maze and the radial arm maze, two cognitive tools that are widely used in rodent studies, require the subject to learn and remember spatial “target” locations. The relationship between PA and memory performance has also been explored in human studies.

Although studies with non-human animals have provided strong evidence as to the beneficial effects of PA on memory, the evidence in the human literature is more equivocal. Recent meta-analyses have reported positive effect sizes relative to acute PA on memory performance (Chang et al., 2012; Lambourne & Tomporowski, 2010; Roig et al., 2013). Among these meta-analyses, Chang et al. (2012) is unique in that the authors assessed primary (e.g., exercise intensity, general cognitive task type) and secondary (e.g., type of exercise activity, specific cognitive tasks) moderators of the acute PA-cognition relationship relative to the experimental paradigm (i.e., cognition assessed during, immediately following, or after a delay). Results indicate that the direction of the effect of PA on memory may depend on the specific aspect of memory being assessed by the neuropsychological tool. For example, while positive effects were observed with measures of free recall and visual short-term memory obtained after an acute bout of PA (d=.485 and d=.234, respectively), negative effects were observed for measures of auditory learning (Auditory Verbal Learning Task) and sequential memory (d= -.250 and d= -.169, respectively). Interestingly, the free recall and auditory learning measures both assess the participant's ability to learn and remember a list of words. However, for measures of "free recall", participants were required to remember lists of words that were presented visually, while measures of auditory learning required participants to listen to the word lists. The findings from this meta-analysis are suggestive of

mechanistic differences that may occur with different types of memory (e.g., auditory vs. visual).

The meta-analysis by Lambourne and Tomporowski (2010) provides information that is helpful to consider when designing a study to assess the acute PA-cognition relationship. When assessed during PA, cognition was impaired in acute PA bouts 20 minutes or less in duration and improved in longer bouts. This suggests that PA duration of more than 20 minutes is required to observe a beneficial acute PA-cognition relationship. While benefits to cognition were observed in bouts with durations greater than 20 minutes, larger benefits were observed when cognition was assessed within 15 minutes of completion of the PA. This suggests that the beneficial effects may be transient in nature, and observations must occur within a window of benefit. Additionally, findings from this review illustrate that the acute PA-cognition relationship is not equal across cognitive domains. Larger benefits were seen with assessments of memory than with assessments of information processing. Taking these findings into consideration, in order to observe positive acute PA-cognition relationships researchers should use PA durations greater than 20 minutes, assess memory domains of cognition, and assess post PA cognition within a 15-minute window.

While meta-analyses performed by Lambourne and Tomporowski (2010) and Chang et al. (2012) provide valuable information pertaining to moderating factors of the acute PA-cognitive performance relationship, Roig et al. (2013) extended the literature base by performing a meta-analysis that explored the effect of acute

PA on different domains of memory storage (i.e., short-term and long-term memory). Results showed positive overall effects of acute PA on both short-term and long-term memory. However, these findings were moderated by multiple factors relating to the experimental design of the included studies. Additionally, these moderating variables differed as a function of memory type (i.e., short-term or long-term). For measures of short-term memory, the effect of acute PA was moderated by memory subtype (i.e., verbal-auditory, visuo-spatial), time of retention (i.e., during exercise, after exercise, immediately after exercise, after a delay), mode of exercise (i.e., treadmill, bicycle, walking), duration of exercise (i.e., short, medium, long), and intensity of exercise (i.e., light, moderate, vigorous, maximal). Effects were largest in designs that assessed visuo-spatial memory subtypes, used walking as the mode of exercise, had short exercise durations, and light exercise intensities. For measures of long-term memory, the effect of acute PA was moderated by memory subtype (i.e., verbal-auditory, visuo-spatial, procedural), time of encoding (i.e., before exercise, after exercise), time of retention (i.e., immediately after exercise, after a delay), mode of exercise (i.e., treadmill, bicycle, other), duration of exercise (i.e., short, medium), intensity of exercise (light, moderate, vigorous-maximal), fitness level of subjects (i.e., average, excellent), and age of subjects (i.e., young-adult, aged). Effects seem to be largest in designs that assessed measures of verbal-auditory or procedural memory, presented the memory stimuli post-PA, assessed memory after a post-PA delay, used acute PA conditions that were comprised of bicycling or other

modes of PA, short or medium durations, light or vigorous-maximal intensities, in young or aged adults with average fitness. This meta-analysis provides evidence of certain factors that should be considered when assessing the acute PA-memory relationship. The moderating variables identified as relevant in this review suggest that factors such as the mode, duration, and intensity of the PA condition may influence the resulting effects on either short-term or long-term memory. Based on these findings, the timing of exposure to the memory stimuli relative to the acute bout of PA seems to be more important, in terms of generating the largest effect size, for measures of long-term memory, while the type of memory asssessed (i.e., verbal-auditory, visuo-spatial, procedural) may be important to consider based on when you expect to observe a significant effect (i.e., short-term or long-term). These outcomes might indicate that in order to observe beneficial effects of acute PA on short-term memory measures of visuo-spatial memory should be used, while measures of verbal-auditory memory would be better suited for observing effects of acute PA on long-term memory.

While meta-analyses provide a method by which to observe the relationships between acute PA and memory in a collection of studies, methodological intricacies require that interpretation of these results be done in the context of the individual studies. One reason for this is related to issues of third-order causation that may mask or result in a misinterpretation of results. This is due to the lack of power that most meta-analyses have to assess multiple moderators of the observed effects (e.g., three-way interactions). Therefore, most meta-analyses

only statistically assess main effects of a given relationship (e.g., PA-cognition relationship, conditional on the timing of cognitive assessment), leaving potential two-way (e.g., PA-cognition relationship, conditional on both the timing of cognitive assessment and domain of cognition assessed) interactions to be assessed subjectively. Therefore, an interpretation of meta-analytic results without a developed contextual understanding of the literature greatly reduces their efficacy in providing guidance for the design of future studies. The complex nature of the acute PA-cognition relationship lends itself to issues surrounding such subtleties (e.g., PA intensity, timing of cognitive tasks). To this end, a selection of individual studies exploring the acute PA-memory relationship will be presented next to allow for a closer examination of critical variables influencing the effects.

Labban and Etnier (2011) observed benefits to auditory memory in a randomized control trial that explored the effects of an acute bout of moderate intensity PA on cognition in healthy, young adults. One aim of this study was to assess the effect of acute PA on memory relative to the timing of exposure to the memory stimulus (i.e., encoding of Guild memory subtest which is an auditory verbal memory test). Participants in the PA groups were either exposed to the memory stimulus prior to or after an acute bout of moderate intensity PA, and a non-PA group was used as a control. Results showed that participants in the group exposed to the memory stimulus after PA were able to recall a significantly greater number of memory elements (i.e., paragraphic elements) compared to

controls. Though large effect sizes were observed between PA groups, this difference did not reach statistical significance. The authors explained that based on results from a post-hoc power analysis, this difference might have reached significance with an increased sample size by 11 participants.

In order to recall a memory stimulus, the stimulus must first be encoded and then it must be consolidated. The creation and subsequent recollection of a memory stimulus may be illustrated as a three-step process; encoding, consolidating, and recalling. Findings from Labban and Etnier (2011) suggest that acute PA aids in early stages of memory formation (i.e., encoding) and not the later stages (i.e., recall). Therefore, based on these observations, PA benefits the learning of new information, not, per say, the recollection of previously learned information. However, the meta-analysis performed by Chang et al. (2012) reported a significant benefit in crystallized intelligence (e.g., one's general knowledge) following an acute bout of PA. This suggests that when looked at meta-analytically, acute PA in fact does aid in memory recollection. More studies should be conducted to elucidate these contradictory findings.

Coles and Tomporowski (2008) performed a study to assess the effect of an acute bout of moderate intensity PA on memory. The within-subjects design utilized a 40-minute bout of PA on a cycle ergometer, a 40-minute period of sitting on the cycle ergometer (no PA), and a 40-minute period sitting in a chair (rest) as the experimental conditions. Memory was assessed prior to (pre) and following (post) the experimental conditions by two different measures of visual

memory. The Brown-Peterson test, a measure of visual short-term memory, required the participants to remember 20 memory sets comprised of three letter trigrams (e.g., “KYZ”). The memory sets were presented for two seconds and were followed by a delay period of varying durations (i.e., 3, 9, or 18 seconds). After the delay, participants were required to recall the memory set. The free recall task assessed visual memory by presenting participants with a 40-item word list, one word at a time for five seconds. Upon viewing the last word, there was a 100-second consolidation period, after which they were required to recall as many words as possible (immediate recall). Delayed recall was assessed following a 12-minute delay. Results showed that post-test scores on the Brown-Peterson test were worse than pre-test scores, showing decay in memory for all conditions. Acute PA did not have an effect on memory as assessed by the Brown-Peterson or free recall tests. However, when each condition was assessed with related-samples t-tests, post-test primacy and recency free recall scores were significantly worse than pre-test scores after the two non-PA conditions, and there was no significant change after the PA condition. Acute PA seemed to stop the delay-induced decline in memory performance. Though caution should be used when interpreting these results from multiple t-tests due to the increased type-I error rate, these results point out the complexity of the acute PA-memory relationship in that moderate intensity PA seems to elicit different effects on two seemingly similar measures of visual memory. More work

is needed to determine if this is truly an observation of differential cognitive effects or is merely reflective of differences in tool sensitivity.

Pesce, Crova, Cereatti, Casella, and Bellucci (2009) performed a study to assess the effects of two qualitatively different types of acute PA on free recall memory performance in adolescents. Using a within-subjects design, the authors assessed memory after performance of different acute PA conditions of moderate to vigorous intensity (i.e., circuit training, team games) and after a period of no PA. Performance on the free recall task required the participants to view a 20-item word list presented one word at a time, for five seconds per word. Words were printed on posters, and the researchers manually displayed each poster. As with the study by Coles and Tomporkowski (2008), the presentation of the words was followed by a 100 second consolidation period, after which the participants were instructed to write down as many words as they could remember (immediate recall). This was followed by a 12-minute class discussion, with the aim of inhibiting possible rehearsal of the words, after which the participants were asked to write down as many words as they could remember (delayed recall). Results indicated that team games benefited primacy and recency measures of immediate recall compared to no PA, and performing either type of PA (i.e., team games or circuit training) benefited recency measures of delayed recall. Since statistical analyses showed no difference in intensity levels performed during the two PA conditions, this study provides evidence that the qualitative experience of acute PA might affect observed memory benefits.

Results from this study are in disagreement with that performed by Coles and Tomporowski (2008). While Coles and Tomporowski (2008) observed that an acute bout of moderate intensity cycling prevented a decline in delayed free recall scores compared to non-PA conditions, Pesce et al. (2009) observed an improvement in immediate and delayed free recall scores in response to PA.

Etnier, Labban, Piepmeier, Davis, and Henning (2014) explored the effect of an acute bout of PA on memory relative to a non-PA condition (control) in a sample of children. Participants in the PA group performed the Progressive Aerobic Cardiovascular Endurance Run (PACER) as part of the regularly scheduled fitness assessments used by their Physical Education class in a private K-12 school. The Auditory Verbal Learning Task was administered immediately after completion of the PACER. A modified version of this task was used to facilitate its completion in small groups. The memory task required the participants to listen to lists of words prior to recalling as many words as possible by writing them on a sheet of paper. Results showed that those in the PA group performed at a higher level on the memory task than controls. These effects were specifically observed for measures of short-term memory, in that those in the PA group recalled a greater number of words during the five learning trials of the Auditory Verbal Learning Task as well as a greater number of words after the presentation of a list of interference words. Acute PA did not have an effect on measures of long-term memory, as assed by a 24-hour recognition task. These

results suggest that acute PA may have a differential impact on memory type (i.e., short-term or long-term memory).

The reviewed meta-analyses and individual studies clearly indicate that acute PA produces beneficial effects on measures of memory in children and young adults. However, these studies illustrate the complexity of the acute PA-memory relationship and provide examples of experimental methodologies that may be important to consider when designing future studies. There is experimental evidence that acute PA aids in the earlier stages of memory processes (e.g., encoding) (Labban & Etnier, 2011). Findings from Etnier et al. (2014), Pesce et al. (2009), and Roig et al. (2013) demonstrate that the specific memory duration of interest (e.g., short-term, long-term) should be taken into consideration when selecting neuropsychological measures to assess subtypes of memory (e.g., auditory, visual) as this may affect findings. For example, while Etnier et al. (2014) and Pesce et al. (2009) both used measures of memory requiring participants to perform a verbal recall (i.e., recall a list of previously introduced words), differences in the administration of the memory stimuli (i.e., Auditory - Etnier et al., 2014; Visual - Pesce et al., 2009) may explain the effect of PA on both short-term and long-term memory for the study performed by Pesce et al. (2009) but on only short-term memory for the study performed by Etnier et al. (2014).

Executive Function

"Executive function" is an umbrella term for complex cognitive processes that require the use of the brain's frontal lobe in order to perform conscious manipulations of mental material. In order to perform well on these types of tasks, a central "executive" is enacted to exhibit control over lower order cognitive functions. By performing latent variable analyses, Miyake, Friedman, Emerson, Witzki, Howerter, and Wager (2000) determined that set-shifting of mental sets (shifting), monitoring and updating of working memory (updating), and inhibition of prepotent responses (inhibition) are three distinct cognitive processes that can be used to assess components of executive function. For example, some executive functioning tasks require updating in order to plan or solve a multi-step problem set, some require the inhibition of an automatically selected response for a consciously selected response, while others require the ability of shifting from irrelevant to relevant tasks. While each of these task types assess components of executive functioning, the results from the individual tests should be interpreted as performance on the separate components that make up executive functioning (e.g., shifting, updating, inhibition) and not as executive functioning performance as a whole. Without making this distinction, researchers may report observed differences in executive function performance that on the surface seem contradictory (e.g., one study observes benefits while another observes detriments), but are in reality observations of different cognitive processes. This type of oversight unnecessarily complicates the literature base.

While acute PA has been shown to affect aspects of cognitive processing, such as simple reaction time (Pontifex, Hillman, Fernhall, Thompson, & Valentini, 2009), it has also been shown to affect higher-order executive functions. The specificity of the effect of PA on benefiting executive function was illustrated in a meta-analysis performed by Colcombe and Kramer (2003). Findings from this meta-analysis suggested that while chronic PA benefits multiple cognitive domains, it seems to exhibit the largest effects on tasks requiring executive function. Recently, meta-analyses and experimental studies have shown that acute PA also yields benefits on the performance of executive function tasks (Chang et al., 2012; Chang, Chu, et al., 2011; Chang et al., 2012; Chang, Tsai, et al., 2011; Lambourne & Tomporowski, 2010). The importance of these findings emanates from the ability of acute PA to positively affect consciously directed mental effort.

Chang et al. (2011) performed a randomized control trial to explore the effect of an acute 30-minute bout of moderate to vigorous intensity cycling on measures of executive function in young adults. Planning and problem-solving components of executive function were assessed with the Tower of London task prior to and upon completion of the PA or control condition. The Tower of London task requires participants to rearrange three colored beads that are placed on three towers, so that their pattern matches that of a pre-identified “goal” pattern in as few moves as possible. Results showed that those in the PA group completed the task with a lower total number of moves (total moves) and completed more

trials using only the minimum possible moves (correct score) compared to the control group. This suggests that an acute bout of moderate to vigorous intensity aerobic PA improved executive function.

In addition to the evidence for the acute PA-executive function relationship in studies using aerobic PA (i.e., cycling, running), there is also support for a beneficial effect of acute PA on executive function in studies using resistance PA (i.e., weight lifting). Chang et al. (2012) performed a study to assess the effect of an acute bout of moderate intensity resistance PA on executive function in middle-aged to older adults. This within-subjects study utilized the Tower of London task to assess executive function prior to and following a non-PA control condition and a 70% 10-repetition max (10-RM) condition. After an initial session to determine their individual 10-RM (i.e., the amount of weight they could lift a maximum of 10 times), participants attended two experimental sessions on separate days at least 48 hours apart. Results showed that resistance PA improved three aspects of performance on the Tower of London task (i.e., total correct score, total move score, total initial time). The total correct score is the number of trials completed by using the minimum possible moves while the total move score is the sum of all moves from all trials. The authors explain that these scores may represent the performance of cognitive processes related to planning quality and efficiency as well as mental manipulation. Scores of total initial time represent the total amount of time between the onset of the trial and the first move of each trial. The authors explain that this score is related to planning and

problem-solving performance with longer initial times being beneficial. While total initial time decreased after the non-PA condition (i.e., pre to post), total initial time, total correct, and total move scores all increased following the PA condition, with PA posttest scores being greater compared to PA pretest as well as non-PA posttest scores. These results show that improvements in executive function following acute bouts of PA are not relegated to aerobic modes of PA.

Meta-analytic and experimental evidence indicates that benefits in higher order cognitive processes, such as executive function, may be observed following acute PA. Additionally, the studies performed by Chang et al. (Chang et al., 2012; Chang, Tsai, et al., 2011) illustrate that these cognitive benefits are seen with either aerobic or resistance PA. These results suggest the possibility that aerobic and resistance PA may influence executive function through similar mechanisms. This has implications for the design of future studies aimed at exploring potential additive effects of PA modality (e.g., aerobic and resistance combined) on cognition, as well as similar mediating factors (e.g., physiological mechanisms).

Intensity

The effect of PA intensity should be taken into consideration when exploring the acute PA-cognition relationship. Meta-analytic and experimental evidence suggests that acute PA intensity affects its relationship with cognition. The meta-analysis performed by Chang et al. (2012) showed that when assessed immediately following acute PA, very light, light, and moderate intensities of PA

(<50%, 50-63%, and 64-76% maximal heart rate [HRmax], respectively) generate the largest positive effects on cognition while hard, very hard, or maximal intensities (77-93%, >93%, and 100% HRmax, respectively) result in very small positive effects or null effects. However, when assessed after a delay of more than one minute, very light intensities result in negative effects on cognition while light, moderate, hard, and very hard intensities result in large positive effects; with “hard” intensities eliciting the largest effect. The authors posited that the interaction between the PA intensity and the timing of cognitive assessment may suggest the existence of underlying biological mechanisms affecting cognition. The findings also provide evidence of the possible nature of the PA-biological mechanism-cognition relationship. The results imply a “Goldilocks zone” in which these PA-induced mechanisms benefit cognition. Outside of this zone, either too small or too large, and the mechanisms hinder cognition. For example, a “very light” PA intensity may induce just enough of a biological response to benefit cognition for a short period of time (i.e., less than one minute). However, a “hard” PA intensity may induce too great of a biological response to benefit cognition immediately after exercise (i.e., the response surpassed the upper limits of the Goldilocks zone) and requires a short delay (i.e., one minute) to fall back into the zone.

Experimental studies have provided evidence of the relationship of acute PA intensity on cognition, however the evidence is equivocal as to the magnitude and direction of the effect. Studies have shown support for a seemingly linear

relationship between acute PA intensity and cognition, with higher intensities generating the largest benefits as compared to lower intensities in measures of memory (Etnier et al., in preparation; Winter et al., 2007), inverted-U relationships, with moderate intensities generating the largest benefits as compared to lower and higher intensities in measures of executive function (Chang, Chu, et al., 2011; Ferris et al., 2007) or both depending on the difficulty of the specific measure used to assess cognition (i.e., cognitive processing, executive function) (Chang & Etnier, 2009).

Etnier et al. (in preparation) performed a within-subjects study to assess the relationship between acute PA intensity and memory in healthy young adults. Participants completed three acute PA sessions, each of a different intensity. The first session consisted of a graded maximum exercise test (VO_2max) on a treadmill to identify the participant's ventilatory threshold (VT), which was used to set the intensity of subsequent sessions. The second and third sessions consisted of a 30-minute bout of acute PA at a randomly selected intensity level (i.e., 20% below VT, 20% above VT). After each of the three PA conditions (VO_2max, 20% below VT, 20% above VT), the participants performed the Auditory Verbal Learning Task and a 24-hour recognition task to assess long-term memory. The recognition task required participants to identify previously learned words from a list containing learned words as well as new, unlearned words. Results indicated that 24-hour recognition (i.e., long-term memory) was improved following the VO_2max condition compared to the 20% below VT

condition. Though there were no significant differences between the 20% above VT condition and any other condition, performance after this condition fell between the other two conditions. This suggests a dose-response relationship between PA intensity level and cognition as assessed by a long-term memory task, with maximal PA exhibiting the largest benefits to memory.

Winter et al. (2007) performed a within-subjects designed study to assess the effects of acute PA intensity on cognitive performance (learning and memory) in healthy young adults. Participants completed four sessions. Session one consisted of a field-based fitness test that assessed heart rate and lactate concentration to aid in the determination of intensity levels for the acute PA conditions. Sessions two, three, and four were presented in a randomized order and consisted of a 15-minute rest period (control), a 40-minute bout of steady-state running (moderate condition), and two 3-minute bouts of sprinting at an increasing speed (intense condition). Cognition was assessed after the experimental conditions by a language-learning task requiring participants to associate novel words with pictures of objects (e.g., word: “glump” / picture: car). Participants performed 5 learning sets for a total of 600 trials. Results showed that the rate of learning was increased following the intense condition compared to the moderate condition or control. Following the intense condition, maximal learning (i.e., a ceiling effect) occurred at trial four, while the ceiling was reached at trial five for the other conditions. This suggests that a shorter bout of high

intensity PA is more beneficial to relational learning than a longer bout of moderate intensity PA.

Chang et al. (2011) performed a within-subjects study to explore the dose-response effect of acute resistance PA intensity on executive function, as assessed by the Tower of London task, in middle-aged and older adults. Participants attended an initial session where their individual 10-RM was identified. On four separate days, participants performed four experimental conditions consisting of different PA intensities (i.e., 40%, 70%, or 100% of 10-RM) or a non-PA control condition (i.e., movie). The Tower of London task was completed after each session. Results indicated a curvilinear effect of PA intensity on Tower of London performance, in that better total move and correct move scores were observed after performing 40% and 70% 10-RM intensities compared to control or 100% 10-RM. These results suggest an inverted-U relationship between performance and acute PA intensity, with moderate levels of intensity eliciting beneficial effects on executive function.

Ferris et al. (2007) performed a within-subjects study to assess the effect of acute PA intensity on cognitive performance (e.g., executive function) in a sample of healthy young adults. Participants completed three PA sessions on a cycle ergometer. Session one consisted of a VO_2max test, which was used to determine the intensity of the subsequent PA conditions as well serving as the maximum intensity condition. Sessions two and three consisted of performing a 30-minute bout of low (20% below VT) or high (10% above VT) PA intensities in

a randomized order. Cognitive performance was assessed using the Stroop task prior to (pre) and following (post) each PA condition. Additionally, participants completed a practice session of the Stroop task prior to the first cognitive assessment. The Stroop task contains three sections. Each section required participants to read a list of items as quickly as possible. In this study, participants were given a 45-second time limit. Section one (word) required the participant to read a list of words comprised of three colors (i.e., red, blue, green), section two (color) contained items that were written as "XXXX" in different font colors (i.e., XXXX, XXXX, XXXX) and required participants to name the font color used, and section three (word-color) required participants to name the font color of a list of incongruent words and colors as quickly as possible (e.g., red, blue, green). While the word and color sections are typically interpreted as measures of cognitive processing speed, the word-color condition is a measure of inhibition (Etnier & Chang, 2009; Miyake et al., 2000). Analyses used to assess performance did not include all PA conditions. Pre-post performance was assessed for the VO_2max condition and separate analyses were performed to assess performance in the low and high intensity conditions. Therefore, information pertaining to statistical differences between the VO_2max condition and the low and high intensity conditions is not available. Results showed that performance on the word and color sections of the Stroop task improved following all of the PA intensities and performance on the word-color section improved only following the high intensity PA condition. This suggests

that while all intensities of PA (i.e., low, high, max) benefited measures of cognitive processing speed, high intensity PA benefits measures of executive function (i.e., inhibition). The VO_2max condition, which was not statistically compared to the other conditions, was qualitatively different compared to the other two PA conditions (e.g., total time), and a table of means was not provided in the results. An inspection of the provided figures (i.e., bar graphs) illustrating performance on the Stroop task pre and post each PA condition, seems to support an inverted-U relationship between acute PA intensity and executive function performance, with an improvement in performance following high intensity PA and no improvement following maximum or low intensities. However, the lack of a statistical test inhibits inferences including all three PA conditions. Further research is warranted to explore the potential intensity-dependent effect of acute PA on cognitive performance and how this may differentially influence distinct domains of cognitive performance (e.g., processing, executive function).

Chang and Etnier (2009) performed a between-subjects study to assess the relationship between acute resistance PA intensity and cognition on two measures of executive function (i.e., Stroop task, Paced Auditory Serial Addition Task). Participants completed two sessions, the first of which was used to determine the 10-RM to aid in establishing the appropriate weight to create specific intensity levels for session two (i.e., 0% [control], 40%, 70%, 100% 10-RM, randomized assignment). Assessment of cognitive performance was obtained prior to and following the PA condition. The Paced Auditory Serial

Addition Task contains four sections of increasing difficulty and was used as a measure of cognitive processing and attention. To perform this task, participants listened to a series of single-digit numbers and were required to say the sum of the last two numbers they heard (i.e., each consecutive pair). Results showed a positive linear relationship between PA intensity and performance on the color section (e.g., cognitive processing) of the Stroop task and a quadratic relationship between PA intensity and performance on the word-color section (e.g., inhibition). Quadratic relationships were also observed between PA intensity and performance on sections two, three, and four of the Paced Auditory Serial Addition Task. These results suggest that performance on more simple tasks assessing cognitive processing, such as naming the color of a list of items, benefited from increasingly greater PA intensities, while performance on more difficult tasks assessing higher-order cognitive processes, such as summing consecutive pairs of numbers presented at a rapid pace, benefited more from a moderate PA intensity than higher or lower intensities. These results suggest that the PA intensity-cognition relationship changes from positive and linear with simple tasks to quadratic (i.e., inverted-U) with more difficult tasks.

The effects observed in the previous studies suggest that the beneficial effect of acute PA on cognition is intensity dependent, and the nature of this effect may be conditional on the specific cognitive domain assessed and the difficulty of the specific task used to assess cognition. By looking at studies that have assessed the effect of PA intensity on measures of lower order (e.g., cognitive processing)

or higher order (e.g., memory, executive function) domains of cognition, we are able to observe how the PA-cognition relationship differs as a function of PA intensity, and may be conditional upon the difficulty of the cognitive task. Performance on memory tasks (i.e., long-term verbal recognition, short-term relational) or less demanding assessments of cognitive processing is improved following an acute PA bout at higher intensities (Chang & Etnier, 2009; Etnier et al., in preparation; Winter et al., 2007). However, performance on demanding assessments of cognitive processing and executive function tasks is most improved by an acute PA bout of moderate-high intensities (Chang, Chu, et al., 2011; Ferris et al., 2007). These findings are important in that they suggest PA intensity-mechanism-cognition relationship may be conditional on the assessed cognitive domain. Additionally, these findings provide information vital to the design of future studies interested in exploring the dose-response nature of the acute PA-intensity-cognition relationship. While higher or maximal PA intensity levels should be used in order to observe the largest effects of acute PA on measures of memory (i.e., long-term verbal recognition, short-term relational), or easier cognitive processing tasks, more moderate intensity levels should be used to observe the largest effects on measures of executive function or more difficult cognitive processing tasks.

Chronic Physical Activity and the Brain

Chronic PA has been shown to benefit cognition through improved neurological adaptations (Colcombe et al., 2006; Dishman et al., 2006; Erickson,

Voss, et al., 2011; van Praag et al., 2005). Rodent studies have explored the relationship between chronic PA-induced improvements in cognition and the neurological adaptations allowing for enhanced cognition. These adaptations include an increase in the number of new neurons (brain cells), synapses (neural connections), and blood vessels in areas of the brain responsible for the cognitive processes being tested (e.g., executive function, memory) (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990; van Praag et al., 2005).

Black et al. (1990) performed a study to assess the neurological effects of qualitatively different types of chronic PA in rodents. The subjects were placed into either an acrobatic, forced, voluntary, or no PA condition for 30 days. Those in the acrobatic condition performed five daily trials that required them to traverse seven obstacles including bridges, see-saws, and balance beams. The forced PA condition required the subjects to perform a one-hour quick-paced walk on a treadmill (i.e., 10m/min). Those in the voluntary PA condition were given access to a running wheel at all times. Those in the non-PA condition were given minimal learning or PA opportunities. The PA conditions were qualitatively different in that the acrobatic condition required the rodents to continually learn how to traverse new and different obstacles, while the skills required to perform the forced and voluntary PA conditions required little learning to master. Electron microscopy was used to assess morphological differences in the cerebellar structure between conditions. Results showed that those in the acrobatic and voluntary PA conditions experienced significant neurological changes compared

to those in the non-PA condition. However, the nature of the adaptations differed as a function of the qualitative nature of the PA condition, with those in the acrobatic condition experiencing synaptogenesis (i.e., increase in neural connection), those in the voluntary PA condition experiencing angiogenesis (i.e., increase in blood vessels), and those in the forced PA condition experiencing non-significant changes in neural structure. These results suggest that while voluntary PA led to an enhancement in blood supply to the brain, potentially benefiting neural metabolic capacities, acrobatic PA led to an enhancement in the connectivity of the brain, potentially benefiting cognitive processing. These results suggest that the qualitative nature of PA have differential effects on the neural environment. Though not tested in this study, it is reasonable to hypothesize that these different adaptations to PA may result in domain specific differences in the performance of cognitive tasks (e.g., neural processing speed, problem solving).

Van Praag et al. (2005) performed a study to assess the effect of one month of chronic PA (voluntary wheel running) on the neural structure and cognitive performance of older and younger rodents (voluntary PA) compared to age-matched controls (no PA). The rodents in the PA group were given access to a running wheel for 45 days. Additionally, they were injected with bromodeoxyuridine daily for the first week in order to label newborn neural cells. Cognitive performance (i.e., spatial learning and memory) was assessed with the Morris Water Maze over the course of five days (i.e., day 35 to 39). The Morris

Water Maze consisted of a pool of opaque water with a hidden platform placed 1cm under the water's surface. Each day, four training trials (i.e., learning) were performed and a probe trial (i.e., memory) was performed four hours after the last training trial on the fifth day. Learning trials began with placing the rodent into the pool and ended when the rodent either found the platform or after 40 seconds. The memory trial consisted of removing the platform before placing the rodent in the pool for 40 seconds. Measures of latency and swim path length were used as assessments of learning, with lower scores interpreted as indicative of better learning. Measures of time (i.e., seconds) in the target zone and target quadrant were used to assess memory. Immunohistochemistry and immunocytochemistry measures were used to assess differences in hippocampal structure. Results showed that younger and older rodents in the voluntary PA group as well as younger rodents in the no PA group performed better (i.e., shorter latency and path length) on the learning trials as compared to older rodents in the no PA group. While younger rodents in the voluntary PA group outperformed all other groups on measures of memory (i.e., time in target zone and target quadrant), older rodents in the voluntary PA group performed better than older rodents in the no PA group on measures of memory (i.e., time in target quadrant). Hippocampal analyses showed that younger rodents in the voluntary PA group experienced greater neurogenesis compared to all other groups, and younger rodents in the no PA group and older rodents in the voluntary PA group experienced greater neurogenesis compared to older no PA rodents. Importantly,

levels of neurogenesis in older rodents in the voluntary PA group were the same as younger rodents in the no PA group. This illustrates the positive effect of PA in reducing age-related declines in neurogenesis. These results suggest that voluntary chronic PA benefits the form and function of the brain as observed by measures of learning, memory, and hippocampal neurogenesis.

These two rodent studies provide strong evidence for the beneficial effect of chronic PA on measures of brain structure and cognitive performance, and, specifically, within older samples. Recently, neuroimaging tools have allowed researchers to observe the effects of chronic PA and fitness on the human brain through measures of increased brain volume (Colcombe et al., 2006; Colcombe et al., 2003; Erickson, Voss, et al., 2011). These techniques have helped to identify specific regions of the brain that are affected by chronic PA, and this information is used to help researchers understand how chronic PA may produce benefits to the health and performance of the human brain as it ages.

Two studies by Colcombe and colleagues (Colcombe et al., 2006; Colcombe et al., 2003) have produced a foundation of evidence for the positive effects of fitness and chronic PA on the structure of the human brain in samples of older adults. Colcombe et al. (2003) performed a cross-sectional study to explore correlations between brain volume, age, and aerobic fitness in healthy older adults. Participants underwent magnetic resonance imaging (MRI) scanning, and the subsequent images were assessed by voxel-based morphography to determine brain volume and composition (i.e., white and gray matter density).

Analyses were then performed to assess associations between age, fitness (i.e., Rockport protocol estimated VO_2max), and brain morphology. Results showed a negative association between age and brain volume, with older adults having lower brain volumes. These age-related reductions occurred in gray matter of the prefrontal, superior parietal, and middle/inferior temporal cortices. Age-related reductions were also oberved in anterior white matter tracts, and to a lesser extend, in posterior tracts. Importantly, results showed that fitness had a beneficial association with age-related reductions in brain volume. A higher level of fitness was associated with less age-related decreases in brain volume. Due to the cross-sectional nature of this study, further interpretation of these results is not advisable. However, these results provide a foundation of evidence to build hypotheses related to the effect of chronic PA interventions on brain struction and cognitive performance in older adults.

Colcombe et al. (2006) performed a randomized control study to assess the effect of a 6-month aerobic PA intervention on measures of brain volume. Participants, consisting of 59 older adults, were randomized into either an aerobic PA or a non-aerobic PA (i.e., stretching and toning) group. Additionally, 20 younger adults were used as non-PA controls for brain volume as it was hypothesized that there would be no change in volume after a 6-month period. All participants underwent MRI scanning prior to and upon completion of the intervention. Results showed that the sedentary younger adults experienced no significant pre-post changes in brain volume. Older adults in the aerobic PA

group experienced significant increases in gray matter volume in the anterior cingulate cortex, supplementary motor cortex, right inferior frontal gyrus, and left superior temporal gyrus; and increases in anterior white matter tract volume. The results showed that a 6-month aerobic PA intervention went beyond an "ameliorating effect", as was hypothesized in the previous cross-sectional study (Colcombe et al., 2003), and actually increased brain volume. This is a powerful observation, as it suggests that PA may be a viable option for improving brain health in older adults.

As part of the Cardiovascular Health Cognition Study, a population-based longitudinal study focused on exploring cardiovascular disease and stroke in older adults (i.e., 65 years and older), Erickson et al. (Erickson et al., 2010) assessed the relationship between PA, brain volume, and cognitive impairment over a 9-13 year period. Baseline measures included MRI scanning to assess brain volume, the Minnesota Leisure-Time Activities Questionnaire to assess PA behavior (i.e., number of blocks walked in a one-week period), and clinical neuropsychological assessments of cognitive performance (e.g., mild cognitive impairment). After a 9-13 year period, measures of brain volume and cognitive performance were assessed a second time. Results showed that greater levels of PA behavior at baseline (i.e., more blocks walked) predicted increases in gray matter in the frontal, temporal, and occipital lobes, including the entorhinal cortex and hippocampus, nine years later. The areas of PA-predicted gray matter increase were also associated with lower risks of developing mild cognitive

impairment at posttest. Scores of PA behavior were broken down into quartiles (blocks walked: Q1=0-12, Q2 =12-24, Q3 =25-70, Q4 =72-300) in order to explore the existence of critical PA levels needed to receive benefits to brain volume. Only those in the fourth quartile experienced benefits to brain volume from PA. These findings suggest that greater levels of leisure-time PA (e.g., walking) benefit the neural and cognitive health of older adults. Additionally, results suggest that these benefits may be working through PA-induced neurogenesis in regions of the brain vital for performance on tasks used to assess cognitive impairment. Additional analyses, using statistical techniques to assess mediation, are required in order to determine the mechanism(s) of this relationship.

Erickson et al. (2011) performed a randomized control trial to assess the effect of a 1-year PA intervention on hippocampal volume and spatial memory ability in a sample of older adults. Participants were randomized into either a moderate intensity aerobic PA group or a stretching and toning PA group. Each group performed the assigned PA three days per week for a total of one year. Measures of spatial memory and hippocampal volume (MRI) were assessed at baseline, mid-point (i.e., six months), and upon completion of the 12-month intervention. VO_2max tests were performed prior to and upon completion of the 12-month study to assess changes in levels of aerobic fitness. Spatial memory was assessed using a computerized paradigm that required the participants to view one, two, or three black dots appearing in random locations on a computer

screen for a short period of time (500ms). After 500ms, the screen went blank and remained blank for three seconds. The participants were then shown a red dot and had two seconds to determine whether it was or was not in the location of one of the previously viewed black dots. Results showed a significant moderating effect of PA on hippocampal volume. Those in the aerobic PA group experienced a 2.12% and 1.97% increase in the left and right hippocampus, respectively, while those in the stretching and toning PA group experienced a 1,4% and 1.43% decrease in hippocampal volume. Analyses of aerobic fitness results showed that increases in VO_2max were associated with increases in hippocampal volume. Additionally, those in the stretching and toning PA group with higher VO_2max levels at baseline experienced less reduction in hippocampal volume compared to those with lower VO_2max levels. Results for performance on the spatial memory task showed that both groups improved as a function of time. There was a positive association between hippocampal volume and spatial memory at baseline and posttest. However, associations between spatial memory and changes in hippocampal volume were only apparent in the aerobic PA group. These results suggest that a 12-month chronic aerobic PA intervention has a positive effect on the hippocampal volume of older adults. It was shown that older adults with higher levels of aerobic fitness had higher hippocampal volumes than lower fit individuals, and increases in hippocampal volume experienced by those in the aerobic PA group translated to improved performance on a task of spatial memory. A major limitation of this study is its

lack of a true non-PA control group. Therefore, it is not possible to assess the variance observed between the two qualitatively different PA groups in relation to a group that did not perform any PA (i.e., sedentary condition).

The reviewed studies illustrate the beneficial effect of chronic PA on brain structure, and how these neural changes may translate to improvements in performance on cognitive tasks. The use of chronic PA paradigms is a logical manner of exploration because the structure of the brain is not likely to change in response to an acute bout of PA (e.g., neurogenesis, synaptogenesis, angiogenesis). In a review, Bischofberger (2007) explains that it may take several weeks to complete the process of neurogenesis, with synaptogenesis reaching maximal density at six to eight weeks. These findings suggest the existence of a mechanism(s) that aids in the brain's adaptation to chronic PA resulting in enhanced cognition and increased neural volume.

Brain-Derived Neurotrophic Factor

As part of the family of neurotrophins, brain-derived neurotrophic factor (BDNF) is a growth factor vital to the health and performance of the nervous system. While BDNF has been widely studied for its role in a diversity of health-related conditions, including type 2 diabetes (Krabbe et al., 2007), multiple sclerosis (Tongiorgi et al., 2012), and depression (Brunoni, Lopes, & Fregni, 2008; Yoshida, Ishikawa, Niitsu, et al., 2012), a major area of interest is in its effect on neural plasticity and cognitive performance (Cirulli et al., 2004; Horch et al., 1999; Mu et al., 1999). The hippocampus has been identified as a major area

of BDNF expression (Neeper, Gomez-pinilla, Choi, & Cotman, 1996). As the hippocampus is a brain region vital to learning and memory, this area of expression may explain why studies have observed the importance of BDNF for the performance of cognitive tasks requiring learning and memory. The importance of BDNF for memory is evident in its participation in long-term potentiation and long-term depression, cellular models of memory (Cunha, Brambilla, & Thomas, 2010).

Mu, Li, Yao, and Zhou (1999) performed a randomized control study to explore the effects of endogenous BDNF deprivation on the cognitive performance (e.g., spatial learning and memory) of adult rats. Rats were either exposed to a BDNF antibody (BDNF deprivation group) or immunoglobulin G (control group). These compounds were continuously and directly delivered into the rats' brains for seven days. After exposure, measures of learning and memory were assessed using the Morris Water Maze. Learning trials took place twice per day over the course of four and a half days with memory being assessed after the last learning trial. Learning was assessed by measures of escape latency (i.e., time to find the hidden platform) during each trial and memory was assessed by measures of focused swimming (i.e., percent distance swam in the target quadrant) during a 2-minute period without the escape platform. Analyses revealed that those in the BDNF deprivation group experienced significantly slower learning (increased escape latency) and worse

memory (less focused swimming) compared to controls. These results provide support for the vital role of BDNF in learning and memory.

Cirulli, Berry, Chiarotti, and Alleva (2004) used a similar form of the Morris Water Maze to assess memory in adult rats. However, while the previously reviewed study explored the effects of endogenous BDNF *deprivation*, the focus of this study was on the exogenous *administration* of BDNF directly to the hippocampus. After the rats performed six learning trials (i.e., finding the escape platform), one group received an administration of BDNF (exogenous BDNF group) and another group received an administration of saline solution (control group). The next day, the rats performed a probe memory trial followed by six reversal learning trials. The reversal task was a new learning task that required the rats to find the escape platform that had been moved to the opposite quadrant from its placement during the initial learning trials performed on the previous day. Results showed that the learning performance of both groups experienced a similar amount of improvement over the six learning trials and there was no difference in their performance on the memory probe task. However, those in the exogenous BDNF groups experienced significantly quicker learning in the reversal trials compared to those in the control group as assessed by shorter escape latency and more directed swim paths. These results suggest that the exogenous administration of BDNF improved the rats' cognitive flexibility, in that they were better able to learn a new version of a previously performed task.

The invasive nature of experimental designs used to explore BDNF in non-human animals is a limiting factor in that it inhibits the ability to conduct similar research with humans. However, an important aspect of BDNF is its ability to cross the blood-brain barrier (Pan et al., 1998; Poduslo & Curran, 1996). This allows BDNF expressed in the brain to leave the brain and enter the periphery. This molecular property allows for the sampling of BDNF through the periphery by drawing blood samples from veins in the arm or hand, and the assessment of BDNF concentrations in serum or plasma. Additionally, direct research with non-human animals (Klein et al., 2011), as well as indirect research with humans (Rasmussen et al., 2009) has shown that peripherally assessed BDNF positively correlates with central BDNF. Since the hippocampus is a major area of expression for central BDNF (i.e., neurally expressed BDNF), as well as an area of the brain directly involved with learning and memory, this is an important factor when interpreting future results from mediational analyses exploring the PA-BDNF-cognition relationship.

Associations have been observed between levels of peripherally assessed measures of BDNF concentration and brain volume. Erickson et al., (2010) performed a cross-sectional study to assess the association between BDNF and age-related decreases in brain volume in humans. Participants consisted of 142 healthy adults between the age of 59 and 81 years. MRI scans were used to assess hippocampal volume. Blood samples were collected two weeks prior to the MRI scans in order to assess peripheral concentrations of BDNF, and

cognitive performance was assessed one week prior to the MRI scans. Cognitive performance was assessed using the same computerized spatial memory paradigm as previously described in the study performed by Erickson et al. (2011). Older participants were found to have significantly lower peripheral concentrations of BDNF, smaller hippocampus volumes, and worse performance on spatial memory tasks compared to younger participants.

Initially, BDNF, like all neurotrophins, is translated from mRNA as an immature (pro) isoform that must be enzymatically modified in order to take on its mature (m) isoform. In a review, Lu, Pang, and Woo (2005) described the "Yin and Yang" approach taken by the two isoforms, with both being vital to neural health and performance, yet interacting with different receptors that signal pathways resulting in opposing functions. The dichotomous roles of proBDNF and mBDNF are seen with proBDNF eliciting cell death (apoptosis) as well as dendritic and synaptic retraction, and mBDNF eliciting cell growth (neurogenesis), as well as dendritic and synaptic spine formation. This takes place through different signaling pathways, with proBDNF binding to $p75^{NTR}$ receptors and mBDNF binding to TrkB receptors, both of which are expressed in the hippocampus (Bernabeu & Longo, 2010; Drake, Milner, & Patterson, 1999).

The BDNF isoforms have been observed to affect neural activity through their association with long-term potentiation and long-term depression (Barker, 2009; Cunha et al., 2010). While proBDNF has been identified as being important for eliciting long-term depression, mBDNF has been found to be important for

eliciting long-term potentiation. Long-term potentiation has been shown to elicit growth in dendritic and synaptic spines, while long-term depression has been shown to elicit dendritic and synaptic retraction. This is an important concept as it shows that specific BDNF isoforms may not only play a role in the acute activity of a cell (i.e., long-term potentiation and long-term depression), but this acute activity may be directly related to changes in cellular morphology.

Physical Activity and Brain-Derived Neurotrophic Factor

Chronic and acute PA paradigms have both been shown to affect peripherally assessed BDNF concentrations (Etnier et al., *in preparation*; Ferris et al., 2007; Griffin et al., 2011; Rasmussen et al., 2009; Winter et al., 2007; Zoladz et al., 2010; Zoladz et al., 2008). Chronic PA paradigms have been used to explore the effect of chronic PA on basal BDNF concentrations, as well as the effect of chronic PA on the acute PA-induced BDNF response. Results from studies using chronic PA paradigms to explore the effect of PA on basal (i.e., resting) BDNF concentrations have been equivocal. A 6-month high intensity aerobic PA training program was shown to have a non-significant effect on basal BDNF concentrations in a sample of older adults with amnesic mild cognitive impairment (Baker et al., 2010), and a 1-year moderate intensity aerobic PA training program was shown to have a non-significant effect on basal BDNF concentrations in a sample of healthy older adults. However, a 5-week moderate-high intensity training program was shown to eliicit a significant increase in basal BDNF concentrations in a sample of young healthy men (Zoladz et al., 2008). In

the same study, Zoladz et al. (2008) also observed differences in basal BDNF concentrations as a function of athletic status, with athletes having higher basal BDNF concentrations compared to non-trained participants. These studies suggest that the effect of chronic PA on basal BDNF concentrations may be conditional on the age of the participants, though more research is needed to assess this hypothesis. Results from studies using chronic PA to explore its effect on an acute PA-induced BDNF repsonse are unclear. Findings from Zoladz et al. (2008) show that a VO_2max test on a cycle ergometer (i.e., acute PA) elicited a non-significant change in BDNF concentration in a sample of healthy young men prior to a 5-week training period, but induced a significant increase upon completion of the training. However, findings from Griffin et al. (2011) show that a VO_2max test on a cycle ergometer (i.e., acute PA) elicited a significant change in BDNF concentration immediately post PA in a sample of healthy young men prior to a 5-week training period. A non-significant acute PA-induced response in BDNF concentration was observed after three weeks of training, and a significant response was observed in a non-training group immediately following the VO_2max test and remained significant at the 30-minute post acute PA timepoint. After completing the 5-week training period, a significant acute PA-induced response in BDNF concentration was observed 30 minutes following the VO_2max test, and a significant response was observed in a non-training group immediately following the VO_2max test and remained significant at the 30-minute post acute PA timepoint. The results of these two studies illustrate the needs for

more research into the conditional effects of chronic PA training on the acute PA-induced BDNF response.

Results from studies using strictly acute PA paradigms have largly shown support for a positive relationship between acute PA and BDNF concentations in a varietly of PA modalities including a 15-min step protocol (Tang, Chu, Hui, Helmeste, & Law, 2008), VO_2max tests (Etnier et al., in preparation; Ferris et al., 2007; Griffin et al., 2011; Rojas Vega et al., 2006), sub-maximal endurance protocols (Etnier et al., in preparation; Ferris et al., 2007; Rasmussen et al., 2009), and a sub-maximal sprint protocol (Winter et al., 2007). However, reporting of methodological procedures used in the analysis of BDNF make some findings difficult to interpret. For example, Brunelli et al. (2012) observed a significant increase in BDNF concentration following a VO_2max test on a cycle ergometer, but the change was determined to be non-significant once BDNF concentration data were adjusted for changes in plasma volume. Of the studies described, Brunelli et al. (2012) reported making a plasma-based adjustment and Winter et al. (2007) reported making a blood volume-based adjustment to BDNF concentration data. However, unlike Brunelli et al. (2012), Winter et al. (2007) did not provide a summary of non-adjusted results. While there is support for an acute PA-induced increase in BDNF concentration, greater methodological detail must be provided in published manuscripts in order to create a more cohesive literature base concerning the acute-PA BDNF relationship.

Studies exploring the acute PA-BDNF relationship typically use enzyme-linked immunosorbent assay (ELISA) kits to assess BDNF concentrations. The specific ELISA kits used in these studies either do not differentiate between BDNF isoforms (i.e., Millipore, Promega), or assess mBDNF with an identified cross-reactivity with proBDNF (i.e., R&D). As well as exploring the effect of acute PA on BDNF concentration (previously described), Brunelli et al. (2012) also assessed the effect of acute PA on BDNF isoform proportios in peripheral blood mononulear cells. Participants attended two separate PA sessions separated by one week. The first session required participants to perform a VO_2max test on a cycle ergometer. Information obtained during this session was used to determine the intensity for the subsequent session (individual anaerobic threshold). The second session required particpants to perform a 30-minute bout of cycling at an intensity level that corresponded with the previously identified individual anaerobic threshold. For each session, blood samples were taken prior to the PA (pre), upon completion of the PA (post), and 30 (post-30) and 60 (post-60) minutes following the PA. Proportions of BDNF isoforms were assessed using Western Blot analyses and were quantified using ImageJ software. Results indicated a main effect of time for proBDNF, with a significant increase in proBDNF levels from pre to post-30 that was retained at post-60 in the VO_2max condition and a significant increase from pre to post-60 in the submaximum PA condition. A significant PA by time interaction was observed for mBDNF levels, with a significant increase from pre to post followed by a significant decrease

from pre to post-60 in the VO_2max condition, and a significant increase from pre to post-30 that was retained at post-30 in the submaximum PA conidtion. These findings show that, while the relationship between PA and proBDNF isoform is not significantly dependent upon PA intensity or time of assessment, with significant increases observed in each PA condition, the relationship between PA and the mBDNF isoform is conditional on the intensity of the PA and the time of assessment, with significant increases observed in both PA conditions and a significant decrease in the VO_2max condition. In order to create mBDNF, the proBDNF isoform has to be enzymatically cleaved to remove a section of the protein. Therefore, these findings suggest that acute PA has an effect on the cleavage of proBDNF into mBDNF that is dependent on the PA intensity and amount of time elapsed since stopping the PA. Due to the dichotomous effects of the BDNF isoforms in the brain (e.g., long-term potentiation, long-term depression), the PA-dependent cleaving of mBDNF to proBDNF illustrates a potential mechanism by which PA may affect the neural environment.

There is strong support for the importance of BDNF in the form and function of the brain, and the specific BDNF isoforms created by post-translational modifications (i.e., enzymatic cleaving) have been shown to affect opposing signaling pathways implicated in a well-established cellular model of memory (i.e., long-term potentiation and long-term depression). Importantly, the correlation between centrally and peripherally assessed BDNF concentrations has allowed for the exploration of the acute PA-BDNF relationship in humans.

The results from these studies have shown support for acute PA-induced increases in peripheral concentrations of BDNF, as well as moderation of BDNF isoform proportion, in humans. These observations lay the foundation and justification for an exploration into the relationship between acute PA, BDNF, and cognition.

The Physical Activity-BDNF-Cognition Relationship

Chronic PA

Multiple reviews have addressed the importance of BDNF in the chronic PA-cognition relationship. In their review, Cotman and Berchtold (2002) discuss the role of chronic PA on increasing the hippocampal expression of BDNF mRNA and protein concentrations in rodents and its tenable role in enhancing brain function, neuronal plasticity, and learning. In a review exploring an evolutionary theory of diet and chronic PA for brain health, Vaynman and Gomez-Pinilla (2006) illustrate how chronic PA-induced increases in BDNF expression may be connected to the brain's ability for rapid neurotransmission through its regulation of Synapsin-1, a protein that assists in tethering pre-synaptic vesicles to the neuron's cytoskeleton, and performance on learning and memory tasks. Similarly, the review by Lista and Sorrentino (2010) also directs attention to the importance of BDNF in the packaging of neurotransmitters, its positive relationship to long-term potentiation and learning, as well as its negative relationship to neurodegenerative diseases (i.e., Alzheimer's disease). Additionally, their examination revealed support for BDNF potentially mediating

the chronic PA-BDNF-cognition relationship in terms of its effect on neurogenesis.

Experimental observations also provide support for the PA-BDNF-cognition relationship. Vaynman et al. (2004) performed an elegantly designed rodent study to explore the mediational role of BDNF in the chronic PA-BDNF-cognition relationship. Rodents were assigned to either a 1-week period of voluntary wheel running PA (PA condition) or a 1-week sedentary condition (SED condition). Additionally, half of the rodents from each condition were injected with a BDNF receptor blocking molecule (i.e., TrkB-IgG) and the other half were injected with standard control protein. This created four groups of subjects (PA-Block, PA-Open, SED-Block, SED-Open). The Morris Water Maze was used to assess learning and memory upon completion of the 1-week period, and hippocampi were also collected to assess BDNF mRNA. Results showed that those in the PA-Open group had both greater learning and memory performance and mRNA concentrations compared to those in all other conditions. Additionally, there was no difference between those in the PA-Block condition, the SED-open, and SED-Block conditions. These results show that blocking BDNF from binding with its TrkB receptor eliminates the benefits in learning and memory gained from performing a 1-week period of PA. Additionally, the results show that blocking BDNF also eliminates increases in PA-induced BDNF expression in the hippocampus. This finding supports the role of BDNF in regulating its own expression. Observations from this study strongly support the mediational role of

BDNF in the chronic PA-BDNF-cognition relationship. Though statistical mediational analyses were not performed, the experimental design used in this study allows for inferences of mediation.

The rodent literature presents findings that support the chronic PA-BDNF-cognition relationship. However, results from human studies have been more equivocal. Baker et al. (2010) performed a 6-month randomized control trial to explore the chronic PA-BDNF-cognition relationship in sedentary older adults with amnesic mild cognitive impairment. One of the aims of this study was to understand the effect of chronic PA on biomarkers associated with Alzheimer's disease (e.g., BDNF). Participants were randomly assigned to either a high intensity aerobic PA group or a stretching group (control), and performed their activity for 45-60 minutes per day, four days per week. Those in the PA condition performed aerobic PA at an intensity that reached 75-85% heart rate reserve by the sixth week of training. Various modes of PA were allowed (i.e., treadmill, stationary cycle, elliptical machine) and was self-selected by the participant. The first eight training sessions were supervised by the trainer, after which supervision was provided once per week to ensure proper form. Measures of cognitive performance and BDNF were assessed at baseline, three months, and six months. Measures of peak oxygen capacity (VO_2peak) were assessed at baseline and upon completion of the 6-month training period. Each testing session was performed after a 12-hour fast between 8am and 10am. Multiple measures of executive function (i.e., Trail Making Test, Stroop Task, Task

Switching, Verbal Fluency, Symbol Digit Modalities) and memory (i.e., Story Recall, List Learning, Delayed-Match-To-Sample) were used to assess cognition. Results show sex specific effects of chronic PA on cognition, with women in the PA group experiencing improved performance on executive function tasks (i.e., Stroop Task, Symbol Digit Modalities, and Verbal Fluency) compared to those in the stretching group. Additionally, improvements in VO_2peak were associated with improvements in executive function. There were no significant improvements in executive function for men and no significant improvements in memory performance for men or women. Results also indicated that there were no significant changes in BDNF concentration as a result of the chronic PA training. The findings from this study suggest that a 6-month high intensity chronic PA training program in older adults with amnesic mild cognitive impairment may results in sex-specific improvements in executive function. The lack of control over the chronic PA training program hinders the interpretation of these findings. Supervision of the PA performances was not available at each training session, and the mode of PA was not standardized across participants. Therefore, it is not possible to determine whether the results observed were specifically due to the performance of chronic PA training compared to stretching or another factor (e.g., PA modality, improper PA performance). This lack of control may help explain the non-significant change in BDNF concentration and memory performance.

Erickson et al. (2011) performed a randomized control trial to assess the chronic PA-BDNF-cognition relationship in older adults. The design of this study was previously explained in detail. Participants performed the assigned condition (i.e., moderate intensity aerobic PA group, stretching and toning group) three days per week for one year. Measures of cognition, BDNF, and brain volume (MRI) were assessed at baseline, six months, and 12 months. Results indicated that those in the chronic PA group experienced an increase in hippocampal volume while those in the stretching and toning group experienced a decrease in hippocampal volume. Analyses confirmed that those in the PA group experienced greater increases in VO_2max compared to the stretching and toning group, and that larger changes in VO_2max correlated with larger changes in hippocampal volume. Additionally, for those in the stretching and toning group, higher VO_2max scores at baseline were correlated with less severe losses in brain volume over the course of the 1-year study. This suggests that higher levels of aerobic capacity may act as a protective factor against age-related declines in brain volume. Measures of cognition (i.e., spatial memory) improved for both groups. While VO_2max scores were positively correlated with memory performance, changes in VO_2max score from baseline to posttest were not correlated with memory performance. Additionally, hippocampal volume was positively correlated with memory performance, and for those in the chronic PA group, increases in hippocampal volume from baseline to posttest were positively correlated with memory performance. Analyses of BDNF concentrations showed

that there was no difference between groups as a result of the training period. Correlation analyses were performed to assess the association between changes in BDNF and brain volume in both groups. Results showed that changes in BDNF concentration positively correlated with changes in hippocampal volume only for those in the chronic PA group BDNF. Changes in BDNF concentration did not correlate with changes in volume for control neural areas (e.g., caudate nucleus) or for those in the stretching and toning group. However, changes in BDNF concentration did not correlate with memory performance for either group. These results provide strong evidence for the use of chronic PA to increase hippocampal volume in older adults. However, observations showed no difference in cognitive performance between the chronic PA group and stretching and toning group. Thus, this study does not support the use of chronic PA to improve cognitive performance. Additionally, the nature of the statistical analyses used to assess the chronic PA-BDNF-cognition relationship (i.e., simple correlation) provides statistics only suitable for rudimentary inferences.

The mixed findings from the rodent and human literature surrounding the chronic PA-BDNF-cognition relationship illustrate its complex nature. Rodent studies have an advantage over human studies in that an extreme level of control over the subjects may be employed. Additionally, similar measures of PA (i.e., voluntary wheel running), BDNF (i.e., hippocampal tissue), and cognition (i.e., Morris Water Maze) are frequently used between studies. In comparison, human studies often use a variety of PA modalities (i.e., walking, cycling, elliptical),

cognition (e.g., executive function, memory), and BDNF (i.e., serum, plasma). While the differences between rodent and human experimental designs are important to consider when interpreting the results, this may only partially explain the dissimilarity in findings between species. Other factors, including nutrition and participant age should be explored in future research into the effects of chronic PA on basal BDNF concentrations and cognitive performance. Furthermore, the use of statistical mediation techniques and non-PA control groups would improve the ability to infer causality in the chronic PA-BDNF-cognitive performance relationship.

Acute PA

Currently, only eight studies have been performed to assess the acute PA-BDNF-cognition relationship in humans (Etnier et al., *in preparation*; Ferris et al., 2007; Griffin et al., 2011; Lee et al., 2014; Skriver et al., 2014; Tonoli et al., 2014; Tsai et al., 2014; Winter et al., 2007). The justification of assessing this relationship is based on the potential role of BDNF as a mediator in the acute PA-cognition relationship. However, none of the authors performed statistical mediation analyses to test BDNF as a mediator. The overall findings of these studies are mixed in that they support an acute PA-induced increase in BDNF concentration, but only one of the studies observed a significant relationship between PA-induced changes in BDNF concentration and cognition (Winter et al., 2007).

Ferris et al. (2007) performed a study, the design of which was previously described in detail, to assess the role of intensity in the acute PA-BDNF-cognition relationship. Results showed that, while all intensities of PA induced an increase in BDNF concentration, as well as an improvement in the lower-order domain of cognitive processing (i.e., Stroop task: word and color sections), an improvement in the higher-order domain (i.e., executive function) of inhibition (i.e., Stoop task: word-color section) was only observed in the high intensity condition. Changes in BDNF from pre to post PA did not correlate with changes in cognition. These results suggest that while multiple intensity levels of PA benefit lower-order domains of cognition, moderate-high intensity levels benefit higher-order domains. The lack of correlation between BDNF and cognition suggests its lack of involvement with “frontal lobe” tasks. This interpretation is logical since the major area of BDNF expression originates in the medial temporal lobe (i.e., hippocampus). With memory processes being the chief function of the hippocampus, it would be reasonable to assume there would be significant correlations between PA-induced changes in BDNF and cognition as assessed through hippocampal-dependent forms of memory.

Etnier et al. (*in preparation)*, previously described in detail, explored the role of intensity in the acute PA-BDNF-cognition relationship. Results showed that long-term memory was significantly better following an acute bout of maximal intensity PA (i.e., VO_2max) compared to a lower intensity (i.e., 20% below VT). Each condition induced an increase in BDNF concentrations immediately

following the PA, and this concentration returned to baseline levels by the assessments 30 minutes post PA. A significant positive relationship was observed between the post PA maintenance of BDNF concentration (i.e., 30-min post PA BDNF minus immediately post PA BDNF) and long-term memory following the 20% above VT condition. These results suggest that an acute bout of maximal intensity PA benefits long-term auditory memory, and that following an acute bout of high intensity PA, BDNF concentrations within 30 minutes of completing the PA is related to long-term memory performance.

Griffin et al. (2011) performed a study to explore how a 3- or 5-week chronic PA training period affects the acute PA-BDNF-cognition relationship. Participants were assigned to either an acute PA or non-acute PA group and took part in two testing sessions; one prior to and one upon completion of a 3- or 5-week period. During the testing sessions, participants either performed a graded maximal exercise test (VO_2max) on a cycle ergometer or a 30-minute non-acute PA rest period. Cognition was assessed prior to and upon completion of the experimental condition, and BDNF concentration was assessed prior to and upon completion of both cognitive assessments. Measures of relational memory (i.e., Face-Name Matching Task) and executive function (i.e., Stroop Task) were used to assess cognitive performance. The Face-Name Matching Task was performed on a computer and required participants to view ten faces paired with names for 3.5 seconds each. After viewing the entire block of face-name associations, the participants performed a 40-second distraction task to prevent rehearsal of the

previously learned associations (i.e., pushing a button in response to the random presentation of a circle on the screen). Participants were then required to recall the correct name when shown a previously viewed face. Each performance of this task (i.e., four performances total) consisted of novel names and faces. The Stroop Task used in this study differs slight from those previously explained. Participants viewed a series of words (i.e., red, yellow, green, blue) one at a time on a computer screen, and were required to verbally announce the color of the font used to write the word. Congruent word-color combinations (e.g., “yellow” written in a yellow font) were frequently presented while the random presentation of incongruent word-color combinations (e.g., “yellow” written in a red font) was less frequent. After the first testing session, participants from the acute PA group were assigned to either a 3 or 5-week period of chronic PA (i.e., 30-60 minutes of aerobic training 3-5 days per week) or chronic non-PA (i.e., sedentary), while those in the non-acute PA group remained sedentary. Upon completion of the assigned chronic PA or sedentary period, participants took part in a second testing session, the protocol of which matched that of the first testing session. Those who were originally assigned to the acute PA condition at the first testing session performed the acute PA group at the second testing session. Similarly, those assigned to the non-PA group at the first session did not perform the acute PA at the second session. Results from the first testing session (i.e., prior to the 3 or 5-week period) showed that while relational memory performance improved in both the acute PA and non-acute PA groups, those in the acute PA group

experienced the greatest improvement. Acute PA had no effect on the performance of the Stroop task. An increase in BDNF concentration was observed immediately following the acute PA, and by 30 minutes post acute PA, concentrations were not different than those at baseline. Analyses of VO_2max data revealed that only the 5-week period of chronic PA training induced an increase in aerobic capacity, with a 3-week period having no significant effect on measures of aerobic capacity. The results showed that only the 5-week period of chronic PA training had a significant positive effect on memory. However, the authors do not clearly explain whether the pre-training scores were statistically compared to the post-training scores obtained prior to or upon completion of the bout of acute PA (VO_2max). Thus, it is unclear if this effect was elicited from chronic PA training or the acute bout of PA. Measures of executive function were not collected during the post-training period. Both chronic PA training periods affected the response of BDNF to acute PA. Results revealed that acute PA induced an increase in BDNF concentrations following either a 3-week or 5-week period of sedentary behavior. However, acute PA did not induce a change in BDNF concentrations following a 3-week period of chronic PA, and following a 5-week period of chronic PA training, the acute PA-induced change in BDNF appeared 30 minutes post PA. These results indicate that benefits to relational memory may be experienced following an acute bout of maximal PA and a 5-week period of chronic PA training. Additionally, it was observed that an acute bout of maximal PA induces immediate post-PA increases in BDNF

concentrations when preceded by sedentary behavior. No analyses were performed to assess the relationship between BDNF concentrations, PA, and cognitive performance. Therefore, the interpretation of this data is limited in terms of elucidating the role of BDNF in the PA-BDNF-cognition relationship.

Similar to the studies by Etnier et al. (*in* preparation) and Ferris et al. (2007), Winter et al. (2007), performed a within-subjects study to assess the acute PA-BDNF-cognition relationship relative to PA intensity. The design of the study was already described in detail. Concentrations of BDNF were assessed prior to and immediately upon completion of the PA or rest conditions and again after completion of the initial cognitive assessments. The results showed that the vigorous PA intensity condition elicited benefits in learning speed compared to the sedentary and moderate PA intensity conditions. Additionally, exploratory analyses showed that long-term memory assessed at 1-week post was greater following the vigorous PA intensity condition compared to the moderate PA condition. No differences were observed for long-term memory assessed at six to eight months. Analyses of BDNF concentrations revealed significant differences in the baseline concentrations between conditions, with the vigorous PA baseline being higher compared to the moderate PA condition. No statistical techniques were used to account for the differences in BDNF concentration at baseline (e.g., analysis of covariance). Instead, the authors elected to only interpret any significant time by condition interactions. There was a significant quadratic time by condition interaction, and post hoc analyses revealed “stronger changes” in

BDNF concentration across time in the vigorous condition compared to the non-PA condition. An interpretation of the provided figure of the interaction suggests that while BDNF concentrations in the non-PA condition seem to change very little across time, the BDNF concentrations in the vigorous and moderate PA conditions seem to follow an inverted-U (i.e., quadratic) trend with greater concentrations immediately following the PA relative to baseline and upon completion of the cognitive assessment. These results illustrate the transient nature of acute PA-induced increases in BDNF concentration. Additionally, perseverance of BDNF concentrations post-PA (i.e., concentrations following cognitive assessment minus concentrations following PA) was positively correlated with better learning performance in the vigorous condition. These results suggest that factors in the acute PA-BDNF-cognition relationship may act differently across PA intensity levels, with the most benefit to cognition coming from vigorous intensities. Additionally, this study, along with that performed by Etnier et al. (*in preparation*) shows a correlation between post-PA BDNF perseverance and cognitive performance. However, correlational analyses do not allow for the determination of cause-effect relationships. Therefore, more research is needed to elucidate the acute PA-BDNF-cognition relationship in humans.

The interpretation of data from these studies comes with the same challenges previously mentioned in relation to those exploring the chronic PA-BDNF-cognition relationship. The differing aspects of their experimental designs may

explain the equivocal findings. For example, the studies by Etnier et al. (*in* preparation) and Ferris et al. (2007) utilized PA protocols that ensured the performance of precise intensity levels relative to each participant (e.g., percentage of individualized ventilatory threshold), as well as standard durations of PA. This allowed for the assessment of potential dose-response relationships between PA, BDNF, and cognition. However, Etnier et al. (*in preparation*) and Ferris et al. (2007) differ in their use of PA modality (i.e., treadmill, cycle ergometer) and assessment of cognitive domain (i.e., memory, executive function). While Winter et al. (2007) and Griffin et al. (2011) used PA protocols that are not directly comparable, they both utilized measures of relational memory to assess cognition. Importantly, only Etnier et al. (*in preparation*) and Winter et al. (2007) observed significant associations between PA-induced changes in BDNF concentrations and cognitive performance. These findings suggest that the retention of BDNF concentrations post PA is positively associated with improved memory performance.

The translational nature of research exploring the PA-BDNF-cognition relationship is in its infancy. While the application of cognitive assessment tools used in previous studies would help to create a common thread by which to compare future results, it is also logical to select cognitive assessment tools based on their relevance to a study's theoretical foundation. The rodent literature provides the strongest evidence of the mediating role of BDNF in the PA-BDNF-cognition relationship (Vaynman et al., 2004), and both rodent and human

literature have shown positive effects of chronic PA on hippocampal volume (Erickson et al., 2011). Additionally, chronic PA has been shown to increase BDNF mRNA concentrations in the hippocampus (Neeper et al., 1996; Vaynman et al., 2004), while acute PA had been shown to increase BDNF concentration in the periphery. These results support the use of cognitive assessment tools that measure hippocampus-dependent cognitive domains (e.g., relational memory). This type of memory assessment has been used in many rodent studies in the form of spatial learning and memory tasks (e.g., Morris Water Maze, Radial Arm Maze). While there is support in the literature for the positive effect of acute PA on measures of executive function, it is suggested that the exploration of the acute PA-BDNF-cognition relationship would benefit from the use of a cognitive assessment tool that more closely resembles those used in rodent studies and more accurately reflects the theoretical basis of the relationship which it is testing. Specifically, using measures of spatial memory is important in order to logically translate the literature from animal to human studies. The paradigm shift that is evident in the literature from assessing relational processes (i.e., spatial learning and memory) in rodents to assessing non-relational memory processes in humans is not justified and may explain the less consistent results observed in human studies.

The Acute PA-BDNF Isoform-Cognition Relationship

Research has yet to be performed to assess any potential association between PA, BDNF isoform proportions, and cognition. Proportions of BDNF

isoforms have been identified as potential biomarkers for cognitive impairment (Carlino et al., 2011), neural disease (Tongiorgi et al., 2012), and depression (Zhou et al., 2013). Additionally, BDNF isoforms have been shown to dichotomously affect brain form and function through their roles in signaling pathways for long-term potentiation and cell growth (i.e., mBDNF stimulating TrkB receptors) or long-term depression and cell death (i.e., proBDNF stimulating $p75^{NTR}$ receptors). Importantly, recent research has demonstrated that acute PA may change BDNF isoform proportions based on the PA intensity level performed. A 30-minute bout of submaximal acute PA (i.e. at ventilatory threshold) was shown to increase mBDNF levels at 30 and 60 minutes post PA and proBDNF levels at 60 minutes post PA. A graded maximal exercise test (VO_2max) was shown to increase mBDNF immediately post PA, decrease mBDNF at 60 minutes post PA, and increase proBDNF at 30 and 60 minutes post PA (Brunelli et al., 2012). These changes in isoform proportions were assessed in peripheral blood mononuclear cells and the enzyme-linked immunosorbent assay (ELISA) used to assess BDNF concentration in blood serum did not show changes in concentration from either PA intensity. The authors concluded that this is evidence in support of an intensity-dependent cleavage of the BNDF protein. A limitation of this study is that the assessments of BDNF isoform proportions and concentration were performed in two different biological milieux (i.e., peripheral blood mononuclear cells, blood serum). Additionally, the company that produced the ELISA kit used to measure BDNF

concentrations (Promega, Milan Italy) has not performed testing to determine if the kit preferentially assesses one isoform over another (personal communication with Promega technical support via email). From this data, it is not possible to tell if the reported non-significant change in BDNF concentration is detecting one or both isoforms, or to directly determine the relationship between changes in BDNF concentration and isoform proportion. For example, it may be possible that cleavage of BDNF in serum responds differently to PA intensity than peripheral blood mononuclear cells. Assessing both BDNF concentration and isoform proportion in a consistent medium (i.e., blood serum) would aid in deciphering the PA-BDNF relationship. This would allow for the determination of changes in BDNF expression (i.e., proBDNF + mBDNF = expression) and cleavage (i.e., proBDNF → mBDNF) in relationship to PA intensity.

Research has yet to explore the acute PA-BDNF isoform-cognition relationship. It is not known if acute PA-induced changes in BDNF isoform proportion have an effect on cognitive performance. Explorations of the acute PA-BDNF-cognition relationship have relied mainly on measures of the mBDNF isoform (Etnier, et al., *in preparation*; Ferris et al., 2007; Griffin et al., 2011; Winter et al., 2007). However, the companies who manufactured the specific ELISA kits used by Ferris et al. (2007) and Griffin et al. (2011) have not determined the degree to which the kits assess one isoform over another. Given the extreme difference in function between BDNF isoforms, future research

exploring the acute PA-BDNF-cognition relationship should assess BDNF isoform proportions in order to take into account the basic function of the protein.

Summary

Research has shown clear support for the beneficial effect of acute PA on cognitive performance (Chang et al., 2012; Chang, Chu, et al., 2011; Chang et al., 2012; Chang, Tsai, et al., 2011; Coles & Tomporowski, 2008; Etnier et al., in preparation; Labban & Etnier, 2011; Lambourne & Tomporowski, 2010; Pesce et al., 2009). Additionally, evidence suggests that the effect of acute PA on cognition may be depednent on the PA intensity performed and specific cognitive domain assessed (Chang & Etnier, 2009; Etnier et al., *in preparation*; Ferris et al., 2007; Griffin et al., 2011; Winter et al., 2007).

Observations from chronic PA studies provide evidence for potential biological mechanisms that may exist in the acute PA-cognition relationship. Rodent studies have shown that chronic PA alters the form and function of the brain through neurogenesis, synaptogenesis, and angiogenesis (e.g., Black et al., 1990; van Praag et al., 2005) and human studies have shown similar results as assessed by MRI (e.g., Colcombe et al., 2006; Erickson et al., 2010 & 2011). Due to its PA-induced expression (e.g., Neeper et, 1996; Rasmussen et al., 2009, Zoladz et al., 2008) and its connection to learning and memory (e.g., Cirulli et al., 2004; Mu et al., 1999), BDNF is a prime suspect as a biolocial mechanism for the PA-cognition relationship. Additionally, rodent research has provided strong support for the PA-BDNF-cognition relationship (Vaynman et al., 2004).

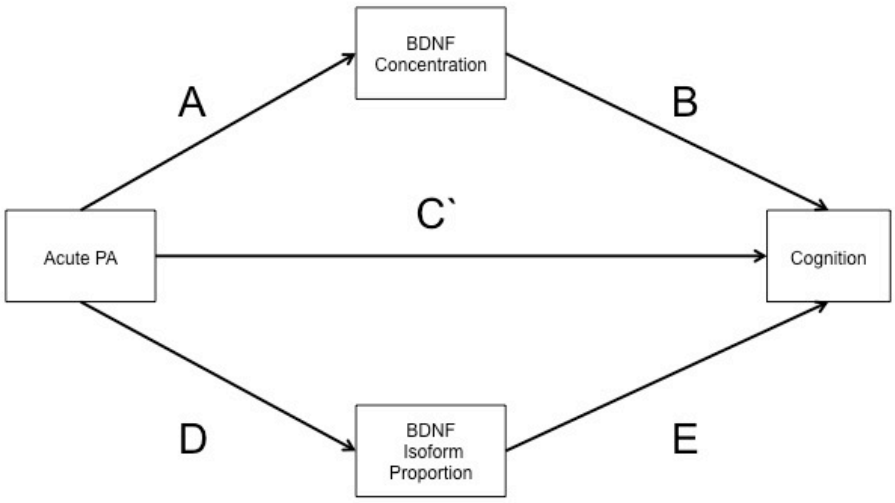
Human research into the PA-BDNF-cognition relationship has been equivocal in both the chronic (e.g., Baker et al., 2010; Erickson, et al., 2011) and acute (Etnier et al., *in preparation*; Ferris et al., 2007; Griffin et al., 2011; Winter et al., 2007) paradigms. However, this may be due to the inconsistency in the selection of neuropsychological tools to assess cognition, as well as a lack of theoretical justification for the selected tools. An additional explaination as to the mixed findings from human studies, is the lack of detailed assessment of the BNDF protein. Currently, reseach has not taken into account the effects of potential PA-induced changes in BDNF isoform proportions on cognition. This is a fundamental gap in the literature as it ignores the basic function of the potential mechanism. Thus, research is needed to help close this gap.

The goal of this research was to increase our knowledge pertaining to one potential mechanism of the acute PA-cognition relationship. The performed research was innovative because it represented a new and substantive departure from the status quo, namely the approach of using analyses to assess the acute PA-BDNF isoform proportion-cognition relationship. This would allow BDNF isoform proportions to be tested as a mechanism for the enhancing effect of acute PA on cognition.

The contribution of the performed research was expected to be the confirmation of a mechanism responsible for the acute PA-induced enhancements in cognition. Gaining an understanding of the mechanisms that enhance cognition is vital to the development of strategies aimed at exploiting

these benefits. The contribution of the performed research is significant because it will facilitate the design of future experiments. Researchers, will be able to build from this work to identify mechanisms that are sensitive to PA and impact specific areas of cognition (e.g., memory) as well as to be able to identify moderators of PA that provide the biggest impact on cognition (e.g., PA intensity).

Figure 1. Theoretical Model



This model (figure 1) illustrates the proposed relationships between acute PA, BDNF concentration, BDNF isoform proportions, and cognition with BDNF concentration and isoform proportions mediating the relationship between acute PA and cognition. Specifically, the indirect effects of acute PA on cognitive performance (AB, and DE) will explain significant portions of the variance in cognition while controlling for the direct effect of acute PA on cognition (C'). This model will be statistically tested to determine the appropriateness of inferring BDNF concentrations and isoform proportions as mediating the relationship betweenacutePAandcognition.

CHAPTER III

METHODS

This purpose of this research was twofold. The primary aims were to examine the potentially intensity-dependent nature of the acute PA-cognition relationship and the acute PA-BDNF relationship. The secondary aim was to assess total BDNF concentration, as well as BDNF isoform proportions, as mediators of the acute PA-BDNF-cognition relationship. The approach used a between-subjects randomized control design to assess BDNF and cognition relative to an acute bout of vigorous intensity PA (vigPA), low intensity PA (lowPA), or control (noPA) condition. Assessments of BDNF were performed prior to (pre), immediately following (post), 30 minutes after (post-30), and 60 minutes after (post-60) each condition. Cognitive performance was assessed immediately following (post) and 30 minutes after (post-30), and 24 hours (post-24) after each condition.

Participants

Healthy male adults between 18-30 years old were recruited for this study from the Greensboro area. Our lab had performed preliminary work (Etnier et al.,

in preparation) to assess the effect of PA intensity on measures of BDNF concentration and verbal memory. The within-subjects study was performed with a sample size of 16 physically active adults and indicated effect sizes for BDNF concentration ($\eta^2_{partial} = .46$) and memory performance as assessed by the Auditory Verbal Learning Task ($\eta^2_{partial} = .20$). With these effect sizes, power analyses were performed using G*Power (version 3.1) and determined an appropriate sample size to obtain a power of .80. Results suggested adequate sample sizes for BDNF concentration (n=12) and memory (n=42). Therefore, it was determined that a sample size of N=60 would provide more than adequate power. However, this power analysis was based only on measures of BDNF concentration, not isoform distribution. As this work was the first, to our knowledge, to assess the relationship between PA intensity, BDNF isoforms, and cognition, additional information was needed in order to perform a more robust power analysis, and that was one of the outcomes that resulted from this study. Participants were required to be healthy enough to perform a maximum exercise test. Therefore, inclusion criteria required that participants' responses on the AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire indicated that they were at "low risk" according to the American College of Sports Medicine guidelines (ACSM, 2014). Additionally, participant's responses on the National Health and Nutrition Examination Survey (NHANES) were required to indicate that they met the American College of Sports Medicine's recommendation of performing at least 150 minutes of moderate intensity PA per

week. These requirements were to ensure the safety of the participants and minimize negative emotional reactions to the maximal exercise test, which might have been observed in persons who were less physically active.

Cognitive Assessments

Past studies exploring the acute PA-cognition relationship and the acute PA-BDNF-cognition relationship have utilized a variety of cognitive assessments and have assessed multiple cognitive domains. In order to allow findings from this study to be directly related back to previous findings from the literature, as well as assess additional aspects of cognitive performance, this study used multiple neuropsychological tools to assess cognitive performance.

Memory

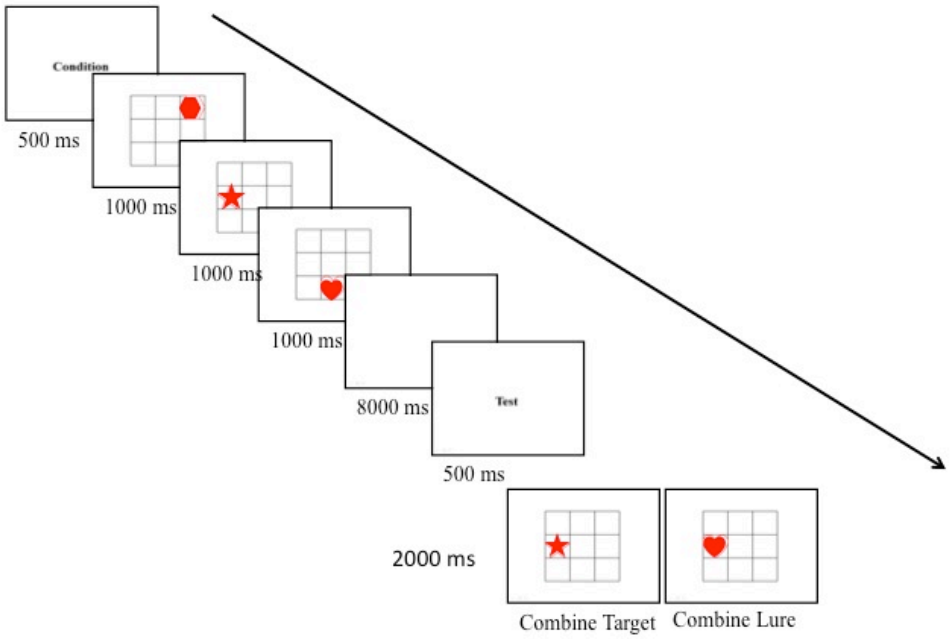
Relational memory was assessed with a computerized memory task (Figure 2.). This task required participants to remember how specific shapes related to specific spatial locations on a 3X3 grid. Participants were required to view various object-location combinations on a computer screen, and then as quickly as possible, to identify an object-location combination as either new (i.e., not previously viewed) or old (i.e., previously viewed). This type of task required the memory of multiple aspects of a stimulus (i.e., spatial and object) and performance of this task has been shown to require hippocampal activity. To complete the task, participants performed 32 memory trials. This number of trials was selected based on previous research (Mitchell, Johnson, Raye, & D'Esposito, 2000). Each trial (figure 2) began with a cue presented on the

computer screen (i.e., “object + location”) for 500 milliseconds (ms). Three different objects from a group of 8 possible objects (e.g., hexagon, heart, cross, circle, diamond, star, rectangle, or triangle) were then sequentially presented in three different locations on a 3X3 grid, excluding the center of the grid, for 1,000ms each. Participants then viewed a blank screen for 8,000ms and a second cue screen (i.e., “READY”) for 500ms. Finally, participants viewed a screen for 2000ms that presented an object-location combination that either was (i.e., target) or was not (i.e., lure) previously viewed in the current memory trial. Participants were required to identify targets and lures by pressing the “P” and “Q” keys on the keyboard, respectively, as quickly as possible. Collection of responses continued through the viewing of a blank 2000ms intertrial interval screen. Performance was assessed by accuracy of memory (i.e., number of correctly identified object-location combinations divided by total trials) and reaction time (i.e., total time [ms] to respond). The entire memory protocol (i.e., 32 trials at 16 seconds per trial) took approximately nine minutes to complete.

The Rey Auditory Verbal Learning Task was used to assess short-term and long-term memory. This task has been previously used in a study exploring the acute PA-BDNF-cognition relationship (Etnier et al., *in preparation*). Only seven studies have explored the PA-BDNF-cognition relationship, each using a different measure of cognitive performance. Variations between studies, such as cognitive measures, have hindered the ability to directly compare findings between

studies. Therefore, inclusion of the Auditory Verbal Learning task allows for a direct comparison with the study performed by Etnier et al. (*in preparation*).

Figure 2. Relational Memory Trial



This figure (figure 2) illustrates an example of a single trial in the computerized relational memory task. In this figure, the frames have been identified as "Combine Target" and "Combine Lure" to illustration purposes.

To perform the task, participants completed five learning trials, one distraction trial, one short-term memory trial, and two long-term memory trials. A learning trial was comprised of participants hearing a pre-recorded list of 15 words (list A) presented at the rate of one word per second, and then verbally recalling as many words from the list as possible within a 2-minute period. This was repeated four additional times to total five learning trials. The distractor trial was comprised

of participants hearing a pre-recorded list of 15 different words (list B) presented at the rate of one word per second, and then verbally recalling as many of these new words as possible. The short-term memory trial took place immediately after the distractor trial, and required participants to recall as many words as possible from list A without hearing them again. The long-term memory trials took place after a 30-minute and 24-hour period, and required the participants to recall as many words as possible from list A without hearing them again. Learning performance was calculated by summing the number of correctly recalled words across the five learning trials. Short-term and long-term memory performance was operationalized as the number of correctly recalled words in each trial. Completion of this protocol will took approximately 15 minutes.

Experimental Protocols

Apparatus & Tools

A LODE Corival Recumbent Cycle-Ergometer (Lode BV, Groningen, The Netherlands) was used for the physical activity. The cycle was equipped with a dual-screen display that provides information to the participant as well as the researcher. The screen facing the seat of the cycle showed participants information pertaining to the total time and distance (kilometers) cycled as well as their cadence (i.e., rotations per minute). The screen facing away from the seat allowed the researcher to assess and adjust the resistance load (i.e., watts) as well as assess cycling cadence. When synched to a Polar heart rate monitor, this display also allowed the researcher to assess heart rate.

Polar heart rate monitors and T-31 coded chest straps (Polar USA) were used to assess heart rate. The T-31 chest strap automatically synchs with the LODE cycle to allow measures of heart rate to be assessed directly from the dual screen display as well as a wrist-watch computer. This was a benefit to the researcher as it increased the efficiency of collecting heart rate data while monitoring the participants' performance.

The Borg Ratings of Perceived Exertion scale was used as a measure of the participants' subjective assessment of PA intensity. This valid and reliable 15-point scale (Borg, 1990) consisted of scores that range from 6 (no exertion at all) to 20 (maximal exertion). This tool was easy for the participants to use, even while performing PA at very high intensities, and is a standard measurement tool of subjective PA intensity in the exercise literature.

A SensorMedics metabolic cart (Vmax, SensorMedics, Yorba Linda, CA) was used to assess resting and maximal aerobic capacity (i.e., VO_2resting, VO_2max) by measuring air exhaled by the participants. Assessment of VO_2resting was obtained by having the participants sit on the cycle without pedaling, for a 5-minute rest period. Assessment of VO_2max was obtained by having the participants perform a graded cycling test. VO_2max was determined when at least two of three criteria have been reached (RER > 1.1, plateau in VO_2, RPE > 17) (ACSM, 2014). Measures of VO_2resting and VO_2max were used to determine individual VO_2reserve scores for each participant (VO_2reserve = VO_2max - VO_2resting). VO_2reserve scores was in turn used to define the intensity levels of

subsequent PA conditions (i.e., lowPA: 35% VO_2reserve [± 5%], vigPA: 85% VO_2reserve [± 5%]).

VO_2max & PA Protocols

Testing took place on two days, separated by at least 72 hours. During the period between session one and session two, participants were told to continue their normal pattern of PA. Therefore, activities which took place between sessions were not controlled. In order to control for diurnal and dietary variations, all testing took place in the morning, after a 10-12-hour fast, and all participants were instructed to "try to get 8 hours of sleep" during the previous night. Due to observations taking place in the morning, the Pittsburgh Sleep Index questionnaire was used to assess and differences in quality of sleep between assigned treatment conditions. During session one, participants read and signed an informed consent approved by the Institutional Review Board and filled out a short demographics questionnaire. Next, measures of height and weight were assessed, and participants were instructed on how to wear the heart rate monitor. Then, participants sat on a reclined seat while an IV was placed into an arm vein. Next, participants were instructed on how to use the Borg RPE scale. The seat of the cycle as adjusted so that the participant's leg was straight when the heel of their foot was on the pedal. This ensured the proper distance when the ball of the foot was placed on the pedal. Then, the facemask from the SensorMedics metabolic cart was fit onto the participant. The participant

remained seated for a 5-minute period in order to obtain assessments of resting heart rate and VO_2resting.

Participants then performed a graded maximal exercise test (VO_2max) to exhaustion on a recumbent cycle ergometer. The VO_2max test protocol was an adapted version of the YMCA cycle ergometer protocol (Golding, Myers, & Sinning, 1989) and consisted of a warm-up, test period, and cool-down. The warm-up consisted of peddling at a cadence equal to or greater than 50 rotations per minute (RPM), against a resistance load of 25 watts, for a total of 3.5 minutes. The test period consisted of peddling at a cadence equal to or greater than 50RPM, against a resistance load of 100 watts, for the first two minutes. Every two minutes, the resistance load was increased by 50 watts. The test continued until the participant was unable to continue peddling at a cadence equal to or greater than 50RPM. The cool-down consisted of peddling at a self-selected cadence, against a resistance load of 25 watts for 5 minutes. Measures of heart rate and ratings of perceived exertion were assessed at 1-minute intervals during warm-up and cool-down and 2-minute intervals during the test period. Data from the VO_2max test was used to identify the appropriate intensity level for the subsequent PA condition (lowPA: 35% VO_2reserve [$\pm$ 5%], vigPA: 85% VO_2reserve [$\pm$ 5%]) and assess potential differences in the acute PA-induced BDNF response between participants.

Participants were randomly assigned to one of three PA conditions (i.e., noPA, lowPA, vigPA), were told which condition they would be performing after

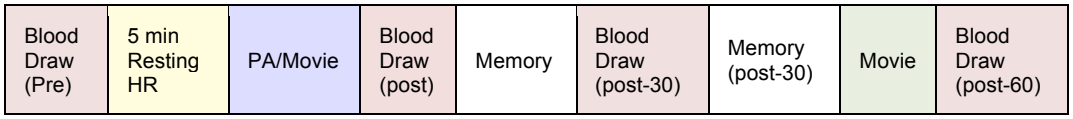
the first blood draw of session two was completed. Research has shown PA durations longer than 20 minutes to elicit the greatest cognitive benefits (Chang et al., 2012). Therefore, the PA protocols consisted of a 5-minute warm-up, a 25-minute bout of low or vigorous intensity PA, and a 5-minute cool-down. The warm-up began with a resistance load of 25 watts, and increased at 2-minute intervals until the participant reached the target intensity (lowPA: 35% VO_2reserve [$\pm$ 5%], vigPA: 85% VO_2reserve [$\pm$ 5%]) (ACSM, 2013). The participant performed the target intensity PA for 25 minutes and then performed a 5-minute cool-down at a self-selected cadence at 25 watts. Measures of heart rate and rating of perceived exertion were assessed at 1-minute intervals during warm-up and cool-down, and 5-minute intervals during the target intensity period. The noPA condition consisted of watching a nature documentary for 35 minutes (control). Measures of heart rate were assessed at 1-minute intervals for five minutes, at 5-minute intervals for 25 minutes, and 1-minute intervals for five minutes. This will be done in order to directly compare measures of heart rate from the three conditions.

Blood Draws and BDNF Assessment Protocol

Blood samples were drawn from venipuncture (i.e., by placing a small, flexible plastic catheter) at four time points during each session (i.e., pre, post, post-30, post-60) (See Figure 3). Each blood draw took approximately five minutes. Each sample was approximately 10mL a total of 40mL per session, and a grand total of 80mL. Blood was collected in serum separator tubes, allowed to clot for at

least 20 minutes, centrifuged at 3000 X g for 20 minutes, and the serum as aliquoted and stored in a -80 degree C freezer.

Figure 3. Session Map



Concentrations of mBDNF were assessed using enzyme-linked immunosorbent assay (ELISA) kits developed by Aviscera Bioscience INC and following the procedures recommended for each kit. While other companies sell ELISA kits to assess BDNF, Aviscera Bioscience manufactures the only commercially available ELISA kits designed to specifically assess the proBDNF and mBDNF isoforms with no cross-reactivity between isoforms. Other commercially available kits either measure mBDNF with an established 13% cross-reactivity with proBDNF (R&D Systems, Quantikine Human BDNF Assay), or have not empirically tested the levels of isoform cross-reactivity (Millipore, ChemiKine Brain Derived Neurotrophic Factor Sandwich ELISA; Promega, BDNF Emax ImmunoAssay System). The kits manufactured by Aviscera Bioscience INC have been used in previous studies assessing BDNF isoform concentrations (Yoshida, Ishikawa, Iyo, & Hashimoto, 2012; Yoshida, Ishikawa, Niitsu, et al., 2012). However, due to faulty manufacturing, concentrations of proBDNF could not be assessed using the proposed Aviscera Bioscience INC ELISA kits. Even though the entire lot of purchased kits was specifically produced for this study,

passed the quality control standards set forth by Aviscera Bioscience INC, was properly stored, and serum samples were analyzed prior to the expiration date the inconsistency and poor quality of resulting data indicated problems with the standards. The proBDNF kits have been returned to Aviscera Bioscience INC and we are currently awaiting communication from them concerning the creation, integrity, and shipment of a new lot of kits. Upon receiving the new lot of kits, serum samples will be assessed for concentrations of proBDNF, and this data will be included into the analyses of this study. As Aviscera Bioscience INC is currently the only commercial producer of proBDNF ELISA kits, no other option exists for obtaining alternative ELISA kits.

Upon receiving and performing analyses using the proBDNF kits, additional assessments will be possible, including assessments of total BDNF concentration (proBDNF + mBDNF = total BDNF), as well as individual BDNF isoform concentrations being converted into a single score representing the proportion of mBDNF to the total concentration of both isoforms. This will be done by taking the mBDNF concentration and dividing it by the sum of proBDNF and mBDNF concentrations. This will provide a number that represents the proportion of mBDNF relative to total BDNF concentrations.

Safety Procedures

To ensure the safety of the researchers and participants during the study, all researchers involved with PA sessions were CPR/AED certified, an AED was be accessible at all times, and a cellphone was available to call campus

police/paramedics if needed. Water was be available for consumption. Researchers obtained training and demonstrate proficiency in phlebotomy prior to collecting blood samples from the participants.

Cognitive Task Protocol

Research has shown that the largest cognitive benefits from acute PA are observed within the first 15 minutes following the end of the PA (Chang et al., 2012). Therefore, cognitive assessment took place immediately following completion of the blood draws at the post PA time point. The order in which the cognitive tasks were performed remained consistent between conditions. First, the Auditory Verbal Learning Task learning trials and short-term memory trial were performed (performance time: approx. 11 minutes). There was a 30-minute waiting period that must be performed before conducting the first long-term memory trial. During this time the relational memory task was performed (performance time: approx. 10 minutes). Participants sat on the cycle and watched a nature documentary until the end of the session. The final long-term memory trial was performed over the phone following a 24-hour waiting period.

Statistical Analyses

Statistical analyses were performed using SPSS (V. 22.0). Where appropriate, Mauchly's test of sphericity was utilized to ensure sphericity assumptions were met. If these assumptions were not met, a Huynh-Feldt adjustment was used for degrees of freedom. Upon observing statistical significance, Tukey's HSD post-hoc pairwise comparison was conducted.

Sample Characteristics

A one-factor Analysis of Variance (ANOVA) was performed to assess differences in demographic (i.e., age, BMI), fitness (i.e., VO_2max), PA behavior (i.e., 2-week PA history) and sleep quality (i.e., 1-month sleep history) between conditions.

Manipulation Check

A 3X4 (condition by time) ANOVA was performed to assess differences in HR levels between treatment conditions, and a 2X4 (condition by time) ANOVA was performed to assess differences in RPE levels between PA conditions. Averages from the 5-minute warm-up, 25-minute treatment condition (i.e., PA, control), and 5-minute cool-down periods were used to assess differences in objective and subjective intensity across time as a function of condition. Separate one-factor ANOVAs were performed to assess differences in workload: total kilometers (KM) and maximum Watts between conditions. A 2X2 (workload by condition) ANOVA was performed to assess differences between target workload (i.e., Watts) that was assigned and actual workload that was performed. A 2X2 (workload by condition) ANOVA was performed to assess differences between HR experienced at target workload and performed workload.

BDNF Concentrations

Two separate 3X4 (condition by time) repeated-measures ANOVAs were performed to assess differences in mBDNF concentrations between treatment

conditions and across the four assessments (pre, post, post-30, post-60) for both sessions.

Cognitive Performance

For the Relational Memory task, two separate one-factor ANOVAs were performed to assess differences in memory accuracy (i.e., number of correctly identified object-location combinations divided by total trials) and reaction time for correct trials (i.e., average total time [ms] for correct responses). For the Auditory Verbal Learning Task, a 3X5 (condition by time) repeated measures ANOVA was performed to assess learning (i.e., summation of words recalled in each Trial, 1-5). Two separate one-way ANOVA's were performed to assess primary measures of short-term memory (i.e., words recalled in Trial 7) and secondary measures of short-term memory (words recalled in Trial 6). A 3X2 (condition by time) repeated measures ANOVA was performed to assess long-term memory recall (i.e., words recalled in Trials 8 [post-30min] and 9 [post-24hr]), and a one-way ANOVA was performed to assess long-term memory recognition (i.e., number of words recognized post-24hr).

CHAPTER IV

RESULTS

Sample Characteristics

Results from the analyses showed non-significant differences in age, BMI, 2-week PA history, Sleep Quality, or VO_2max between conditions ($p>.05$). This confirms the success of the random assignment to treatment conditions. See Table 1 for means, standard deviations, and F statistics.

Table 1. Differences in Sample Characteristics Between Conditions

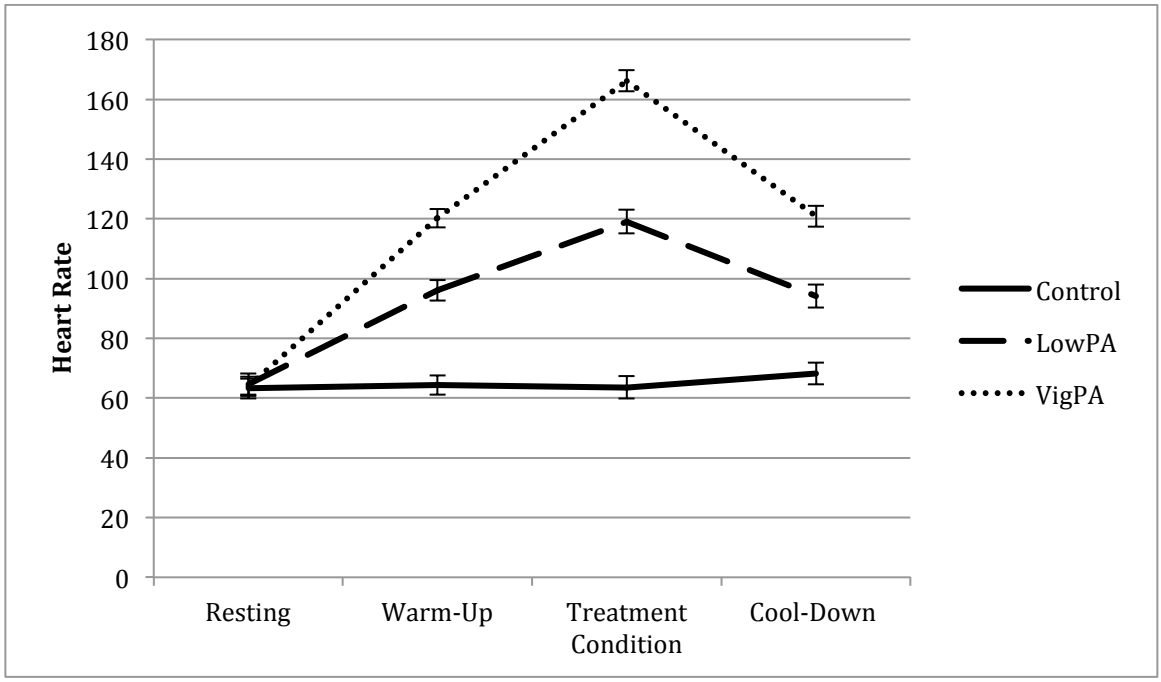
	Control Condition (N=11)		LowPA Condition (N=8)		VigPA Condition (N=10)				
Variable	Mean	SE	Mean	SE	Mean	SE	df	F	p
Age	21.18	.882	22.26	1.13	21.70	.967	2	.355	.705
BMI	26.39	1.88	27.54	2.04	24.01	.887	2	1.24	.340
PA Hist.	8.75	1.40	5.79	.993	6.34	.897	2	1.87	.174
Sleep	5.64	.691	5.00	.681	4.40	.733	2	.821	.451
VO_2max	36.08	1.34	37.80	2.50	39.46	2.17	2	.803	.459

PA Hist= 2-week physical activity history; Sleep=Sleep Quality (Pittsburgh Sleep Index)

Manipulation Check

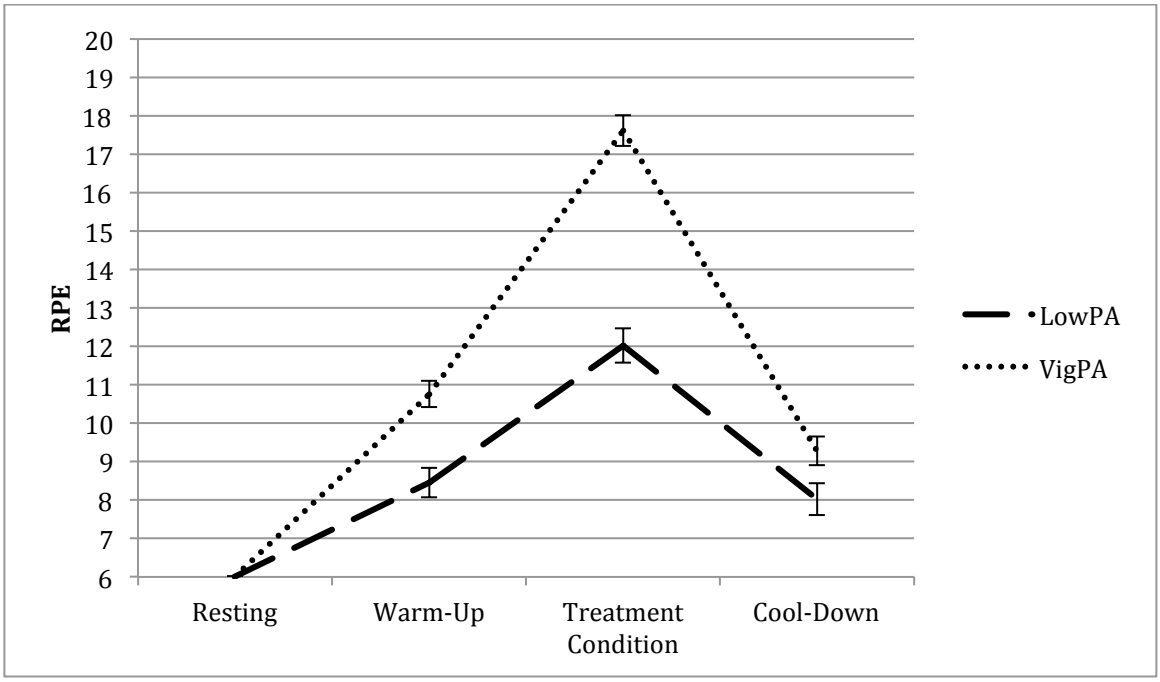
Results from the analyses of HR during session two (i.e., treatment conditions) showed a significant main effect of time ($F_{2.181,52.342}$=344.711, p<.001, $\eta^2_{partial}$ = .935) and condition ($F_{2,24}$=79.657, p<.001, $\eta^2_{partial}$ = .869), as well as a significant time by condition interaction ($F_{4.362,52.342}$=114.967, p<.001, $\eta^2_{partial}$ = .905) (See Figure 4). Additionally, HR was significantly lower for those in the control condition (Mean[SE]=67.289[3.753]) compared to those in the lowPA (Mean[SE]=85.890[3.510]) (p=002) and vigPA conditions (Mean[SE]=102.955[3.510]) (p<.001), while those in the lowPA condition had significantly lower HR compared to those in the vigPA condition (p=.003).

Figure 4. PA Manipulation Check – HR



Results from the analyses of RPE showed a significant main effect of time ($F_{3,48}$=292.956, p<.001, $\eta^2_{partial}$ = .948) and condition ($F_{1,16}$=56.629, p<.001, $\eta^2_{partial}$ = .780), as well as a significant time by condition interaction ($F_{3,48}$=30.781, p<.001, $\eta^2_{partial}$ = .658). A comparison of means shows that RPE was significantly lower for those in the lowPA condition (Mean[SE]=8.625[.227]) compared to those in the vigPA condition (Mean[SE]=10.915[.203]) (See Figure 5).

Figure 5. PA Manipulation Check – RPE



Results from the analyses of workload showed significant differences between conditions ($F_{1,16}$=21.634, p<.001) in measures of total KM, with those in the LowPA condition completing fewer KM (Mean[SE]=12.050[1.010]) than those in the VigPA condition (Mean[SE]=18.280[.885]). There was a significant difference

in maximum Watts between conditions ($F_{1,16}$=25.908, p<.001), with those in the LowPA condition reaching a lower max Watts (Mean[SE]=113.125[10.262]) compared to those in the VigPA condition (Mean[SE]=196.200[12.040]).

Results from the analyses of differences between target Watts that were assigned and Watts that were actually performed by condition showed a significant main effect of workload ($F_{1,16}$=253.973, p<.001, $\eta^2_{partial}$ = .941) and condition ($F_{1,16}$=36.152, p<.001, $\eta^2_{partial}$ = .693), as well as a significant workload by condition interaction ($F_{1,16}$=208.544, p<.001, $\eta^2_{partial}$ = .929). Comparisons of means of target Watts with performed Watts showed that target Watts were higher than performed Watts for those in the VigPA condition (target: Mean[SE]=222.500[9.458]; performed: Mean[SE]=164.100[8.951]) but target Watts and performed Watts were similar for those in the LowPA (target: Mean[SE]=112.500[10.574]; performed: Mean[SE]=109.625[10.007]).

Results from the analyses of HR at target Watts and HR at performed Watts by condition and workload showed a non-significant main effect for workload ($F_{1,16}$=0.484, p=.497, $\eta^2_{partial}$ = .029), a significant main effect of condition ($F_{1,16}$=80.590, p<.001, $\eta^2_{partial}$ = .834), and a non-significant workload by condition interaction ($F_{1,16}$=1.514, p=.236, $\eta^2_{partial}$ = .086). Comparisons of means showed no differences in HR as a function of workload (i.e., difference between HR at target Watts assigned and actual Watts performed) for the LowPA (target: Mean[SE]=118.125[4.472]; performed: Mean[SE]=119.025 [4.066]) or VigPA

(target: Mean[SE]=169.400[3.999]; performed: Mean[SE]=166.160[3.637]) conditions.

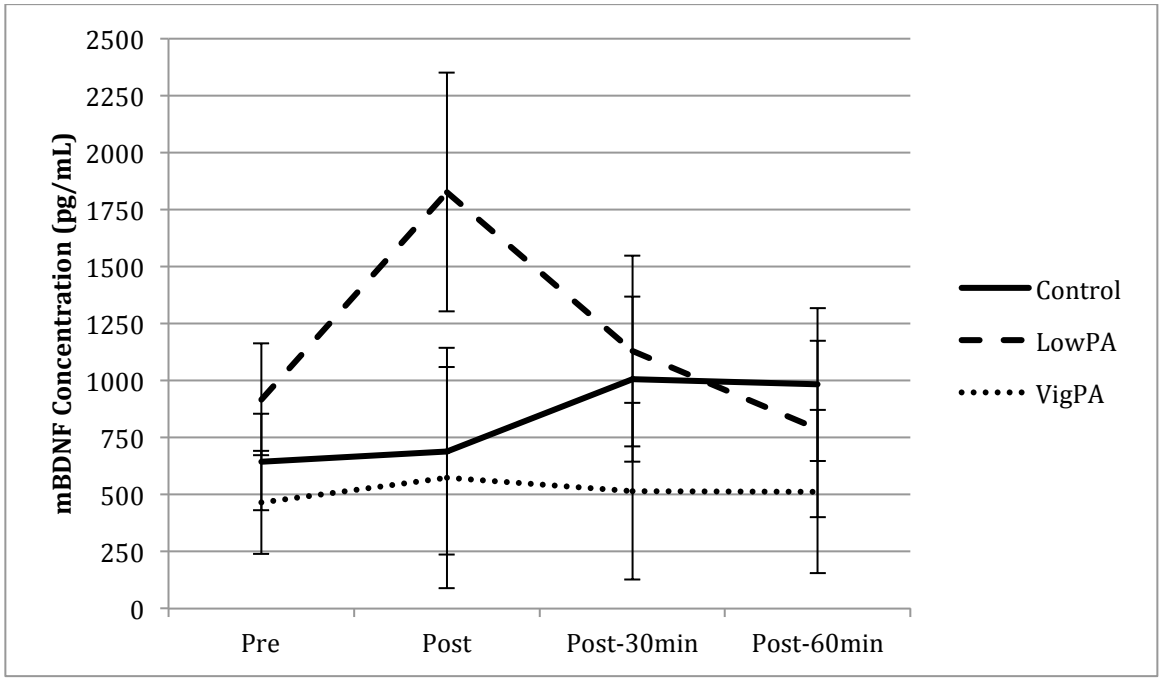
BDNF Concentrations

Results from analyses exploring mBDNF concentration for session one (i.e., VO_2max) showed a non-significant main effect of time ($F_{1.832, 32.968}$=1.718, p=.197, $\eta^2_{partial}$ = .087) and condition ($F_{2, 18}$)=.879, p=.432, $\eta^2_{partial}$ = .089). Additionally, results showed a non-significant time by condition interaction ($F_{3.663, 32.968}$=2.100, p<.151, $\eta^2_{partial}$ = .189) (See Figure 6). See Table 2 for means and standard errors.

Table 2. mBDNF Means and Standard Errors - Session 1: VO2max

	Control Assignment		LowPA Assignment		VigPA Assignment	
Time	Mean	SE	Mean	SE	Mean	SE
Pre	643.591	211.908	917.76	244.69	465.683	226.539
Post	690.018	453.667	1827.008	523.849	574.61	489.99
Post-30	1007.053	362.476	1130.029	418.552	514.648	387.504
Post-60	983.024	335.444	788.488	387.338	513.551	358.605

Figure 6. mBDNF Concentrations - Session 1: VO2max



Results from analyses exploring mBDNF concentrations for session two (i.e., treatment condition) showed a non-significant main effect of time ($F_{1.892,30.278}$=2.656, p=.089, $\eta^2_{partial}$ = .142) and condition ($F_{2,16}$=.848, p=.447, $\eta^2_{partial}$ = .096), as well as a non-significant time by condition interaction ($F_{3.785,30.278}$=1.419, p=.253, $\eta^2_{partial}$ = .151) (See Figure 7). See Table 3 for means and standard errors.

Relational Memory

Results showed non-significant differences in memory accuracy ($F_{2,24}$=1.102, p=.348, $\eta^2_{partial}$ = .084) or reaction time ($F_{2,24}$=.751, p=.483, $\eta^2_{partial}$ = .059) between conditions. See Table 4 for means and standard errors.

Table 3. mBDNF Means and Standard Errors - Session 2: Treatment

	Control		LowPA		VigPA	
Time	Mean	SE	Mean	SE	Mean	SE
Pre	949.794	253.601	830.659	320.782	323.663	292.833
Post	783.826	236.683	882.053	299.383	433.325	273.298
Post-30	791.409	358.44	1195.503	453.394	385.742	413.89
Post-60	544.293	161.594	619.933	204.403	369.728	186.593

Auditory Verbal Learning & Short-Term Memory

For measures of learning, results showed a significant main effect for time ($F_{4,96}$=116.117, p<.001, $\eta^2_{partial}$ = .829), a non-significant main effect for condition ($F_{2,24}$=1.070, p=.359, $\eta^2_{partial}$ = .082), and a non-significant time by condition interaction ($F_{8,96}$=.1.183, p=.317, $\eta^2_{partial}$ = .090) (see Figure 8).

For measures of primary short-term memory (i.e., Trial 7) results showed a non-significant difference between conditions ($F_{2,24}$=.710, p=.502, $\eta^2_{partial}$ = .056). For measures of secondary short-term memory (i.e., Trial 6) results showed a significant difference in performance between conditions ($F_{2,24}$=4.340, p=.025, $\eta^2_{partial}$ = .266). Post hoc analyses indicated that those in the lowPA condition performed significantly better (i.e., recalled more words) than those in the control (p=.049) or vigPA conditions (p=.035) and there was a non-significant difference between those in the vigPA condition and control condition (p=.995) (See Figure 9). See Table 4 for means and standard errors.

Figure 7. mBDNF Concentrations - Session 2: Treatment

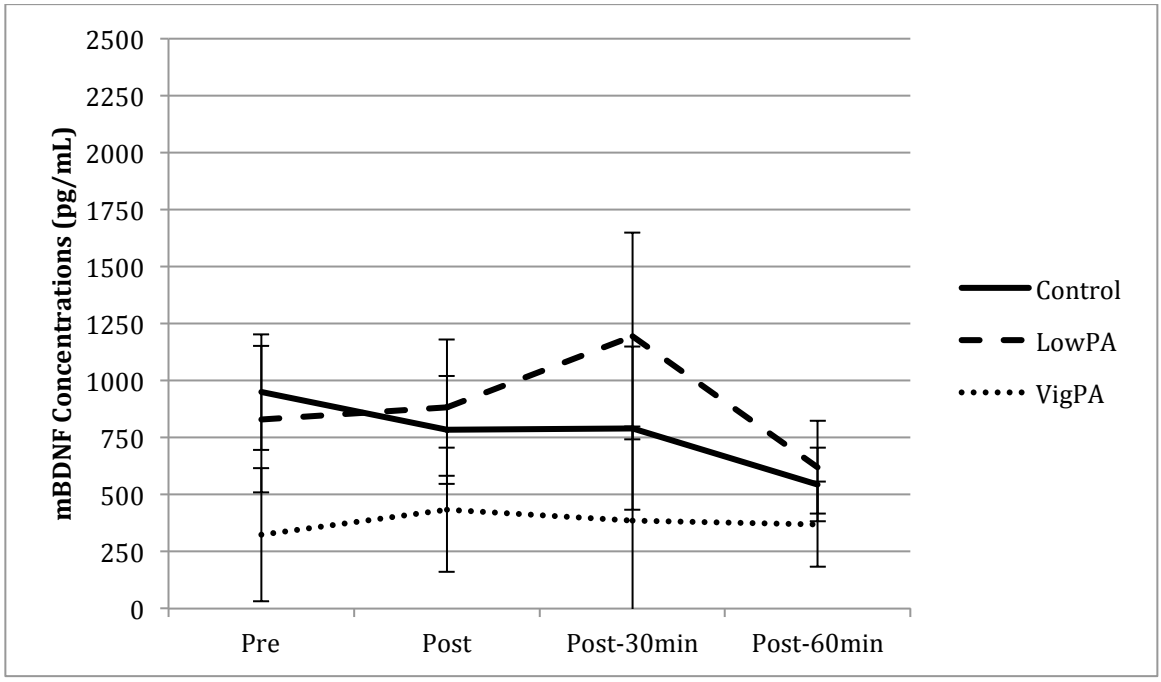


Figure 8. AVLT Learning Trials

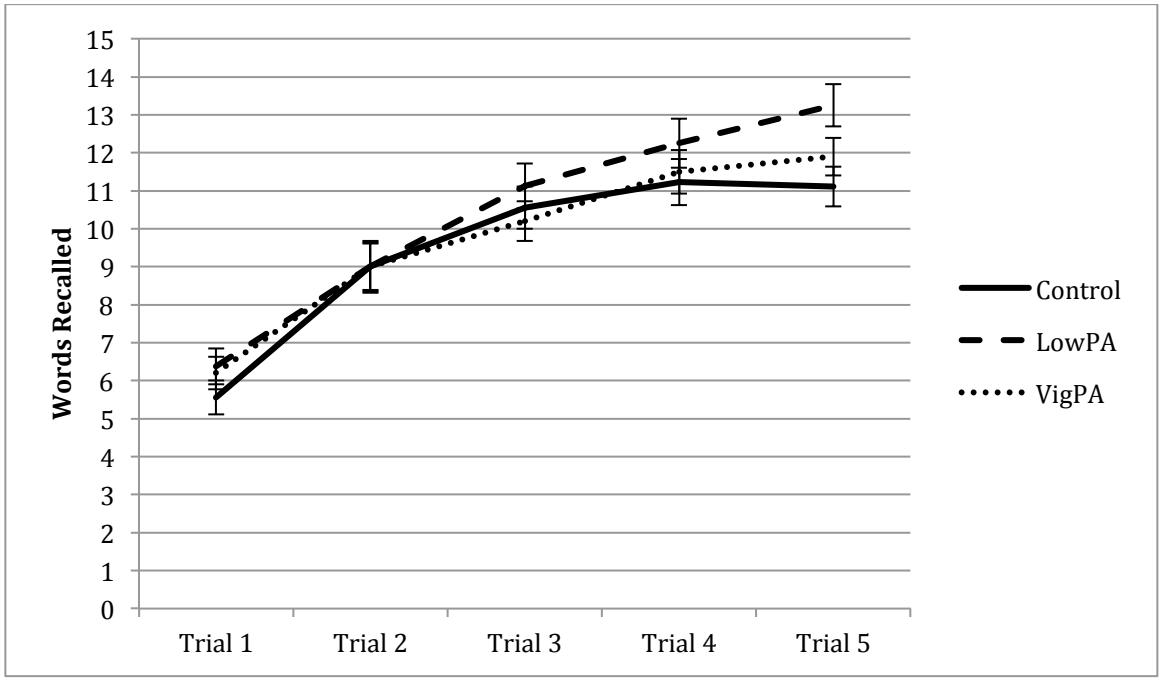
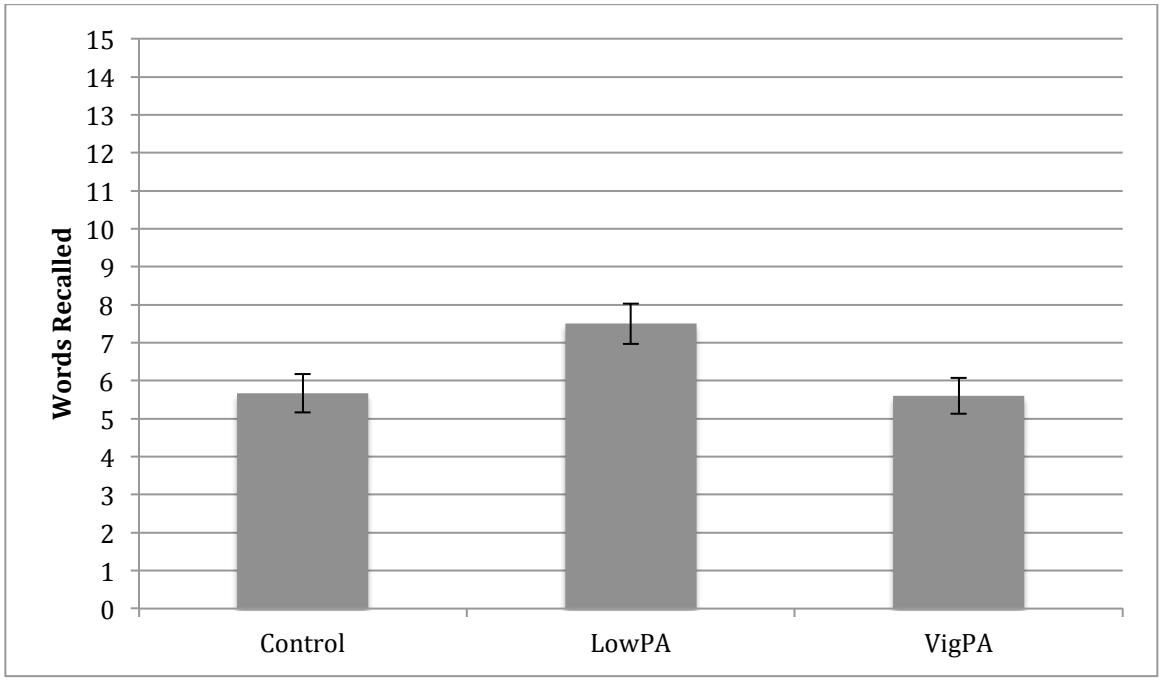


Figure 9. Short-Term Memory: AVLT Trial 6 (List B)

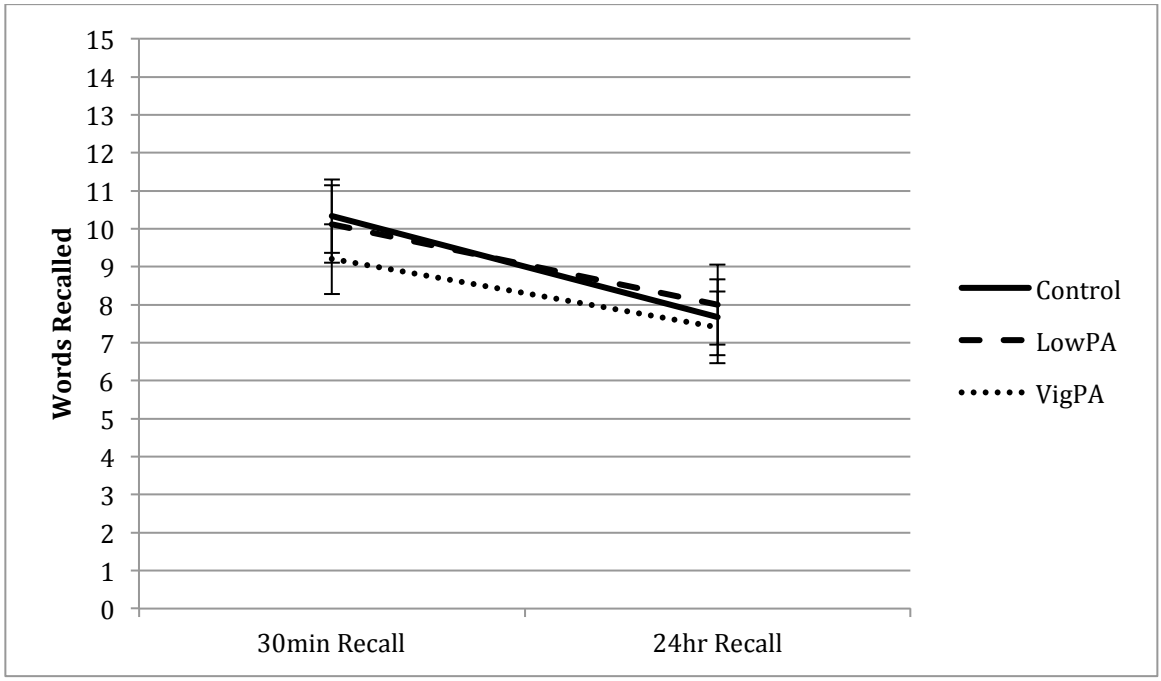


Auditory Verbal Long-Term Memory

For long-term memory recall (i.e., 30min, 24hr), results showed a significant main effect for time ($F_{1,24}$=64.003, p<.001, $\eta^2_{partial}$ = .727), a non-significant main effect for condition ($F_{2,24}$=.206, p=.815, $\eta^2_{partial}$ = .017), and a non-significant time by condition interaction ($F_{2,24}$=.891, p=.423, $\eta^2_{partial}$ = .069) (see Figure 10). See Table 4 for means and standard errors.

For long-term memory recognition, results showed a non-significant difference in performance between conditions ($F_{2,24}$=1.852, p=.179, $\eta^2_{partial}$ = .134). See Table 4 for means and standard errors.

Figure 10. Long-Term Memory: AVLT 30min & 24hr Recall



.

Table 4. Means and Standard Errors: Cognitive Tasks

	Control (N=9)		LowPA (N=10)		VigPA (N=8)	
Variable	Mean	SE	Mean	SE	Mean	SE
Relational Memory						
Accuracy	.854	.030	.918	.031	.878	.028
RT	938.302	56.252	919.000	59.665	1010.128	53.366
AVLT						
Trial 1	5.556	.450	6.375	.477	6.2	.427
Trial 2	9.000	.645	9.000	.685	9.000	.612
Trial 3	10.556	.556	11.125	.589	10.200	.527
Trial 4	11.222	.607	12.250	.644	11.500	.576
Trial 5	11.111	.524	13.250	.556	11.900	.497
Trial 6	5.667	.502	7.500	.532	5.600	.476
Trial 7	10.556	.726	11.125	.771	9.900	.689
30 min Recall	10.333	.968	10.125	1.027	9.200	.919
24hr Recall	7.667	.996	8.000	1.057	7.400	.945
24hr Recognition	29.333	1.474	32.250	1.564	28.300	1.399

CHAPTER V

DISCUSSION

The primary purpose of this research was to explore the acute PA-cognitive performance relationship in a dose-response (i.e., intensity-dependent) paradigm and to use molecular measures (i.e., BDNF concentration) to obtain data for the performance of statistical tests of mediation. An additional purpose of this study was to extend the literature base by exploring the potential intensity-dependent effect of acute PA on measures of specific BDNF isoform concentrations (i.e., proBDNF and mBDNF). A between-subjects randomized control design was used to assess memory performance and BDNF isoform concentrations relative to an acute bout of PA at either a low (35% VO_2reserve [$\pm$ 5%]) or vigorous (85% VO_2reserve [$\pm$ 5%]) intensity or a non-exercise control condition (i.e., watching a movie). Measures of BDNF were also assessed relative to an acute bout of maximal intensity PA (i.e., VO_2max test). Measures of BDNF were assessed prior to (pre), immediately following (post), 30 minutes after (post-30), and 60 minutes after (post-60) each condition. Measures of memory were assessed immediately following post), 30 minutes after (post-30), and 24 hours (post-24) after each condition.

The literature base provides strong support for the beneficial effect of acute PA on measures of cognitive performance as well as an acute PA-induced increase in BDNF concentrations. This evidence comes from individual studies in humans (Chang, Tsai, et al., 2011; Etnier et al., 2014; Ferris et al., 2007; Griffin et al., 2011; Kamijo et al., 2009; Labban & Etnier, 2011; Pesce et al., 2009; Piepmeier et al., 2015; Winter et al., 2007) and non-human animals (Anderson et al., 2000; Carro et al., 2000; van Praag et al., 2005; Vaynman et al., 2004) and meta-analytic reviews (Chang et al., 2012; Etnier et al., 1997; Lambourne & Tomporowski, 2010; Roig et al., 2013). However, findings from the current study do not provide a continuation of this strong support. Multiple measures were used to assess memory, but only one element of memory (i.e., auditory verbal short-term memory) was observed to be differentially effected by the treatment condition (i.e., lowPA). Additionally, acute PA was found to have a non-significant effect on measures of mBDNF concentrations. Furthermore, we were unable to assess measures of proBDNF concentrations due to faulty ELISA kits. This reduced the scope and feasibility of this study to the assessment of a single BDNF isoform (i.e., mature).

Acute Physical Activity Intensity

Results from the manipulation check showed significant differences between intensity levels performed in the three conditions. Results from HR, Maximum Watts, and total KM confirmed differences in objective measures of intensity, while RPE confirmed differences in subjective (e.g., psychological) measures of

intensity. While results showed that those in the VigPA condition performed the acute bout of PA at significantly lower Watts than the target Watts assigned by the protocol (i.e., Watts performed at 85% VO_2reserve [$\pm$ 5%] as assessed during the VO_2max session), analyses of HR levels at target Watts and performed Watts showed no differences. Hence, these results confirm the successful performance of the VigPA condition at an objectively measured intensity level statistically equivalent to that performed at 85% VO_2reserve,

Relational Memory

Results from the relational memory task showed non-significant differences in memory accuracy and reaction time between conditions. High levels of accuracy were observed in all conditions (i.e., >85%). While not a pure “ceiling effect”, the non-significant differences in accuracy suggest that this measure lacks the sensitivity necessary to assess differences in relational memory in an acute PA paradigm using samples consisting of young, healthy, and physically active participants or, alternatively that acute PA does not affect relational memory. Future studies would benefit from utilizing a measure of relational memory that has a higher level of difficulty and greater sensitivity. Non-significant findings relating to reaction time do not agree with past meta-analytic findings. Chang et al. (2012) showed a small but significant positive effect (i.e., d=0.261) of acute PA on choice reaction time. These findings might be due to the nature of the specific cognitive task used in the current study. The nature of the choice reaction time tasks used in the meta-analysis performed by Chang et al. (2012))

was mathematical (i.e., subtraction, addition) while the current reaction time task was spatial. Further research is needed to elucidate the role of task paradigm (e.g., mathematical, spatial) in the acute PA-choice reaction time relationship.

Auditory Verbal Learning Memory Task (AVLT)

Results showed that participants in each condition experienced a significant increase in memory over the course of the five learning trials (i.e., learning). However, this learning was not experienced differentially due to treatment condition (i.e., no condition by time interaction). Additionally, there was no difference in short-term memory as assessed by the primary short-term memory trial (i.e., Trial 7). Furthermore, participants in each condition experienced a significant decrease in memory (i.e., forgetting) over the course of the two long-term memory trials (i.e., post 30min, post 24hr), which was not affected by the treatment condition. These findings do not agree with two past studies which found significant benefits of acute PA on long-term memory as assessed by the AVLT (i.e., Etnier et al., *in preparation*, 2014). However, this may be due to differences in study design. Specifically, the study performed by Etnier et al. (*in preparation*) utilized a different mode of PA (i.e., treadmill), different intensity levels (i.e., ventilatory threshold +/- 20%), a within-subjects experimental design which required the use of three different memory stimuli (i.e., three separate word lists), and included both men and women. Additionally, the study performed by Etnier et al. (2014) utilized a different mode of PA (i.e., Progressive Aerobic Cardiovascular Endurance Run [PACER test]), a different intensity level (i.e.,

maximum), and consisted of 6th grade children. These differences, including the experimental design, specific measurement tool (i.e., three different word lists), participant age and sex, and PA modality and intensity may explain differences in observed effects of acute PA on long-term memory as assessed by the AVLT. The only measure of memory that was significantly affected by acute PA was the secondary short-term memory trial (i.e., Trial 6). Those in the lowPA condition recalled a significantly greater number of words on Trial 6, with non-significant differences between those in the vigPA and control conditions. This was an unexpected finding as Trial 6 is designed to be a "distractor" list with the intent of inhibiting the mental rehearsal of words in the time period between the learning trials (i.e., Trials 1-5) and the primary short-term memory trial (i.e., Trial 7). The AVLT begins with a series of five learning trials, each requiring participants to listen to and recall a list of 15 words (list A). Upon completion of the five learning trials, Trial 6 requires participants to listen to and recall a new list of 15 words (list B). Performing 35 minutes of low intensity PA enabled participants to better recall this novel list of words (list B). Importantly, the improved memory performance on Trial 6 experienced by those in the lowPA condition did not hinder performance of list A recall on subsequent trials. Meaning, that participants did not forget words from list A in order to "make room" to remember additional words from list B. Instead, these participants experienced an increase in the total number of words retained in short-term memory. In addition to its effect on short-term memory, this finding is suggestive of a beneficial effect of low intensity PA on

another domain of cognitive performance; specifically, the inhibition component of executive function. Trial 6 requires participants to momentarily inhibit the mental rehearsal and retrieval of a list of practiced words (list A) in order to learn new list (list B). However, measures of inhibition were not assessed in the current study. Hence, analyses cannot be performed to test for the effect of inhibition on performance of Trial 6 making it unreasonable to generate further inferences into this line of reasoning. Future studies exploring the effect of acute PA on memory performance would benefit from obtaining additional assessments of inhibition in order to explore this relationship. Findings from meta-analytic reviews show that acute PA generates small to moderate effects on the domain of executive function (i.e., Lambourne & Tomporowski, 2010: ES=0.19) as well as the subdomain of inhibition (i.e., Change et al., 2012: ES=0.249).

Brain-Derived Neurotrophic Factor Concentrations

The experimental design of this study allowed for the assessment of BDNF concentrations at four different time points (pre, post, post-30min, post-60min) in relation to four different treatment conditions (VO_2max, lowPA, vigPA, control). The assessment of BDNF in relation to the performance of a VO_2max test was to serve two purposes. Purpose #1) To obtain data pertaining to the acute PA-induced BDNF response in all participants. This goal was to use this data to elucidate differences in BDNF concentration between the three treatment conditions due to a potential time by condition interaction. Purpose #2) To directly relate findings to those of Brunelli et al. (2012) who showed differences in

BDNF isoform proportions in relation to a VO_2max test. While Brunelli et al. (2012) observed differences in BDNF isoform proportions from peripheral blood mononuclear cells, no differences were observed in BDNF concentrations obtained from blood serum. The current study was to extend the findings of Brunelli et al. (2012) by including assessments of BDNF isoform proportions obtained from blood serum.

With respect to the VO_2max-induced BDNF response, results showed non-significant differences in mBDNF concentrations between the three treatment condition assignments. This finding is in support of the successful randomized assignment of participants to treatment conditions in that there were no statistically significant differences in the participants' response to acute maximal PA as a function of their condition assignment. However, results showed non-significant changes in mBDNF concentrations over the four time points (i.e., no main effect for time). Meaning, that participants did not experience a VO_2max-induced BDNF response. While these findings do not agree with a review by Knaepen et al. (2010), which identified a positive relationship between acute PA intensity and BDNF concentration, findings from individual studies have been shown to be equivocal. Notably, Griffin et al. (2011) assessed the VO_2max-induced BDNF response prior to and upon completion of a 3-week cycling training program. While significant increases in BDNF concentration were observed prior to the 3-week training program, the performance of a VO_2max test did not elicit significant changes in BDNF concentration. Zoladz et al. (2008) also

observed non-significant changes in BDNF concentration following a VO_2max. Brunelli et al. (2012) also did not observe significant changes in BDNF concentration in response to the performance of a VO_2max test. Though the half-life of endogenous BDNF is unknown (Sirianni, Olausson, Chiu, Taylor, & Saltzman, 2010), research with rodents has shown that the clearance of exogenous BDNF takes place within several hours of administration (Fukumitsu et al., 2006). Therefore, the lack of a significant PA-induced increase in concentration was not due to disappearance prior to collection of blood samples. However, the non-significant finding may also be due to this being the first study to assess the effect of PA on BDNF concentrations using a mBDNF ELISA kit containing no cross-reactivity to proBDNF. Specifically, it is reasonable to hypothesize that past findings may be partially due to the influence of cross-reactivity with proBDNF in the ELISA kits. Further exploration into acute PA-induced changes in proBDNF concentrations is needed to elucidate these findings.

With respect to the acute PA-induced BDNF response, results showed non-significant changes in mBDNF concentrations across the four time points (i.e., no main effect for time) irrespective of treatment condition (i.e., no main effect for condition). Additionally, changes in mBDNF concentration across the four time points were not dependent on treatment condition (i.e., no time by condition interaction). As previously mentioned, the literature suggests a positive relationship between acute PA intensity and BDNF concentration. Additionally,

findings from recent studies show support for an acute PA-induced increase in BDNF concentration for PA performed at low, moderate, vigorous, and maximal intensities (Ferris et al., 2007; Griffin et al., 2011; Lee et al., 2014; Tonoli et al., 2014; Tsai et al., 2014; Winter et al., 2007). Overall, results from the current study do not agree with the literature base that shows support for an acute PA-induced increase in BDNF concentration. However, it is important to note that previous studies have not used ELISA kits that provide assessments of mBDNF with no cross-reactivity to other isoforms (i.e., proBDNF) in their analyses. Therefore, it is possible that differences between mBDNF concentrations observed in the current study and past studies may be due to isoform proportions. To illustrate this point, Brunelli et al.'s (2012) findings relating to non-significant changes in BDNF concentration, yet significant changes in BDNF isoform proportions, suggest that assessments of both isoforms (i.e., pro and m) are necessary to obtain a more complete understanding of the acute PA-induced BDNF response.

Due to the faulty manufacturing of proBDNF kits by Aviscera Bioscience INC, results only relate to changes in mBDNF concentrations, and not BDNF isoform proportions. In order to reduce potential variance between ELISA kits, a new lot of ELISA kits was created specifically for the current study. As soon as the lot passed Aviscera Bioscience INC's internal quality control standards, it was shipped to UNCG where it was stored in a refrigerator until its use. Multiple attempts were made to generate a viable standard curve in order to analyze

serum samples. However, the resulting data was consistently outside of expected ranges making it ill-advised to use for sample analyses. The resulting data, along with the remaining kits, were returned to Aviscera Bioscience INC for further investigation. Upon testing, it was concluded that the stability of the standards were questionable. New kits are currently being created. Due to these issues, serum samples have not yet been analyzed to assess concentrations of proBDNF. However, upon receiving the newly created proBDNF ELISA kits, serum samples will be analyzed and the resulting data will be incorporated with the findings presented herein. While these circumstances have temporarily limited the scope of the current study, measures of mBDNF may still be used to relate to past research (e.g., Ferris et al., 2007; Griffin et al., 2011; Lee et al., 2014; Skriver et al., 2014; Tonoli et al., 2014; Tsai et al., 2014; Winter et al., 2007)

Conclusion

The present study extends the literature base pertaining to the acute PA-cognitive performance relationship. Findings illustrate a beneficial effect of 35 minutes of low intensity cycling on the performance of a short-term memory task. While multiple aspects of memory were assessed (e.g., relational, short-term, long-term), short-term memory was the only cognitive factor affected by acute PA. An exploration of the acute PA-induced BDNF response showed that acute PA did not significantly alter BDNF concentrations. Thus, analysis into potential mediating effects of BDNF concentration on the acute PA-cognitive performance

relationship was not advised. Additionally, due to manufacturing difficulties by the supplier of the ELISA kits, BDNF analyses were isolated to the mBDNF isoform. Therefore, analyses pertaining to changes in BDNF isoform proportion were not able to be carried out.

Limitations & Future Directions

While various limitations have been previously noted, including manufacturing problems with proBDNF ELISA kits, a major limitation to this study is a lack of power due to the small sample size (Session 1: N=29; Session 2: N=27) and effect sizes. While the obtained effect sizes for measures of mBDNF allow for greater power (i.e., $\geq$.80) with modest increases in sample size (i.e., N$\geq$58), the effect sizes for the majority of the memory measures require much larger samples sizes (i.e., N>100) in order to obtain adequate power (i.e., $\geq$ .80) (See Table 5). Future studies would benefit from utilizing more sensitive measures of cognitive performance, with precedence for obtaining greater effect sizes when performed in an acute PA paradigm.

Table 5. Effect Sizes and Power: Observed and Proposed

		Obtained			Proposed	
Variable	Test	$\eta^2_{partial}$	N	Power	N	Power
Session 1						
mBDNF	Time	.087	29	.321	105	.803
Session 2						
mBDNF	Time	.142	27	.475	66	.805
mBDNF	Condition	.096	27	.170	58	.806
mBDNF	Interaction	.151	27	.375	83	.801
Accuracy	Condition	.084	27	.221	111	.811
RT	Condition	.059	27	.162	159	.806
T1 - T5	Condition	.082	27	.215	111	.800
T1 - T5	Interaction	.090	27	.520	176	.801
T7	Condition	.056	27	.156	168	.807
T8-T9	Condition	.017	27	.078	561	.801
T8-T9	Interaction	.069	27	.185	134	.803
24hr Recog.	Condition	.134	27	.347	66	.804

Relational Memory: Accuracy, RT; AVLT: T1-T5, T8 (post-30min), T9 (post-24hr), 24hr Recog.

As previously mentioned, findings from the meta-analytic review performed by Lambourne and Tomporowski (2010) suggest that the effect of acute PA on cognitive performance is greatest within a 15-minute window of benefit following completion of the PA. This is a limitation and might partially explain the small effect size and non-significant results from the Relational Memory task. Upon completion of the PA, the second blood draw was performed (approximately 5 minutes to complete). Next the participant walked to the computer desk and was given directions on how to complete the first cognitive task. The order of cognitive tasks was kept constant throughout the study, with the Auditory Verbal Learning Task (approximately 11 minutes) always preceding the Relational Memory task. After completing the blood draw, the first cognitive task, and directions for the Relational Memory task, the participant had exceeded

the predicted 15-minute window of benefit (approximately 17-20 minutes). Future research would benefit from counterbalancing the order of cognitive tasks.

A limitation to the current study is the inability to observe the entire life cycle of the BNDF protein in relation to acute PA. The specific tissue(s) of BDNF expression was not identified in the current study. For example, in terms of the creation of BDNF, although there are positive correlations between concentrations of centrally and peripherally assessed BDNF its expression may also occur in non-neural tissues (i.e., skeletal muscle). However, it has been previously shown that approximately 70-80% of peripherally assessed BDNF concentrations are derived from the brain (Rasmussen et al., 2009). Additionally, research has suggested that even though expression of BDNF mRNA is increased in response to skeletal muscular contraction in humans, this does not translate into increased concentrations of peripherally assessed (i.e., blood) BDNF protein (Matthews et al., 2009). Additionally, the specific cellular locations of BDNF uptake (e.g., Trk and p75 receptors in the hippocampus), as well as the disappearance rate of the protein (e.g., half life, clearance) make it difficult to infer the cause of changes (though non-significant) in BDNF concentrations observed across the four time points. For example, although more research is needed to identify the half-life of BDNF to determine its natural rate of degradation (Sirianni et al., 2010), decreases in concentrations could be the result of clearance of the BDNF protein (Fukumitsu et al., 2006). However, even though there are limitations relating to the life cycle of the BDNF protein, this type

of limitation is inherent to all research exploring the acute PA-BDNF-cognition relationship in humans. The direct observation of the various multi-level molecular processes at work is not feasible. As a consequence, the precise molecular cause of the relationship currently remains unknown. Therefore, investigators must rely on the use of randomized control designs and inferential statistical techniques to aid in the elucidation of the acute PA-BDNF-cognitive performance relationship in humans. In this manner, focus is taken away from specific details pertaining to the life cycle of the BDNF protein, and is instead placed on statistical aspects of the acute PA-BDNF-cognitive performance relationship.

A future direction that should be considered is related to the methodology of collecting blood serum samples. Blood samples were collected at four time points (i.e., pre, post, post-30min, post-60min), were allowed to clot at room temperature, and 20 minutes following the final collection were centrifuged to separate serum. This methodology resulted in a variation in total time to clot for samples from each time point. For example, the first sample was allowed to clot for approximately two hours and 20 minutes, the second sample for approximately one hour and 20 minutes, the third sample for approximately 50 minutes, and the fourth sample for approximately 20 minutes. A study performed by Katoh-Semba et al. (2007) suggests that, due to the release of BDNF from blood palettes during clotting, methodological differences of this sort may affect obtained BDNF concentrations. Using BDNF concentrations obtained from

samples allowed to clot for 30 minutes at room temperature (i.e., 26 degrees C) as the control, Katoh-Semba et al. (2007) observed that blood samples allowed to clot at room temperature for four hours resulted in an approximately 50% reduction in BDNF concentration. However, when blood samples were allowed to clot while refrigerated (i.e., 4 degrees C), BDNF concentrations were at their greatest (i.e., closest to 30-minute room temperature control samples) 24-48 hours later. Future studies should consider using the Katoh-Semba et al. (2007) protocol of blood serum preparation. In addition to reducing the degradation of BDNF from blood samples, this protocol is logistically favorable. For example, as previously described all testing for the current study took place in the morning. When observing multiple participants on a single morning, it was logistically difficult to process blood samples from the first participant while preparing to observe the second participant given the number of investigators. By following the Katoh-Semba protocol, samples could have been directly placed in refrigeration following each blood draw and allowed to clot for 24-48 hours. Subsequently, the samples could have been centrifuged the following afternoon without interfering with the morning's participant observations.

REFERENCES

American College of Sports Medicine. (2013). ACSM's Guidelines for Exercise Testing and Prescription (9th ed.). Philadephia, PA: Lippincott Williams & Wilkins.

Anderson, B. J., Rapp, D. N., Baek, D. H., McCloskey, D. P., Coburn-Litvak, P. S., & Robinson, J. K. (2000). Exercise influences spatial learning in the radial arm maze. *Physiology & Behavior*, *70*(5), 425–429. http://doi.org/10.1016/S0031-9384(00)00282-1

Baker, L. D., Frank, L. L., Foster-Shubert, K., Green, P. S., Wilkinson, C. W., McTiernan, A., … Craft, S. (2010). Effects of Aerobic Exercise on Mild Cognitive Impairment. *Archives of Neurology*, *67*(1), 71–79.

Barker, P. a. (2009). Whither proBDNF? *Nature Neuroscience*, *12*(2), 105–6. http://doi.org/10.1038/nn0209-105

Bernabeu, R. O., & Longo, F. M. (2010). The p75 neurotrophin receptor is expressed by adult mouse dentate progenitor cells and regulates neuronal and non-neuronal cell genesis. *BMC Neuroscience*, *11*(1), 136. http://doi.org/10.1186/1471-2202-11-136

Bischofberger, J. (2007). Young and excitable: new neurons in memory networks. *Nature Neuroscience*, *10*(3), 273–5. http://doi.org/10.1038/nn0307-273

Black, J. E., Isaacs, K. R., Anderson, B. J., Alcantara, A. A., & Greenough, W. T. (1990). Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats. *Proceedings of the National Academy of Sciences*, *87*, 5568–5572.

Borg, G. (1990). Psychophysical scaling with applications in physical work and the perception of exertion. *Scandinavian Journal of Work, Environment & Health*, *16 Suppl 1*(suppl 1), 55–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2345867

Brunelli, A., Dimauro, I., Sgrò, P., Emerenziani, G. Pietro, Magi, F., Baldari, C., … Caporossi, D. (2012). Acute exercise modulates BDNF and pro-BDNF protein content in immune cells. *Medicine and Science in Sports and Exercise*, *44*(10), 1871–80. http://doi.org/10.1249/MSS.0b013e31825ab69b

Brunoni, A. R., Lopes, M., & Fregni, F. (2008). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the*

Collegium Internationale Neuropsychopharmacologicum (CINP), *11*(8), 1169–80. http://doi.org/10.1017/S1461145708009309

Carlino, D., Leone, E., Di Cola, F., Baj, G., Marin, R., Dinelli, G., … De Vanna, M. (2011). Low serum truncated-BDNF isoform correlates with higher cognitive impairment in schizophrenia. *Journal of Psychiatric Research*, *45*(2), 273–9. http://doi.org/10.1016/j.jpsychires.2010.06.012

Carro, E., Nuñez, A., Busiguina, S., & Torres-Aleman, I. (2000). Circulating insulin-like growth factor I mediates effects of exercise on the brain. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *20*(8), 2926–33. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11466439

Chaddock, L., Erickson, K. I., Prakash, R. S., Kim, J. S., Voss, M. W., Vanpatter, M., … Kramer, A. F. (2010). A neuroimaging investigation of the association between aerobic fitness, hippocampal volume, and memory performance in preadolescent children. *Brain Research*, *1358*, 172–83. http://doi.org/10.1016/j.brainres.2010.08.049

Chang, Y., & Etnier, J. L. (2009). Exploring the Dose-Response Relationship Between Resistance Exercise Intensity and Cognitive Function. *Sports Medicine*, (31), 640–656.

Chang, Y. K., Labban, J. D., Gapin, J. I., & Etnier, J. L. (2012). The effects of acute exercise on cognitive performance: A meta-analysis. *Brain Research*, *1453*(250), 87–101. http://doi.org/10.1016/j.brainres.2012.02.068

Chang, Y.-K., Chu, I.-H., Chen, F.-T., & Wang, C.-C. (2011). Dose-response effect of acute resistance exercise on Tower of London in middle-aged adults. *Journal of Sport & Exercise Psychology*, *33*(6), 866–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22262709

Chang, Y.-K., Ku, P.-W., Tomporowski, P. D., Chen, F.-T., & Huang, C.-C. (2012). Effects of acute resistance exercise on late-middle-age adults' goal planning. *Medicine and Science in Sports and Exercise*, *44*(9), 1773–9. http://doi.org/10.1249/MSS.0b013e3182574e0b

Chang, Y.-K., Tsai, C.-L., Hung, T.-M., So, E. C., Chen, F.-T., & Etnier, J. L. (2011). Effects of acute exercise on executive function: a study with a Tower of London Task. *Journal of Sport & Exercise Psychology*, *33*(6), 847–65. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22262708

Cirulli, F., Berry, A., Chiarotti, F., & Alleva, E. (2004). Intrahippocampal administration of BDNF in adult rats affects short-term behavioral plasticity in the Morris water maze and performance in the elevated plus-maze. *Hippocampus*, *14*(7), 802–7. http://doi.org/10.1002/hipo.10220

Colcombe, S. J., Erickson, K. I., Raz, N., Webb, A. G., Cohen, N. J., McAuley, E., & Kramer, A. F. (2003). Aerobic fitness reduces brain tissue loss in aging humans. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *58*(2), 176–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12586857

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., … Kramer, A. F. (2006). Aerobic Exercise Training Increases Brain Volume in Aging Humans. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, *61*(11), 1166. Retrieved from http://www.biomedgerontology.oxfordjournals.org/content/61/11/1166.full

Colcombe, S., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychological Science*, *14*(2), 125–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12661673

Coles, K., & Tomporowski, P. D. (2008). Effects of acute exercise on executive processing, short-term and long-term memory. *Journal of Sports Sciences*, *26*(3), 333–44. http://doi.org/10.1080/02640410701591417

Cotman, C. W., & Berchtold, N. C. (2002). Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends in Neurosciences*, *25*(6), 295–301. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12086747

Cunha, C., Brambilla, R., & Thomas, K. L. (2010). A simple role for BDNF in learning and memory? *Frontiers in Molecular Neuroscience*, *3*(February), 1. http://doi.org/10.3389/neuro.02.001.2010

Dishman, R. K., Berthoud, H.-R., Booth, F. W., Cotman, C. W., Edgerton, V. R., Fleshner, M. R., … Zigmond, M. J. (2006). Neurobiology of exercise. *Obesity (Silver Spring, Md.)*, *14*(3), 345–56. http://doi.org/10.1038/oby.2006.46

Drake, C. T., Milner, T. A., & Patterson, S. L. (1999). Ultrastructural Localization of Full-Length trkB Immunoreactivity in Rat Hippocampus Suggests Multiple Roles in Modulating Activity- Dependent Synaptic Plasticity. *The Journal of Neuroscience*, *19*(18), 8009–8026.

Erickson, K. I., Miller, D. L., & Roecklein, K. a. (2011). The Aging Hippocampus: Interactions between Exercise, Depression, and BDNF. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*. http://doi.org/10.1177/1073858410397054

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Heo, S., McLaren, M., … Kramer, A. F. (2010). Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(15), 5368–75. http://doi.org/10.1523/JNEUROSCI.6251-09.2010

Erickson, K. I., Raji, C. a, Lopez, O. L., Becker, J. T., Rosano, C., Newman, a B., … Kuller, L. H. (2010). Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study. *Neurology*, *75*(16), 1415–22. http://doi.org/10.1212/WNL.0b013e3181f88359

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., … Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences of the United States of America*, 1–6. http://doi.org/10.1073/pnas.1015950108

Etnier, J. L., & Chang, Y.-K. (2009). The effect of physical activity on executive function: a brief commentary on definitions, measurement issues, and the current state of the literature. *Journal of Sport & Exercise Psychology*, *31*(4), 469–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19842543

Etnier, J. L., Salazar, W., Landers, D. M., Petruzzello, S. J., Han, M., & Nowell, P. (1997). The Influence of Physical Fitness and Exercise Upon Cognitive Functioning: A Meta-Analysis. *Journal of Sport & Exercise Psychology*, *19*, 249–277.

Etnier, J., Labban, J. D., Piepmeier, A., Davis, M. E., & Henning, D. A. (2014). Effects of an Acute Bout of Exercise on Memory in 6 th Grade Children, 250–258.

Etnier, J., Wideman, L., Labban, J. D., Piepmeier, A. T., Pendleton, D. M., Dvorak, K. K., & Becofsky, K. (n.d.). The effects of acute exercise on memory and brain-derived neurotrophic factor (BDNF): A dose-response study, 1–25.

Ferris, L. T., Williams, J. S., & Shen, C.-L. (2007a). The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Medicine and Science in Sports and Exercise*, *39*(4), 728–34. http://doi.org/10.1249/mss.0b013e31802f04c7

Ferris, L. T., Williams, J. S., & Shen, C.-L. (2007b). The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Medicine and Science in Sports and Exercise*, *39*(4), 728–34. http://doi.org/10.1249/mss.0b013e31802f04c7

Fukumitsu, H., Ohtsuka, M., Murai, R., Nakamura, H., Itoh, K., & Furukawa, S. (2006). Brain-derived neurotrophic factor participates in determination of neuronal laminar fate in the developing mouse cerebral cortex. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(51), 13218–30. http://doi.org/10.1523/JNEUROSCI.4251-06.2006

Griffin, É. W., Mullally, S., Foley, C., Warmington, S. a, O'Mara, S. M., & Kelly, A. M. (2011). Aerobic exercise improves hippocampal function and increases

BDNF in the serum of young adult males. *Physiology & Behavior*, *104*(5), 934–41. http://doi.org/10.1016/j.physbeh.2011.06.005

Horch, H. W., Kru, A., Portbury, S. D., Katz, L. C., Hughes, H., & Carolina, N. (1999). Destabilization of Cortical Dendrites and Spines by BDNF. *Neuron*, *23*, 353–364.

Impellizzeri, F. M., & Bizzini, M. (2012). Systematic review and meta-analysis: A primer. *The International Journal of Sports Physical Therapy*, *7*(5), 493–503.

Kamijo, K., Hayashi, Y., Sakai, T., Yahiro, T., Tanaka, K., & Nishihira, Y. (2009). Acute effects of aerobic exercise on cognitive function in older adults. *The Journals of Gerontology. Series B, Psychological Sciences and Social Sciences*, *64*(3), 356–63. http://doi.org/10.1093/geronb/gbp030

Katoh-Semba, R., Wakako, R., Komori, T., Shigemi, H., Miyazaki, N., Ito, H., … Nakayama, A. (2007). Age-related changes in BDNF protein levels in human serum: differences between autism cases and normal controls. *International Journal of Developmental Neuroscience : The Official Journal of the International Society for Developmental Neuroscience*, *25*(6), 367–72. http://doi.org/10.1016/j.ijdevneu.2007.07.002

Klein, A. B., Williamson, R., Santini, M. a, Clemmensen, C., Ettrup, A., Rios, M., … Aznar, S. (2011). Blood BDNF concentrations reflect brain-tissue BDNF

levels across species. *The International Journal of Neuropsychopharmacology / Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *14*(3), 347–53. http://doi.org/10.1017/S1461145710000738

Knaepen, K., Goekint, M., Heyman, E. M., & Meeusen, R. (2010a). Neuroplasticity – Exercise-Induced Response of Peripheral Brain-Derived Neurotrophic Factor A Systematic Review of Experimental Studies in Human Subjects. *Sports Medicine*, *40*(9), 765–801.

Knaepen, K., Goekint, M., Heyman, E. M., & Meeusen, R. (2010b). Neuroplasticity – Exercise-Induced Response of Peripheral Brain-Derived Neurotrophic Factor A Systematic Review of Experimental Studies in Human Subjects. *Sports Medicine*, *40*(9), 765–801.

Krabbe, K. S., Nielsen, a R., Krogh-Madsen, R., Plomgaard, P., Rasmussen, P., Erikstrup, C., … Pedersen, B. K. (2007). Brain-derived neurotrophic factor (BDNF) and type 2 diabetes. *Diabetologia*, *50*(2), 431–8. http://doi.org/10.1007/s00125-006-0537-4

Labban, J. D., & Etnier, J. L. (2011). Effects of acute exercise on long-term memory. *Research Quarterly for Exercise and Sport*, *82*(4), 712–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22276413

Lambourne, K., & Tomporowski, P. (2010). The effect of exercise-induced arousal on cognitive task performance: a meta-regression analysis. *Brain Research*, *1341*, 12–24. http://doi.org/10.1016/j.brainres.2010.03.091

Lee, J. K. W., Koh, A. C. H., Koh, S. X. T., Liu, G. J. X., Nio, A. Q. X., & Fan, P. W. P. (2014). Neck cooling and cognitive performance following exercise-induced hyperthermia. *European Journal of Applied Physiology*, *114*(2), 375–84. http://doi.org/10.1007/s00421-013-2774-9

Lee, T. M. C., Wong, M. L., Lau, B. W.-M., Lee, J. C.-D., Yau, S.-Y., & So, K.-F. (2014). Aerobic exercise interacts with neurotrophic factors to predict cognitive functioning in adolescents. *Psychoneuroendocrinology*, *39*, 214–24. http://doi.org/10.1016/j.psyneuen.2013.09.019

Lista, I., & Sorrentino, G. (2010). Biological Mechanisms of Physical Activity in Preventing Cognitive Decline. *Cellular and Molecular Neurobiology*, 493–503. http://doi.org/10.1007/s10571-009-9488-x

Lu, B., Pang, P. T., & Woo, N. H. (2005). The yin and yang of neurotrophin action. *Nature Reviews. Neuroscience*, *6*(8), 603–14. http://doi.org/10.1038/nrn1726

Matthews, V. B., Aström, M.-B., Chan, M. H. S., Bruce, C. R., Krabbe, K. S., Prelovsek, O., … Febbraio, M. a. (2009). Brain-derived neurotrophic factor is

produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia*, *52*(7), 1409–18. http://doi.org/10.1007/s00125-009-1364-1

Mitchell, K. J., Johnson, M. K., Raye, C. L., & D'Esposito, M. (2000). fMRI evidence of age-related hippocampal dysfunction in feature binding in working memory. *Cognitive Brain Research*, *10*(1-2), 197–206. http://doi.org/10.1016/S0926-6410(00)00029-X

Miyake, a, Friedman, N. P., Emerson, M. J., Witzki, a H., Howerter, A., & Wager, T. D. (2000). The unity and diversity of executive functions and their contributions to complex "Frontal Lobe" tasks: a latent variable analysis. *Cognitive Psychology*, *41*(1), 49–100. http://doi.org/10.1006/cogp.1999.0734

Mu, J.-S., Li, W.-P., Yao, Z.-B., & Zhou, X.-F. (1999). Deprivation of endogenous brain-derived neurotrophic factor results in impairment of spatial learning and memory in adult rats. *Brain Research*, *835*(2), 259–265. http://doi.org/10.1016/S0006-8993(99)01592-9

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

Pan, W., Banks, W. a, Fasold, M. B., Bluth, J., & Kastin, a J. (1998). Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology*, *37*(12), 1553–61. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9886678

Pesce, C., Crova, C., Cereatti, L., Casella, R., & Bellucci, M. (2009). Physical activity and mental performance in preadolescents: Effects of acute exercise on free-recall memory. *Mental Health and Physical Activity*, *2*(1), 16–22. http://doi.org/10.1016/j.mhpa.2009.02.001

Piepmeier, A. T., Shih, C.-H., Whedon, M., Williams, L. M., Davis, M. E., Henning, D. H., … Etnier, J. L. (2015). The effect of acute exercise on cognitive performance in children with and without ADHD. *Journal of Sport and Health Science*, *4*, 97–104.

Poduslo, J. F., & Curran, G. L. (1996). MOLECULAR Permeability at the blood-brain and blood-nerve barriers of the. *Molecular Brain Research*, *36*, 280–286.

Pontifex, M. B., Hillman, C. H., Fernhall, B., Thompson, K. M., & Valentini, T. a. (2009). The effect of acute aerobic and resistance exercise on working memory. *Medicine and Science in Sports and Exercise*, *41*(4), 927–34. http://doi.org/10.1249/MSS.0b013e3181907d69

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M. V, Leick, L., Hart, E., … Pilegaard, H. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology*, *94*(10), 1062–9. http://doi.org/10.1113/expphysiol.2009.048512

Roig, M., Nordbrandt, S., Geertsen, S. S., & Nielsen, J. B. (2013). The effects of cardiovascular exercise on human memory: A review with meta-analysis. *Neuroscience and Biobehavioral Reviews*, *37*(8), 1645–1666. http://doi.org/10.1016/j.neubiorev.2013.06.012

Rojas Vega, S., Strüder, H. K., Vera Wahrmann, B., Schmidt, A., Bloch, W., & Hollmann, W. (2006). Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Research*, *1121*(1), 59–65. http://doi.org/10.1016/j.brainres.2006.08.105

Sirianni, R., Olausson, P., Chiu, A., Taylor, J., & Saltzman, M. (2010). The behavioral and biochemical effects of BDNF containing polymers implanted in the hippocampus of rats. *Brain Research*, *19*(1321C), 40–50. http://doi.org/10.1016/j.brainres.2010.01.041.The

Skriver, K., Roig, M., Lundbye-Jensen, J., Pingel, J., Helge, J. W., Kiens, B., & Nielsen, J. B. (2014). Acute exercise improves motor memory: Exploring

potential biomarkers. *Neurobiology of Learning and Memory*, *116C*, 46–58. http://doi.org/10.1016/j.nlm.2014.08.004

Tang, S. W., Chu, E., Hui, T., Helmeste, D., & Law, C. (2008). Influence of exercise on serum brain-derived neurotrophic factor concentrations in healthy human subjects. *Neuroscience Letters*, *431*(1), 62–5. http://doi.org/10.1016/j.neulet.2007.11.019

Tongiorgi, E., Sartori, A., Baj, G., Bratina, A., Di Cola, F., Zorzon, M., & Pizzolato, G. (2012). Altered serum content of brain-derived neurotrophic factor isoforms in multiple sclerosis. *Journal of the Neurological Sciences*, *320*(1-2), 161–5. http://doi.org/10.1016/j.jns.2012.07.016

Tonoli, C., Heyman, E., Buyse, L., Roelands, B., Piacentini, M., Bailey, S., … Meeusen, R. (2014). Neurotrophins and cognitive functions in T1D compared to healthy controls: effects of a high-intensity exercise. *Applied Physiology, Nutrition, and Metabolism*, 1–21.

Tsai, C.-L., Chen, F.-C., Pan, C.-Y., Wang, C.-H., Huang, T.-H., & Chen, T.-C. (2014). Impact of acute aerobic exercise and cardiorespiratory fitness on visuospatial attention performance and serum BDNF levels. *Psychoneuroendocrinology*, *41*(1), 121–31. http://doi.org/10.1016/j.psyneuen.2013.12.014

Van Praag, H., Shubert, T., Zhao, C., & Gage, F. H. (2005). Exercise enhances learning and hippocampal neurogenesis in aged mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *25*(38), 8680–5. http://doi.org/10.1523/JNEUROSCI.1731-05.2005

Vaynman, S., & Gomez-pinilla, F. (2006). Review Revenge of the “Sit” : How Lifestyle Impacts Neuronal and Cognitive Health Through Molecular Systems That Interface Energy Metabolism With Neuronal Plasticity. *Journal of Neuroscience Research*, *715*(April), 699–715. http://doi.org/10.1002/jnr

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *The European Journal of Neuroscience*, *20*(10), 2580–90. http://doi.org/10.1111/j.1460-9568.2004.03720.x

Winter, B., Breitenstein, C., Mooren, F. C., Voelker, K., Fobker, M., Lechtermann, A., … Knecht, S. (2007a). High impact running improves learning. *Neurobiology of Learning and Memory*, *87*(4), 597–609. http://doi.org/10.1016/j.nlm.2006.11.003

Winter, B., Breitenstein, C., Mooren, F. C., Voelker, K., Fobker, M., Lechtermann, A., … Knecht, S. (2007b). High impact running improves learning. *Neurobiology of Learning and Memory*, *87*(4), 597–609. http://doi.org/10.1016/j.nlm.2006.11.003

Yoshida, T., Ishikawa, M., Iyo, M., & Hashimoto, K. (2012). Serum Levels of Mature Brain-Derived Neurotrophic Factor (BDNF) and Its Precursor proBDNF in Healthy Subjects. *The Open Clinical Chemistry Journal*, *5*, 7–12.

Yoshida, T., Ishikawa, M., Niitsu, T., Nakazato, M., Watanabe, H., Shiraishi, T., … Hashimoto, K. (2012). Decreased serum levels of mature brain-derived neurotrophic factor (BDNF), but not its precursor proBDNF, in patients with major depressive disorder. *PloS One*, *7*(8), e42676. http://doi.org/10.1371/journal.pone.0042676

Zhou, L., Xiong, J., Lim, Y., Ruan, Y., Huang, C., Zhu, Y., … Zhou, X.-F. (2013). Upregulation of blood proBDNF and its receptors in major depression. *Journal of Affective Disorders*, *150*(3), 776–84. http://doi.org/10.1016/j.jad.2013.03.002

Zoladz, J. a, Pilc, A., Majerczak, J., Grandys, M., Zapart-Bukowska, J., & Duda, K. (2008). Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men. *Journal of Physiology and Pharmacology : An Official Journal of the Polish Physiological Society*, *59 Suppl 7*, 119–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19258661

Zoladz, J. A., PILC, J., Pilc, A., Majerczak, J., Grandys, M., Zapart-Bukowska, J., … Cotman, C. W. (2010). the effect of physical activity on the brain derived neurotrophic factor: from animal to human studies. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *67*(5), 5568–5572. http://doi.org/10.1523/JNEUROSCI.6251-09.2010