EXERCISE, COGNITIVE TRAINING, AND MEMORY

COMBINED EXERCISE AND COGNITIVE TRAINING ENHANCES HIPPOCAMPAL-DEPENDENT MEMORY

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ABSTRACT

There is an established link between exercise, neurogenesis, and memory. Most of this research has been focused on rodent models, with little known about the effects of exercise on cognition in young adults. In rodents, exercise promotes hippocampal neurogenesis by enhancing cell proliferation in the dentate gyrus, while cognitive training promotes hippocampal neurogenesis by enhancing cell survival. Both physical exercise and cognitive training independently induce hippocampal neurogenesis in rodents, suggesting that these different forms of training may work through complimentary neurological pathways to benefit hippocampal memory in young adults. The present study examined the effects of six weeks of physical exercise and cognitive training on hippocampal-mediated memory processes in young adults to determine whether combined training yields enhanced memory benefits. Sixty-six sedentary young adults (32 females; age range 17-30 years) were randomly assigned to one of four groups: 1) Exercise training group (n=15), 2) Cognitive training group (n=16), 3) Combined exercise and cognitive training group (n=18), or 4) No-contact control group (n=17). Memory performance was assessed before and after the intervention on a putative hippocampal neurogenesis-dependent task, the *Pattern Separation* task. Growth factors that support neuron survival and function, brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF-1) were measured in blood serum using enzyme-linked immunosorbent assays (ELISA). Based on the literature, the combination of exercise and cognitive training was expected to

improve pattern separation performance more than the exercise or cognitive training alone, and display augmented effects for high responders to the exercise training. Additionally, if BDNF and IGF-1 were found to be involved in the mechanisms regulating the observed changes in memory, they too were expected to increase the most from the combined training and be dependent on individual changes in aerobic fitness. Critically, the combination of exercise and cognitive training led to the greatest increase in memory requiring pattern separation [$t(16) = 2.91$, $p < 0.01$] , indicating improved hippocampal-mediated memory function. BDNF and IGF-1 were not associated with this change in memory performance but were associated with the individual's response to the exercise training, such that high responders to exercise had greater BDNF [$F(1, 29) = 7.81$, $p < 0.01$] and IGF-1 [$F(1, 29) = 5.09$, $p < 0.05$] than low responders to exercise. The results suggest that exercise and cognitive training may work through synergistic mechanisms to enhance hippocampal neurogenesis and support pattern separation processing. However, BDNF and IGF-1 may not be mediating this change in memory function.

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

FIGURES

TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BDNF	Brain- derived neurotrophic factor
BPS-O	Behavioural Pattern Separation Object-Task
CA1	Cornus ammonis 1
CA3	Cornus ammonis 3
DG	Dentate gyrus
EC	Entorhinal cortex
HIIT	High Intensity Interval Training
IGF-1	Insulin-like growth factor
VO_2 peak	Peak oxygen consumption

DECLARATION OF ACADEMIC ACHIEVEMENT

I.B. Clark's role:

- Contributed to study concept, design, and measurement selection
- Contributed to participant recruitment
- Lead investigator responsible for preparation of lab settings and materials
- Trained and supervised volunteers who assisted with data collection
- Responsible for data collection, analysis, and interpretation
- Responsible for preparation of manuscript

Role of JJH:

- Lead contributor to study concept, design, and measurement selection
- Obtained study funding
- Contributed to data analysis
- Assisted with interpretation of the data

INTRODUCTION

Memory is critical for new information to be encoded, stored, and later retrieved. These abilities are essential for independent function in everyday life. Therefore, it is important to identify ways to improve and optimize memory function. Exercise and cognitive training are two key factors that improve memory function and are proposed to do this by enhancing neurogenesis in the hippocampus — a brain region that is critical for forming new memories and recalling past events. Exercise promotes neurogenesis by increasing the proliferation of newborn cells in the hippocampus, while cognitive training increases cell survival by providing neurons with the support to mature and integrate into the hippocampal network. Thus, exercise and cognitive training induce hippocampal neurogenesis through different and potentially complimentary mechanisms, suggesting the combination may improve memory function over either training regime alone. The present study examined the interplay between exercise and cognitive training on hippocampal-mediated memory function in young adults with the aim of determining whether combined training yields synergistic benefits to memory function.

Hippocampal Structure and Function

The hippocampus is a subcortical brain structure in the medial temporal lobe that is critical for learning and memory (Burgess, Maguire, & O'Keefe, 2002). It is made of an intricate network of multiple regions including the dentate gyrus (DG) and the cornus ammonis 1 (CA1) and 3 (CA3) of the hippocampus

proper (Rolls, 2013). Each region plays a specific role in memory retrieval, which is determined by its structure and connections with the rest of the network.

The CA3 plays an important role in general memory recall (Marr, 1971; Rolls, 2013). CA3 neurons receive direct input from the entorhinal cortex (EC) through the perforent path system (Rolls, 2013; Rolls, Treves, & Rolls, 1998). The CA3 neurons are able to synapse onto themselves through recurrent collaterals to create a reiterative loop that enables the recall of a previously stored memory from a small incomplete part or memory cue — a process known as *pattern completion* (Marr, 1971; Rolls, 2013). Pattern completion is important for the general recall of memories but it does not encode details; this can lead to errors when the system encounters a new pattern that is highly similar to a previously learned cue. In this case the system may mistakenly encode a new memory as one that was previously stored, leading to catastrophic memory interference whereby the new memory is overwritten by a similar previously stored memory (Rolls, 2013; Rolls et al., 1998; Wiskott, Rasch, & Kempermann, 2006).

Fortunately, there is processing upstream of the CA3 that de-correlates incoming information from the EC to create precise and separate memory traces for highly similar patterns; this is a process known as *pattern separation* and involves the DG (Marr, 1971; Rolls, 2013). The DG is anatomically well positioned for the task of pattern separation because it receives inputs from the EC and subsequently sends projections to the CA3 through a mossy fibre network.

The mossy fibre network creates sparse connections between the DG and the CA3 such that randomized inputs create many dispersed synaptic connections that project to the CA1 and then to the cortex for distinct memory traces that keep highly similar memories separate (Rolls, 2013).

A unique feature of the DG is its capacity to generate newborn cells into adulthood (Brown et al., 2003; Spalding et al., 2013). It is this feature that is believed to give the DG its ability to encode details needed to separate potentially overlapping memories (Becker, 2005). In rodents, the DG generates approximately 10,000 new cells per day, a number that is likely magnified 10 to 100 times in humans given the much larger hippocampal size (McDonald & Wojtowicz, 2005). Newborn cells begin as stem-like radial glia cells in the subgranular zone of the dentate gyrus, and then mature into neural progenitor cells and neuroblasts before differentiating to immature granule cells (Kempermann, Jessberger, Steiner, & Kronenberg, 2004). Although most of the immature granule cells die, ~ 40% (in rodents) make functional connections with the CA3 and then become mature granule cells after 4-6 weeks (McDonald & Wojtowicz, 2005). It is the granule cells that integrate into the mossy fibre network; this process facilitates pattern separation processing by creating new sets of random connections between the DG and CA3 that yield more unique synapses needed to reduce interference and increase the distinctions between highly similar inputs (Becker, 2005; Rolls, 2013). Accordingly, when there is a high degree of neurogenesis in the DG, the mossy fibre network is expected to encode details

creating a bias towards pattern separation processing. Conversely, under conditions of low neurogenesis, the direct perforant path between the EC and CA3 may dominate creating a bias towards pattern completion processing, leading to more general memories and increased errors in recalling the details of a specific memory. Thus, it is extremely important to identify interventions that enhance the proliferation and survival of the neurons in the DG to improve pattern separation performance. From here on, neurogenesis in the DG will be referred to as hippocampal neurogenesis.

Exercise and Neurogenesis

Exercise is a promising intervention that increases hippocampal neurogenesis and improves hippocampal-mediated memory function (Hillman, Erickson, & Kramer, 2008; van Praag, 2008; Voss, Vivar, Kramer, & van Praag, 2013). Rodent studies consistently show that aerobic exercise promotes neurogenesis by increasing cell proliferation in the DG, and suggests these newborn cells mediate the improved learning and memory associated with exercise (Creer, Romberg, Saksida, van Praag, & Bussey, 2010; Pereira et al., 2007; van Praag, Christie, Sejnowski, & Gage, 1999a; van Praag, Shubert, Zhao, & Gage, 2005; Voss et al., 2013). van Praag et al. (1999a) found that compared to controls, mice in a exercise condition had more newborn cells in the DG and performed better on a hippocampal-dependent memory task (van Praag et al., 1999a).

Creer et al. (2010) further displayed the functional importance of exercise-induced cell proliferation to hippocampal-mediated memory function by showing that an increase in newborn cells in the DG improved pattern separation performance. In this experiment, sedentary and running rodents were tested on a spatial discrimination task where identically shaped stimuli were presented in close or distal proximity. The stimuli in close proximity represented items with high interference that rely on pattern separation processing. Conversely, the stimuli in distal proximity represented items with low interference that do not rely on pattern separation processing. When stimuli were presented in distal proximity there was no difference in performance between groups. However, when the stimuli were presented in close proximity the exercised mice were better able to distinguish between similar items (Creer et al., 2010). Consistent with the literature, this suggests exercise-induced hippocampal neurogenesis enables unique synapses to separately encode highly similar inputs resulting in improved pattern separation processing.

Directly quantifying neurogenesis in humans is not yet possible, so putative neurogenesis-dependent tasks are commonly used as an index of hippocampal neurogenesis. Kirwan and Stark's (2007) Behavioural Pattern Separation-Object task (BPS-O) is a visual recognition memory task that targets pattern separation processing in the DG. The task involves differentiating between previously learned images versus novel but highly similar images, which generate a high degree of memory interference. Following an incidental learning phase, the

recognition phase asks participants to judge whether the image is an exact copy of an image already seen (exact repetitions; correct response = 'old'), highly similar but not identical to an image already seen (lures; correct response = 'similar'), or completely new (foils; correct response = 'new') (Kirwan & Stark, 2007). In this task, pattern separation and pattern completion are competing processes such that correctly identifying a lure as 'similar' indicates successful pattern separation, whereas incorrectly identifying a lure as 'old' indicates pattern completion. Participants with higher levels of hippocampal neurogenesis are better able to distinguish between highly similar images, whereas those with lower levels of hippocampal neurogenesis are more likely to misidentify lures as 'old' (Déry et al., 2013). This is also supported by age-related declines in hippocampal neurogenesis, as aging causes a shift from pattern separation to pattern completion processing in healthy individuals (Stark, Yassa, Lacy, & Stark, 2013; Toner, Pirogovsky, Kirwan, & Gilbert, 2009; Yassa & Stark, 2011).

If the shift in pattern separation versus pattern completion processing is dependent on hippocampal neurogenesis, exercise should selectively improve pattern separation processing. Déry et al. (2013) used a modified version of BPS-O to examine how a six-week aerobic exercise training intervention impacted hippocampal neurogenesis-dependent memory function. Interestingly, the results suggest the memory benefits depend on aerobic fitness gains. Participants with greater changes in fitness (i.e., high exercise responders) were significantly better at pattern separation (identifying the lures as similar) and concurrently made

fewer pattern completion errors than those with lower changes in fitness (i.e., low exercise responders) (Déry et al., 2013). This indicates that the impact of exercise on pattern separation processing depends on the individual's aerobic response to exercise, which may reflect the degree of hippocampal neurogenesis.

Markers of Neurogenesis

There is an established link between exercise, hippocampal neurogenesis, and memory function, however the underlying mechanisms are not fully understood. Brain derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1) are neural growth factors that support the proliferation, development, and survival of new neurons in the DG and may mediate the effects of exercise on memory.

Brain-Derived Neurotrophic Factor (BDNF)

Aerobic exercise up-regulates BDNF mRNA and protein in rodent hippocampi, and may be a potential mechanism explaining how exercise-induced neurogenesis improves hippocampal-mediated functions. Vaynman et al. (2004) examined whether exercise-induced improvements in learning and memory were dependent on BDNF by blocking BDNF binding in the hippocampi of exercising rodents. Exercise enhanced performance on a hippocampal-dependent task and was associated with increased BDNF mRNA levels; those with the highest BDNF expression had the best hippocampal performance. However, inhibiting BDNF binding in exercising rodents blocked the benefits of exercise on hippocampal function and actually reduced their function to the levels of sedentary controls

(Vaynman, Ying, & Gomez-Pinilla, 2004). This indicates that BDNF may be essential to obtain memory benefits from exercise.

BDNF cannot be measured non-invasively in the hippocampi of humans, but it is estimated the brain contributes to 70-80% of circulating BDNF in the periphery (Rasmussen et al., 2009). Consequently, central BDNF can be inferred by measuring BDNF in serum to predict adult-hippocampal neurogenesis and associated hippocampal function (Klein et al., 2011). Exercise increases circulating serum levels of BDNF in healthy humans and may be dependent on exercise intensity (Voss et al., 2013). Ferris et al (2007) found that BDNF serum concentrations increased more following a maximal aerobic test than endurance exercise in young adults (Ferris, Williams, & Shen, 2007). Additionally, Whiteman et al (2014) found a strong interaction between aerobic fitness and serum BDNF in predicting performance on a hippocampal-dependent memory task that targets the DG in the same manner as the pattern separation task. In that experiment, serum BDNF predicted better memory performance in young adults with high aerobic fitness (Whiteman et al., 2014), suggesting BDNF may be increasing neurogenesis to improve hippocampal function but may be dependent on aerobic fitness.

Insulin-like Growth Factor 1 (IGF-1)

IGF-1 is another growth factor that may support exercise-induced neurogenesis and the subsequent benefits on hippocampal function. Peripherally generated IGF-1 can cross the blood brain barrier to bind with receptors in the

hippocampus and impact neurogenesis. Exercise stimulates the uptake of IGF-1 from the bloodstream to the hippocampus causing an increase in both the proliferation and survival of adult-born neurons (Carro, Nuñez, Busiguina, & Torres-Aleman, 2000; Trejo, Carro, & Torres-Alemán, 2001). Trejo et al. (2001) found that exercising rodents had increased hippocampal neurogenesis following exercise; however, blocking the entrance of circulating IGF-1 into the brains of exercising rats inhibited exercise-induced hippocampal neurogenesis (Trejo et al., 2001). Additionally, rodents with low serum IGF-1 had reduced neurogenesis and impaired hippocampal-function compared to controls. Interestingly, the administration of IGF-1 subcutaneously was able to restore neurogenesis and hippocampal function to control levels (Trejo et al., 2007; Trejo, Llorens-Martin, & Torres-Alemán, 2008). This indicates the importance of IGF-1 for hippocampal neurogenesis and function. Importantly, Whiteman et al (2014) found a positive correlation between serum IGF-1 and aerobic fitness in young adults (Whiteman et al., 2014), suggesting like in rodents, exercise also enhances IGF-1 and that this process depends on the individual's aerobic fitness level.

IGF-1 and BDNF may work together to increase hippocampal neurogenesis and hippocampal function. Exercise-induced up-regulation of IGF-1 is associated with increased levels of BDNF in the brain, indicating that BDNF may be a potential downstream target of IGF-1 that is responsible for adult hippocampal neurogenesis (Berchtold, Chinn, Chou, Kesslak, & Cotman, 2005; Trejo et al., 2001; Voss et al., 2013). Ding et al. (2006) found that exercising

rodents had significantly higher levels of both IGF-1 and BDNF mRNA than sedentary controls. However, blocking the action of IGF-1 in the hippocampus reversed exercise-induced increases in BDNF mRNA and hippocampal function, suggesting BDNF and its effects may depend on IGF-1 (Ding, Vaynman, Akhavan, Ying, & Gomez-Pinilla, 2006).

IGF-1 may also mediate exercise-induced neurogenesis through its effect on angiogenesis (Lopez-Lopez, LeRoith, & Torres-Aleman, 2004). Angiogenesis is the formation of new blood vessels from pre-existing vasculature, and may indirectly impact neurogenesis by providing the necessary resources for new neural networks to develop upon (Hillman et al., 2008). Aerobic exercise leads to the development of capillaries, which provide structure for the migration of new neurons and metabolic support essential for the proliferation and survival of new neurons. As capillaries can be imaged with magnetic resonance imaging, measuring cerebral blood volume has also been used as an indicator of neurogenesis. Pereira et al. (2007) relied on the coupling between angiogenesis and neurogenesis to determine if cerebral blood volume could provide an in vivo correlate of hippocampal neurogenesis. After first confirming that exercise selectively increased DG cerebral blood volume and correlated with post-mortem measurements of neurogenesis in rodents, they observed a selective increase in human DG cerebral blood volume following 12 weeks of exercise that correlated with both cardiopulmonary and memory functions (Pereira et al., 2007).

Enrichment

In addition to exercise, environmental enrichment is another lifestyle factor thought to enhance hippocampal neurogenesis, as consistently demonstrated in rodents (Clemenson, Deng, & Gage, 2015; Kempermann, Kuhn, & Gage, 1997; van Praag, Kempermann, & Gage, 1999b). Cell proliferation in the DG is affected by exercise; however, cell survival, maturation, and integration into the mossy fibre network seem to be dependent on environmental enrichment. Enriched environments provide opportunity for cognitive stimulation and are typically comprised of larger cages, moveable toys, mazes, and running wheels. Kemperman et al. (1997) found that although no difference existed in the initial number of proliferating cells in the hippocampus, a greater percentage of those cells survived four weeks later in rodents in enriched environments compared to those in standard conditions. Like exercise alone, this type of environmental enrichment was sufficient to induce behavioural improvements on hippocampal-dependent memory tasks, and is presumably due to the enhanced cell support the enrichment provides to the developing neurons. However, the enriched environment used in this experiment consisted of a combination of cognitive learning opportunities, social interaction, and physical activity (Kempermann et al., 1997). Thus, it is unclear whether the observed neurogenesis was driven by the exercise alone.

Van Praag et al. (1999b) investigated the separate contributions of exercise and environmental enrichment on hippocampal neurogenesis and found that

exercise and environmental enrichment enhanced different aspects of neurogenesis. Exercise increased new cell proliferation, whereas environmental enrichment did not. In contrast, environmental enrichment increased the percentage of newborn cells that survived, whereas exercise did not (van Praag et al., 1999b). This supports the idea that exercise and enrichment act through different and possibly complimentary mechanisms to promote hippocampal neurogenesis. Enriched environments seem to have little influence on the proliferative activity of progenitor cells in the DG, but instead heighten cell survival, which may be essential for behavioural benefits.

A key component of environmental enrichment seems to be cognitive stimulation involving tasks that target DG specific tasks of the hippocampus. Gould et al. (1999) examined whether hippocampal-dependent learning altered the survival of newborn cells in the DG by engaging rodents in hippocampal-dependent or hippocampal-independent memory tasks during the time the immature granule cells usually die. Training on the hippocampal-dependent task elicited a neuroprotective effect, as these rodents had an increased number of surviving cells in the DG compared to rodents that trained on the hippocampal-independent task (Gould, Beylin, Tanapat, Reeves, & Shors, 1999). These results suggest that training tasks that challenge the functionality of the hippocampus may be a critical component of environmental enrichment that helps the cells survive and integrate into the hippocampal network.

Indeed, this is representative of what is observed in human cognitive training. Like environmental enrichment, cognitive training does not typically transfer to other cognitive domains or to other untrained tasks that are closely cognitively related (Owen et al., 2010). The majority of cognitive training literature focuses on training executive functions, processing speed, or global cognition. Memory specific cognitive training has been examined, but often focuses on enhancing memory strategies for older adults rather than training on tasks that recruit the hippocampus (Cavallini, Pagnin, & Vecchi, 2003). Another common approach is to use a battery of regimes for training multiple cognitive functions (Fabre, Chamari, Mucci, Masse-Biron, & Prefaut, 2002; Oswald, Gunzelmann, Rupprecht, & Hagen, 2006) but again does not concentrate on hippocampal-dependent tasks, displaying how the current memory training literature relies very little on hippocampal-dependent learning.

Interestingly, the studies that have used memory training have mixed results with respect to the transfer of cognitive benefits to untrained tasks. In a study by Owen et al. (2010), participants were trained on a battery of cognitive tasks, one of which trained general memory recall and required participants to remember the location of objects on cards. As expected, improvements were observed on all training tasks, but did not generalize to untrained tasks that used similar cognitive functions. Instead, performance actually decreased on an untrained task that like the trained task, targeted general memory recall (Owen et al., 2010). Conversely, Mahncke et al. (2006) trained older adults using a verbal

recall and recognition task, and observed transfer effects to an untrained verbal recall task in addition to the trained task-specific improvements (Mahncke et al., 2006). Critically, neither of these training paradigms focused on tasks that require pattern separation. Given the potential for plasticity in the DG, it is possible that the largest transferable memory benefits would result from training DG specific functions.

Neurotrophic Factors and Enrichment

Like exercise, environmental enrichment also increases BDNF. Rodents housed in enriched environments display increased expression of BDNF mRNA and improved performance on hippocampal-mediated tasks (Falkenberg et al., 1992). Rossi et al (2006) created BDNF knockout rodents to determine whether BDNF was responsible for mediating the effects of enriched environments on the survival of newborn neurons in the DG. As expected, rodents in enriched environments showed a 2-fold increase in hippocampal neurogenesis compared to rodents in standard conditions. However, the effects of environmental enrichment on hippocampal neurogenesis were eliminated in the BDNF knockout rodents and instead cell survival was similar to controls (Rossi et al., 2006). Thus, BDNF may be required for supporting hippocampal cell survival induced by enriched environments.

In contrast to exercise, the effect of environmental enrichment on IGF-1 has not been examined.

Exercise and Enrichment

Taken together, exercise and environmental enrichment promote hippocampal neurogenesis and function through different mechanisms; exercise increases cell proliferation, while enrichment enhances the survival of the cells that are already present. Combining these mechanisms is expected to have the greatest benefits for hippocampal-dependent functions, such as pattern separation. A few studies have examined the effects of combined exercise and cognitive training interventions and have yielded mixed results; however they are limited in some important ways. Mustroph et al. (2012) housed mice for 32 days in a running only condition, an enrichment only condition, a combined running and enrichment condition, or a control condition. Exercise seemed to be the critical component that drove hippocampal neurogenesis and improved hippocampal function. In contrast, the combination of running and enrichment did not increase hippocampal neurogenesis over the running condition alone. However, in this experiment the enriched environment consisted of bedding, drinking solutions, treats, and toys that engaged sensory modalities and thus this form of enrichment did not appropriately target the functions of the DG (Mustroph et al., 2012).

A few studies have also examined the combined effects of exercise and cognitive training in humans. Fabre et al. (2002) found that older adults who engaged in combined exercise and memory training had the greatest improvement on a clinical memory score than either training alone. Although a positive effect of the combined intervention was found, only one of the eight memory training

sessions in the program focused on pattern separation, with the rest focusing on other cognitive functions. Additionally the clinical memory score tested a variety of cognitive functions, which may have accounted for the observed improvement, even though the training did not target the DG (Fabre et al., 2002). Furthermore, combined exercise and cognitive intervention studies in humans have been limited to testing older adults and because hippocampal neurogenesis declines with age (Voss et al., 2013), the effects of combining exercise with cognitive training may be more robust in young adults.

Neurotrophic Factors and Combined Exercise and Enrichment

Both exercise and environmental enrichment independently induce increases in BDNF; thus, the combination may enhance BDNF over either training alone. The combined effects on BDNF were examined in a study by Kobilo et al. (2013) in which rodents were housed in control, running, enriched, or combined running and enriched conditions. Hippocampal neurogenesis and BDNF increased in the combined and running conditions compared to the enriched and control conditions. Surprisingly, no differences in BDNF were observed between the combined and running conditions or the enriched and control conditions, suggesting that exercise was the critical factor driving the increased BDNF (Kobilo et al., 2011). However, the enriched environment consisted of movable sets of wood chunks, crawl balls, and huts that did not enhance hippocampal neurogenesis or target DG dependent functions. Thus, it is

possible that training on hippocampal-dependent functions that are sufficient to induce hippocampal neurogenesis, may elicit concurrent increases in BDNF.

The effects of combined exercise and environmental enrichment on IGF-1 have not been examined. As a result of the literature on exercise and IGF-1, it is presumed that exercise would be the critical factor driving increases in IGF-1 in a combined intervention.

Purpose and Hypothesis

The present study examined the effects of six weeks of exercise and cognitive training on hippocampal-mediated memory processes and neurotrophic factors (BDNF, IGF-1) in young adults to determine whether combined training enhances pattern separation processing above and beyond either training regime alone. Sixty-six sedentary young adults were randomly assigned to one of four groups: 1) Exercise training group, 2) Cognitive training group, 3) Combined exercise and cognitive training group, or 4) No-contact control group. Hippocampal-dependent memory tasks and serum neurotrophic factors (BDNF and IGF-1) were assessed pre and post intervention. Based on the literature, the combination of exercise and cognitive training was expected to enhance hippocampal neurogenesis and improve pattern separation performance. Consistent with the findings by Déry et al. (2013), changes in memory processing were expected to be augmented in high responders to the exercise training (i.e., those with greater aerobic fitness gains). Additionally, if BDNF and IGF-1 prove to be involved in the mechanisms responsible for the observed changes in

memory, they too should show the greatest increases for combined training and be dependent on individual changes in aerobic fitness.

METHOD

Participants

Sixty-six healthy young adults (32 female; mean age= 21.03 years; age range= 17-30 years) participated in the study (adherence rate = 72%). All participants provided written informed consent and met the inclusion criteria of engaging in no more than one hour of vigorous physical exercise per week, which was assessed through verbal confirmation and by participants' answers on the physical activity section of the Yale Activity questionnaire (Dipietro, Caspersen, Ostfeld, & Nadel, 1993).

All participants were compensated $10 per hour for the 4 hours of pre and post intervention testing. Participants who were assigned to groups taking part in the 6-week intervention also received an additional $130 or $120 and course credit.

Procedure

The experimental procedure included pre and post intervention testing, separated by a 6-week intervention. Pre and post testing were comprised of a fasted blood draw, followed by one hour of cognitive testing, and a half hour of fitness testing. Post-testing was at most 48 hours after completing the intervention. The study consisted of a 2 X 2 design in which participants were exercise trained or not, and cognitive trained or not, resulting in participants being

stratified by sex and randomly assigned to one of four groups for the duration of the intervention: 1) Exercise training group, 2) Cognitive training group, 3) Combined exercise and cognitive training group, or 4) No-contact control group. The measures used in the pre and post intervention testing, and the training protocols are described in detail below.

Pre and Post Intervention Testing

Neurotrophic factors

Peripheral blood samples were collected from participants following a 12-hour fasting period. To obtain serum samples, peripheral whole blood was collected in BD Vacutainer SST tubes (BD, Franklin Lakes, NJ, USA). Immediately following blood collection, the tubes were chilled on ice for a half hour and then centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatant was then extracted and aliquoted into cryovials for storage at -80°C until the samples were needed for analysis.

Serum BDNF and IGF-1 proteins were quantified using enzyme-linked immunosorbent assays (ELISA) and were measured according to kit specifications. Samples and standards for each ELISA were run in duplicate and the absorbency of samples were measured at 450 nm with a reference at 540 nm using Multiskan GO UV/Vis microplate spectrophotometer and SKANIT: 3.2 software (Thermo-Fisher Scientific). Serum BDNF was measured using the human BDNF DuoSet ELISA kit (R&D Systems; Minneapolis, MN, USA; cat #DY248) and serum IGF-1 was measured using human IGF-1 Quantikine®

ELISA kit (R&D Systems, cat #DG100). Serum samples measured by the BDNF ELISA were diluted 75x, while serum samples measured by the IGF-1 ELISA were diluted 100x using Reagent Diluent (R&D Systems).

Cognitive Testing

Hippocampal-mediated memory function was assessed using two computer tasks: the *Concentration Memory Game* and the *Pattern Separation Task*. Participants also completed other cognitive tasks of executive functions, reaction time, and spatial memory that were not analyzed as part of this thesis because they do not target the functions of the hippocampus and are not neurogenesis-dependent.

Concentration Memory Game

Participants were presented with a 4 X 3 array of 12 facedown cards. Two copies of six different faces were hidden beneath the cards. In order to target the DG and increase the demand for pattern separation processing on this task, all faces were the same size, grey-scaled, and without hair. Participants first completed an incidental learning phase in which they were instructed to learn the locations of the faces with the aim of finding matched pairs. During each trial, participants selected two cards to reveal the faces below. Regardless of uncovering a matched pair of faces, all cards returned to a facedown position upon the completion of each trial. Correctly matched pairs of faces were displayed on the side. Once participants found all the matched pairs of faces, their memory for the face locations was tested both immediately and following a ~30-minute

delay. Performance on the *Concentration Memory Game* was based on the number of mismatches made on the memory test following the 30-minute delay, with less mismatches indicating better memory performance.

Putative Neurogenesis Dependent Task

An adapted version of Kirwan and Stark's (2007) Pattern Separation task was used to assess hippocampal-mediated memory function as an index of hippocampal neurogenesis. The task began with an incidental learning phase in which 60 full colour items were presented on the screen for 2 seconds each and participants were asked to indicate whether the stimuli were indoor or outdoor objects. Following the learning phase, participants completed a forced-choice visual recognition task. In the recognition phase, a series of 90 items were presented; 30 items were repeated from the previous learning phase 'repetitions', 30 items were highly similar but not identical to ones in the previous learning phase 'lures', and 30 items were completely new 'foils'. The items were presented one at a time in a random order for 2500ms each, with a 500ms inter-trial interval. Participants were asked to judge whether a presented item was old, similar, or new. Correct responses were made when repetitions were classified as old, lures were classified as similar (but not identical to the target stimulus), and foils were classified as new. A correction factor was applied in order to control for response bias across groups using the response made to the new items (Yassa & Stark, 2011).

A manipulation check was applied to make sure participants were performing the task correctly. Classifying foils as 'new' is a relatively simple task with participants scoring near ceiling (M=0.87, SD=0.2). Accordingly, participants who scored more than two standard deviations away from the mean accuracy on classifying foils as new were thought to not have understood or disregarded task instructions. Five participants' data were discarded leaving 59 for subsequent analysis of *General Memory Performance*, *Pattern Separation Performance*, *Pattern Completion Mistakes*, and *Pattern Separation Bias*.

General Memory Performance was defined by recall performance, or the ability to correctly identify a repetition as 'old'. This was assessed by subtracting the proportion of 'old' responses given the presentation of unrelated foils (new) from the proportion of correct 'old' responses given a repetition (old) [p("Old"| Repetition) − p("Old"| Foil)]. The change in *General Memory Performance* was determined by subtracting the proportion of correctly identified repetitions as old pre-intervention from post-intervention.

Pattern Separation Performance was defined by the ability to distinguish previously learned items (repetitions) from highly similar items (lures) and correctly identify lure items as similar. This is presumed to benefit from greater hippocampal neurogenesis. *Pattern Separation Performance* was assessed by subtracting the proportion of 'similar' responses given the presentation of unrelated foils (new) from the proportion of correct 'similar' responses given a lure (similar) [p("Similar"|Lure) − p("Similar"|Foil)]. The change in *Pattern*

Separation Performance was determined by subtracting the proportion of correctly identified lures as similar pre-intervention from post-intervention.

In contrast, misidentifying a lure item as 'old' was indicative of *Pattern Completion Mistakes*. *Pattern Completion Mistakes* were assessed by subtracting the proportion of 'old' responses given the presentation of unrelated foils (new) from the proportion of incorrect 'old' responses given a lure (similar) [p("Old"|Lure) − p("Old"|Foil)]. The change in *Pattern Completion Mistakes* were determined by subtracting the proportion of misidentified lures as 'old' pre-intervention from post-intervention, with less mistakes indicating better performance.

Thus, to assess if there was a shift towards pattern separation processing from pre to post intervention, *Pattern Separation Bias* was calculated by subtracting the change in *General Memory Performance* from the change in *Pattern Separation Performance.* The measure of *General Memory Performance* was determined by memory recall, which relies on the pattern completion processing structures of the hippocampus. As pattern separation and pattern completion are competing processes, we presumed a positive change in *Pattern Separation Bias* from pre to post intervention would indicate a preference for pattern separation processing over pattern completion processing, and greater hippocampal neurogenesis.

Fitness Testing

Peak oxygen consumption (VO_2 peak) was measured to determine participants' aerobic fitness. VO_2 peak was obtained by continuously measuring oxygen consumption during an incremental exercise test to exhaustion on a cycle ergometer (Lode Excalibur Sport V20; Groningen, The Netherlands). Prior to each fitness test, gas analyzers were calibrated using standard reference gases (VitalAire; Missisauga, Canada) and turbine volume was calibrated using a 3L gas syringe (AEI Technologies). Participants cycled at 50W for a one-minute warm up. The workload then increased by 30W every minute until the participant experienced volitional fatigue, or cycle cadence dropped below 50 rpm. Oxygen consumption (mL/kg/min) and power output (W) were recorded every 15 seconds using a metabolic cart with an online gas collection system (MOXU modular VO2 System; AEI Technologies, Pittsburgh, PA). VO_2 peak was obtained from the highest oxygen consumption value and maximum wattage was obtained from the peak power output value. Maximum heart rate was measured using *Polar RS300X* heart rate monitors.

Intervention Training

Exercise Training

Participants in the Exercise training group and the Combined exercise and cognitive training group, engaged in high intensity interval training (HIIT). Participants trained for an average of 17 sessions (SD = 1.02) of 20-minute duration ~ 3 times/ week for 6 weeks. Pre intervention maximum wattage and maximum heart rate were used to create individualized exercise protocols.

Participants completed HIIT on stationary cycle ergometers (*Lifecycle 95Ci*). The protocol consisted of a 3-minute warm up with a resistance of 50W, followed by ten one-minute alternating high intensity and recovery intervals, before finishing with a 2-minute cool down at 50W. The high intensity intervals consisted of one minute of cycling at 90% of participants' maximum wattage between 80 and 120 rpm, while the recovery intervals consisted of one minute of cycling at 50W at a minimum of 50 rpm. Upon completion of each interval, heart rate and ratings of perceived exertion (scale 1-10) were recorded. Heart rate was monitored using *Polar RS300X* heart rate monitors. Wattage for the high intensity intervals was increased every week to maintain a desired target heart rate of 90% peak. Participants progressed through the levels at an individualized pace for the duration of the intervention.

To examine individual differences due to fitness adaptations, participants in the exercise-trained groups (Exercise training group and the Combined exercise and cognitive training group) were split into low responders to the exercise training (n=18; mean Δ VO_2 peak= 0.99 mL/kg/min; SD = 3.89 mL/kg/min) and high responders to the exercise training (n=17; mean Δ VO_2 peak = 12.21 mL/kg/min; SD = 6.59 mL/kg/min) based on a median split of their change in VO_2 peak (4.7 ml/kg/min).

Cognitive Training

Participants in the Cognitive training group and the Combined exercise and cognition training were trained on a hippocampal-dependent task. Spatial

memory and pattern separation processing were targeted using an adaptive-difficulty version of the *Concentration Memory Game*. Participants trained for an average of 17 sessions (SD= 1.86) of 20-minute duration ~3 times/ week for 6-weeks. Like the pre and post intervention testing version of the game, participants were presented with a 4 X 3 array of 12 facedown cards and were instructed to find the six matching pairs of faces. Once all the matched pairs of faces were found, memory for the face locations was tested following a 5-minute delay, in which participants played a filler task (Bejeweled online puzzle game). Performance was based on the number of mismatches made on the memory test following the 5-minute delay, with fewer mismatches indicating better memory performance. If three or fewer mismatches were made on the subsequent memory test, participants would advance to a level of greater difficulty in which the array of cards increased by two face pairs, or four cards. Participants progressed through the levels at an individualized pace for the duration of the intervention.

Combined Exercise and Cognitive Training

Participants in the Combined exercise and cognitive training group participated in both training protocols for 6 weeks, performing the cognitive training immediately prior the exercise training.

Control participants did not train.

Statistical Analysis

Manipulation Checks

To confirm that the groups did not differ on key measures of aerobic fitness, memory performance, and neurotrophic factors at baseline, separate one-way analysis of variance (ANOVAs) were conducted on pre-intervention values with a between-subject factor of group.

To check that the exercise and cognitive training were adaptive processes, repeated measures ANOVAs were conducted on the average wattage of the high intensity intervals for the exercise training, and the average level completed for the cognitive training, with a within-subject factor of week (6) and a between subject factor of group (exercise training: Exercise training group vs. Combined exercise and cognitive training group; cognitive training: Cognitive training group vs. Combined exercise and cognitive training group).

To verify that the interventions induced the expected changes in pre and post measures, separate 2 X 2 ANOVAs were conducted on post- minus pre-intervention values of VO_2 peak and the number of errors on the trained memory task with two between-subject factors of exercise training (trained vs. not trained) and cognitive training (trained vs. not trained).

Group-based analyses

To evaluate the effect of the interventions on the untrained memory tasks and neurotrophic factors for each group, planned one-sample *t*-tests (one-tailed) with a criterion comparison value of zero (i.e., no change) were conducted on post- minus pre-intervention values. It was expected that pattern separation processing would increase, while pattern completion processing would decrease

as a result of the training. Group differences were also assessed using separate 2 X 2 factorial ANOVAs with two between-subject factors of exercise training (trained vs. not trained) and cognitive training (trained vs. not trained).

Individual-differences analyses

To evaluate individual differences in response to exercise training on the untrained memory task and neurotrophic factors, separate 2 X 2 factorial ANOVAs with two between-subject factors of exercise responder (high vs. low) and cognitive training (trained vs. not trained) were conducted on post- minus pre-intervention values.

RESULTS

Manipulation Checks

At baseline, groups did not differ on key measures of aerobic fitness, memory performance, and neurotrophic factors (all $p > 0.15$) (Table 1).

Exercisers and cognitive trainers improved on the trained task. For exercisers, the average wattage of the high intensity exercise intervals increased over time $F(5, 165) = 532.21$, $p < 0.001$ (Figure 1). For the cognitive trainers, the average level completed on the cognitive training task also increased over time $F(5, 160) = 100.95$, $p < 0.001$ (Figure 2).

The interventions induced the expected changes on the pre-post measures such that exercise training increased VO_2 peak [Main effect of exercise training $F(1, 61) = 7.91$, $p < 0.01$] and cognitive training decreased errors on the trained memory task [Main effect of cognitive training $F(1, 59) = 9.34$, $p < 0.01$].

Group-based analyses

Untrained memory tasks. Table 1 presents the mean performance on the untrained memory tasks for the four groups. Figure 3 depicts the *Pattern Separation* Bias for the untrained memory task. Combined exercise and cognitive training elicited the only significant changes in performance on the untrained memory tasks with increased *Pattern Separation Performance* [$t(16) = 1.73$, $p = 0.05$], less *Pattern Completion Mistakes* [$t(16) = -1.52$, $p = 0.07$], and decreased performance for *General Memory Performance*: $t(16) = -2.32$, $p < 0.05$]. This resulted in an increased pattern separation bias [$t(16) = 2.91$, $p < 0.01$] for the Combined exercise and cognitive training group only. None of the other groups had significant changes in performance on the untrained memory task and there were no significant differences between groups.

Neurotrophic factors. Table 1 presents the mean concentration of BDNF and IGF-1 for the four groups. No groups had significant changes in BDNF or IGF-1 concentrations and there were no significant differences between groups.

Individual-differences analyses

Untrained memory tasks. Table 2 presents the mean performance on the untrained memory tasks for high versus low exercise responders that were or were not cognitively trained. High exercise responders that were cognitively trained had a greater increase in performance for the untrained memory tasks than high exercise responders that were not cognitively trained [Interaction of exercise responder X cognitive training for *Pattern Separation Performance*: $F(1, 29) =$

8.01, $p < 0.01$; *Pattern Completion Mistakes*: $F(1, 29) = 3.68$, $p = 0.065$; *Pattern Separation Bias*: $F(1, 29) = 6.64$, $p < 0.05$]. Post-hoc independent samples *t*-tests comparing change in performance for high exercise responders that were cognitively trained or not [*Pattern Separation Performance*: $t(13) = 3.39$, $p < 0.01$; *Pattern Completion Mistakes*: $t(13) = -1.98$, $p < 0.05$; *Pattern Separation Bias*: $t(13)= 3.09$, $p < 0.01$]. There was no significant difference in memory change for low exercise responders that were or were not cognitively trained. No significant differences were observed for general recall performance on the untrained memory task between high and low exercise responders that were or were not cognitively trained.

Neurotrophic factors. Figure 4 depicts the mean change in BDNF and IGF-1 concentrations for high versus low exercise responders that were or were not cognitively trained. High exercise responders had greater increases in BDNF and IGF-1 serum concentration levels than low exercise responders [Main effects of exercise responder for BDNF: $F(1, 29) = 7.81$, $p < 0.01$; IGF-1: $F(1, 29) = 5.09$, $p < 0.05$].

Table 1. Mean values for aerobic fitness, memory performance, and neurotrophic factors across groups.

	Combined exercise and cognitive training group			Exercise training group			Cognitive training group			No-contact control group		
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ
VO_2 Peak	32.28 (1.75)	37.87 (2.47)	5.60*** (1.27)	36.74 (1.77)	44.19 (2.00)	7.45** (2.47)	36.58 (2.32)	39.03 (2.81)	2.45 (1.81)	33.71 (2.51)	33.20 (3.19)	0.23 (1.70)
Trained Memory Task Errors	5.84 (1.28)	2.16 (0.40)	-3.68** (1.36)	5.27 (0.96)	7.69 (1.67)	2.87 (2.01)	6.8 (1.73)	3.25 (1.23)	-3.53* (1.74)	6.5 (1.43)	6.33 (1.27)	0.21 (1.64)
General Memory	0.86 (0.03)	0.80 (0.04)	-0.07* (0.03)	0.88 (0.02)	0.88 (0.01)	0.00 (0.03)	0.83 (0.03)	0.87 (0.03)	0.03 (0.03)	0.76 (0.07)	0.77 (0.06)	0.00 (0.03)
Pattern Separation	0.41 (0.05)	0.53 (0.05)	0.10* (0.06)	0.53 (0.06)	0.60 (0.04)	0.07 (0.04)	0.52 (0.06)	0.60 (0.05)	0.07 (0.05)	0.42 (0.05)	0.46 (0.06)	0.04 (0.04)
Pattern Completion Mistakes	0.37 (0.04)	0.28 (0.02)	-0.08$^{\#}$ (0.05)	0.32 (0.04)	0.29 (0.03)	-0.03 (0.03)	0.32 (0.05)	0.26 (0.04)	-0.06 (0.05)	0.34 (0.03)	0.33 (0.05)	-0.01 (0.04)
BDNF	27.20 (2.54)	25.70 (2.25)	-1.16 (2.61)	31.66 (2.12)	28.20 (2.65)	-3.30 (3.89)	23.66 (2.71)	26.34 (2.41)	2.68 (2.93)	28.73 (2.32)	32.07 (1.83)	3.34 (3.02)
IGF-1	191.01 (13.24)	179.64 (14.09)	-12.03 (6.62)	203.82 (17.16)	208.04 (13.74)	9.03 (8.25)	183.30 (10.40)	181.81 (10.38)	-1.48 (8.19)	179.70 (10.28)	185.41 (8.63)	5.71 (7.60)

$^{*}p < 0.05$
$^{**}p < 0.01$
$^{***}p < 0.001$
$^{\#}p = 0.07$

Figure 1. Mean wattage of the high intensity interval over the 6-week exercise training period for the Exercise training group and the Combined exercise and cognitive training group.

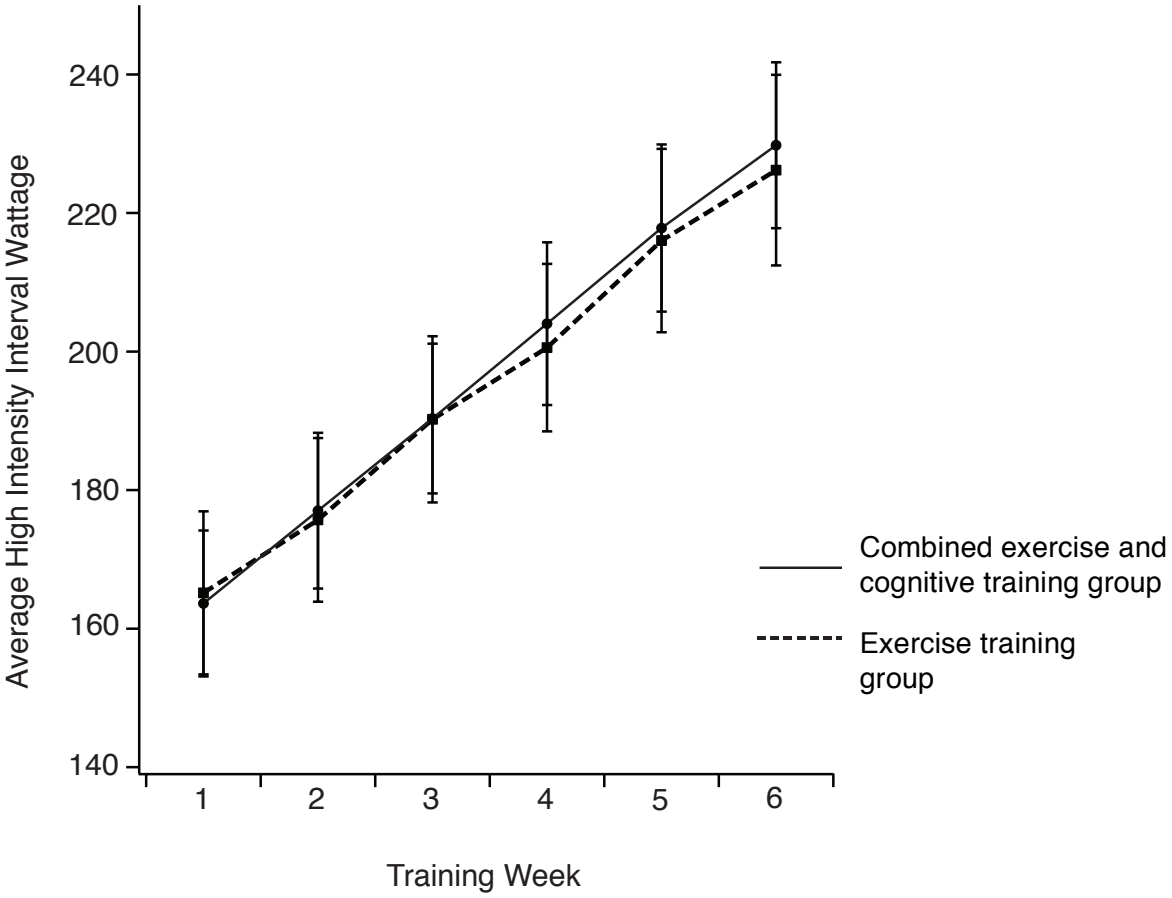


Figure 2. Mean level completed and standard error on the Concentration Memory Game of the cognitive training protocol for the Cognitive training group and the Combined exercise and cognitive training group.

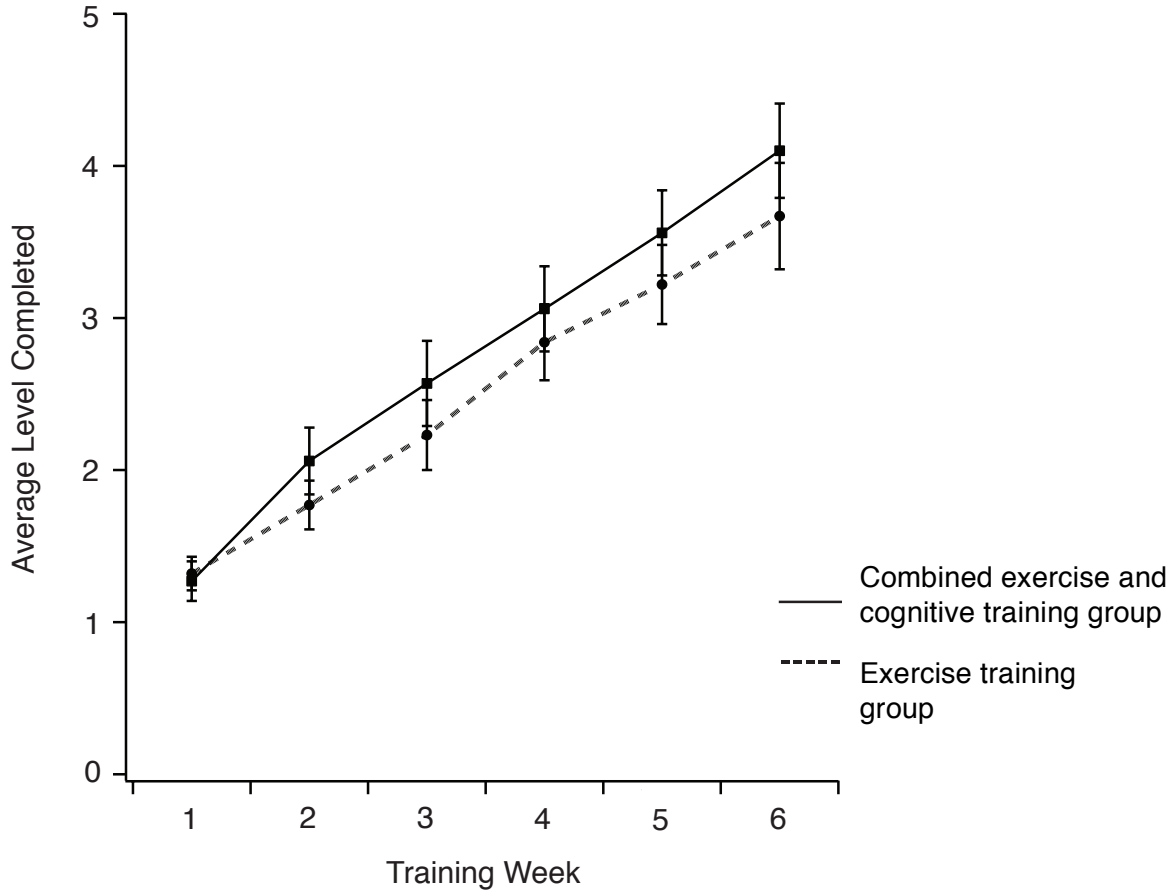


Figure 3. Mean Pattern Separation Bias and standard error for the untrained neurogenesis-dependent memory tasks across groups. * $p < 0.01$.

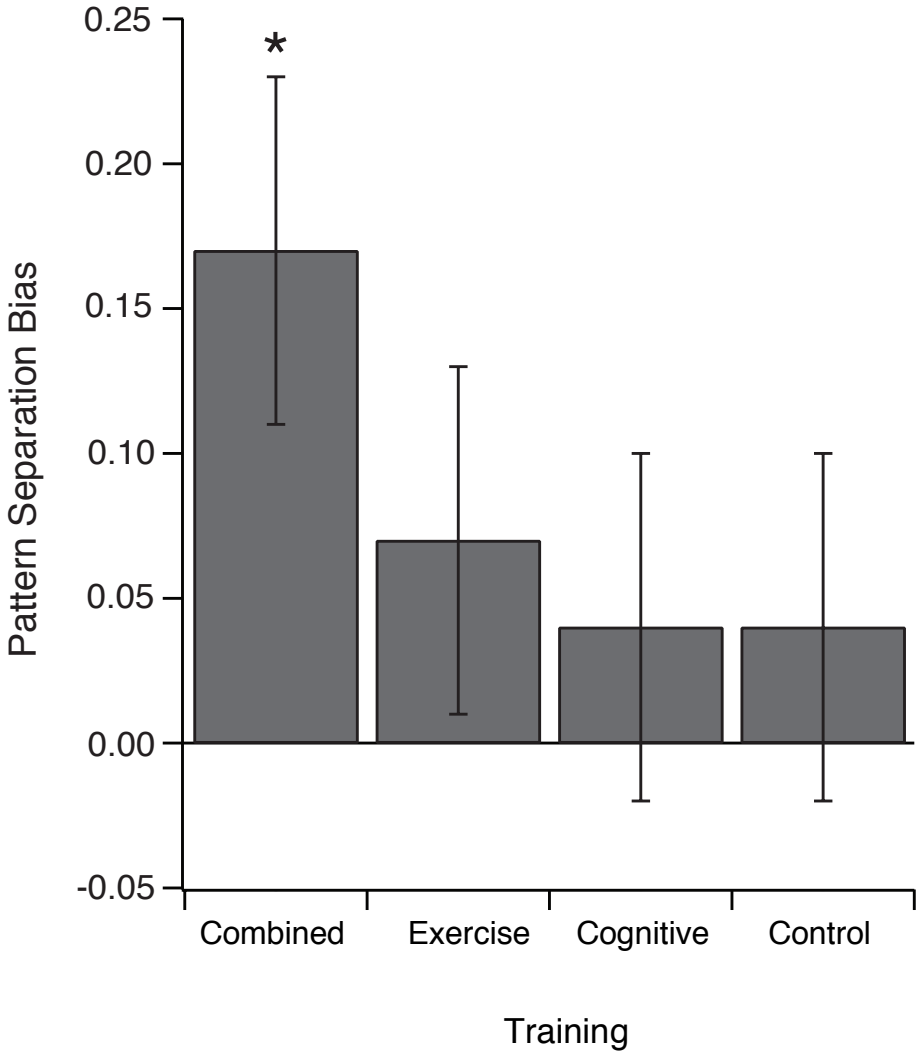
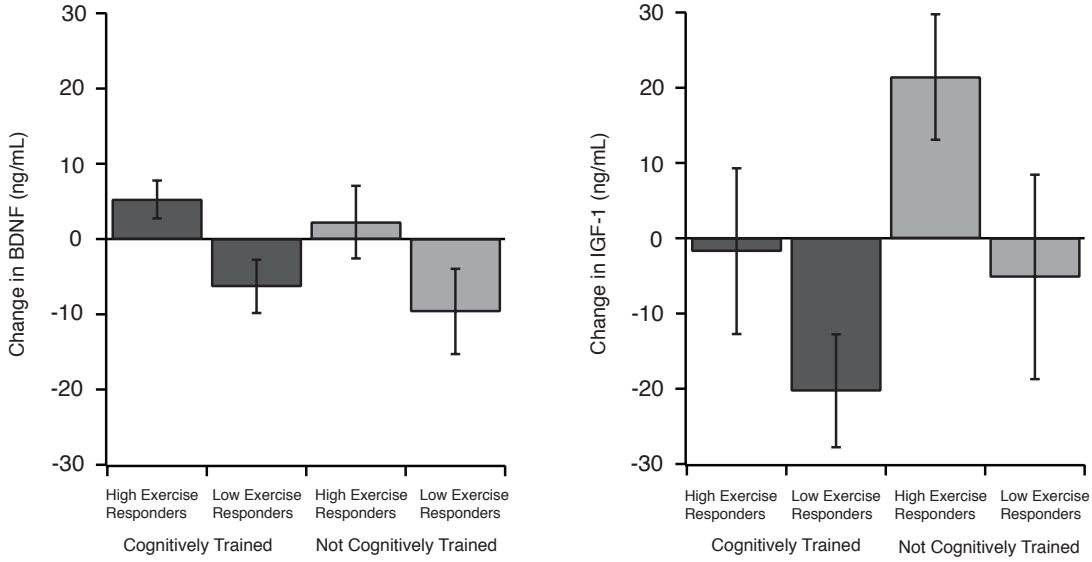


Table 2. Mean performance on the untrained neurogenesis-dependent memory task for high versus low exercise responders that were or were not cognitively trained.

	High exercise responders						Low exercise responders					
	Cognitively trained			Not cognitively trained			Cognitively trained			Not cognitively trained		
	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ	Pre	Post	Δ
General Memory	0.86 (0.04)	0.81 (0.07)	-0.07 (0.05)	0.86 (0.04)	0.86 (0.02)	0.00 (0.04)	0.86 (0.03)	0.80 (0.06)	-0.06 (0.03)	0.90 (0.03)	0.90 (0.01)	0.00 (0.04)
Pattern Separation	0.38 (0.07)	0.63 (0.04)	0.23** (0.08)	0.54 (0.05)	0.53 (0.04)	-0.01 (0.07)	0.43 (0.07)	0.46 (0.08)	0.03 (0.06)	0.53 (0.11)	0.70 (0.04)	0.18 (0.07)
Pattern Completion Mistakes	0.38 (0.08)	0.21 (0.02)	-0.16# (0.07)	0.31 (0.04)	0.32 (0.04)	0.01 (0.06)	0.36 (0.04)	0.33 (0.02)	-0.03 (0.05)	0.33 (0.08)	0.25 (0.04)	-0.08 (0.06)
Pattern Separation Bias	-0.48 (0.08)	-0.18 (0.05)	0.30* (0.09)	-0.32 (0.07)	-0.33 (0.06)	-0.01 (0.07)	-0.24 (0.05)	-0.33 (0.04)	0.09 (0.07)	-0.37 (0.12)	-0.19 (0.04)	0.18 (0.08)

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$
# $p = 0.065$

Figure 4. Mean change in BDNF and IGF-1 and standard error for high versus low exercise responders that were or were not cognitively trained.



DISCUSSION

The present study examined the effects of exercise and cognitive training on hippocampal-mediated memory processes and neurotrophic factors (BDNF, IGF-1) to determine whether the combination of exercise and cognitive training enhances pattern separation processing more than either training alone. Sedentary young adults were randomly assigned to one of four six-week programs: 1) exercise training, 2) cognitive training 3) combined exercise and cognitive training, or 4) a no-contact control group. Critically, the combination of exercise and cognitive training led to the greatest increase in memory requiring pattern separation processing but also the greatest decrease in memory requiring pattern

completion processing. BDNF and IGF-1 were not associated with this change in memory performance but were affected by the individual's response to the exercise training. Thus, exercise and cognitive training may work through complimentary neurological pathways to enhance neurogenesis and bias hippocampal function towards pattern separation processing. However, BDNF and IGF-1 may not be mediating the observed changes in memory.

The combination of exercise and cognitive training significantly improved pattern separation memory performance, whereas exercise and cognitive training alone did not. This is consistent with our hypothesis and suggests that exercise and cognitive training may work through complimentary mechanisms to enhance hippocampal neurogenesis and provide synergistic benefits to pattern separation processing. This is the first study in humans to show enhanced hippocampal-mediated function from combined exercise and cognitive training. Exercise and cognitive training likely promote hippocampal neurogenesis and function through different mechanisms. In animal models, exercise increases cell proliferation whereas cognitive training supports cell survival (Clemenson et al., 2015; Voss et al., 2013); thus, together a greater number of new cells may be able to survive and integrate into the hippocampal network, accounting for the observed behavioural benefits.

The combined benefit also reveals that cognitive training elicited transfer benefits to the untrained neurogenesis-dependent task. Consistent with the literature, the greatest benefits of the cognitive training were observed for the

trained task, but benefits for the untrained task were also observed, which relied on the trained pattern separation functions. Cognitive training does not typically benefit untrained tasks, even when they engage the same cognitive functions (Owen et al). Critically, the cognitive transfer effects observed here only occurred when combined with exercise training, which is a novel finding, and suggests exercise may prime the brain to benefit from the cognitive training.

The improved pattern separation performance from combined exercise and cognitive training was accompanied by a decrease in pattern completion mistakes. This has been seen before (Déry et al., 2013), and makes sense given the impact of exercise and cognitive training on hippocampal neurogenesis in animal models and the role that the DG plays in memory processing (Rolls, 2013). Increased DG neurogenesis enables more granule cells to integrate into the mossy fibre network, and may have facilitated pattern separation processing by creating new sets of random connections between the DG and CA3 for unique memory traces (Becker, 2005). Encoding information more precisely would improve one's ability to distinguish between highly similar inputs and would result in fewer errors of misidentifying similar patterns as the same.

Interestingly, improved pattern separation processing may actually impair pattern completion processing, as demonstrated by decreased general recall performance. There was an observed decline in the ability to correctly identify an image that had been seen before, which relies on pattern completion processing by the perforent path system (Rolls, 2013). This highlights that there is a delicate

balance between details and generalizability of memories that seems to be dependent on the level of neurogenesis. Under conditions of high neurogenesis the DG and mossy fibre network may dominate memory processing over the perforant path. Thus when presented with an image, instead of encoding it as a general memory, the DG encodes specific details of that image. The downside is that if this biased system is presented with a partial cue of an 'old' image, it may not be able to generalize that image to the previous one and thus be unable to recall it. This has obvious consequences for general memory recall, as it would interfere with the ability for sparse or incomplete cues to be effective for retrieval, which is a critical feature of memory function that allows us to generalize across past experiences (Rolls, 2013). This implies a possible trade-off between pattern separation and pattern completion processing indicating improved pattern separation performance compromises general memory recall (O'reilly & McClelland, 1994), and explains the observed bias towards pattern separation processing. Thus, maintaining a balance between the two processes, pattern separation and pattern completion, may be ideal for the most efficient memory recall.

Additionally, those with the highest fitness changes also showed the greatest change in memory performance. Consistent with the study by Déry et al. (2013), aerobic responses to the exercise training seem to be important for hippocampal neurogenesis, and indicates that individual differences exist. The effects of the training on pattern separation processing were augmented when

looking at the differences between high and low responders to exercise who were also cognitively trained. Specifically, high responders to exercise who did cognitively train had better pattern separation performance, made less pattern completion errors, and had an increased bias towards pattern separation processing, than high responders to exercise who did not cognitively train. No differences existed between low responders to exercise who did or did not cognitively train. In contrast to what was seen at the group level, when looking solely at the participants who were exercise trained, no significant differences were observed for general memory recall performance, limiting the negative effects of increased neurogenesis. As typically seen, individuals in the present study displayed a wide range of responses to the exercise training, which could be explained by genetics or lifestyle factors such as diet, stress, and recovery that were not examined (Mann, Lamberts, & Lambert, 2014). It is possible exercise creates a supply of newborn cells within the DG that is dependent on fitness change, in which the cognitive training can then support and enhance the cells' survival to elicit the greatest improvements in hippocampal-dependent memory. This suggests that changes in aerobic fitness may be a critical factor in promoting hippocampal neurogenesis and supporting general memory function.

One potential way that aerobic fitness may support memory is through neurotrophic factors BDNF and IGF-1, which support the proliferation and survival of new neurons in the hippocampus. Indeed, high responders to exercise had significantly greater serum concentrations of BDNF and IGF-1 than low

responders to exercise, displaying that changes in neurotrophic factors may be driven by fitness adaptations (Ferris et al., 2007; Whiteman et al., 2014). Specifically, exercise induces stress and causes cortisol to bind to glucocorticoids in the hippocampus (McEwan 2007). High levels of cortisol reduce BDNF expression in the dentate gyrus and cause cell death (Schaff, Hoetelmans, de Kloet, & Vreugdenhil, 1997). However, repeated exercise-related stress that elicits fitness adaptations, may increase BDNF levels and the proliferation of newborn cells in the DG as a way to buffer the damage caused by the stressor (ie., exercise). Thus, successful physiological adaptation would lower the stress response to exercise and increase the neurotrophic factors, as observed with the high responders to exercise. In contrast, the inability to physically adapt would elevate the stress response to exercise and reduce neurotrophic factors, as observed with the low responders to exercise. It is possible the growth factors support exercise-induced neurogenesis through this mechanism, however the survival and integration of newborn cells into the network may require appropriate cognitive input and could explain the improved memory associated with exercise in the combined training. Alternatively, BDNF and IGF-1 may be catalysts for exercise-induced neurogenesis and not directly account for the observed behavioural benefits, suggesting there may be other factors regulating the synergistic memory benefits of combined exercise and cognitive training.

Limitations

Despite the important contributions the present study has made to understanding the effects of exercise and cognitive training on hippocampal-mediated functions, limitations do exist. For each training session, the duration of training of the combined exercise and cognitive training group was twice as long as that of the participants in the exercise or cognitively trained groups. Additionally, the combined training always consisted of the cognitive training followed by the exercise training. Based on our results, it is possible that exercising before cognitive training may have enhanced performance on the cognitive training task. Finally, as the study was conducted on a human sample, we were unable to directly measure hippocampal neurogenesis and unable to measure hippocampal BDNF and IGF-1. Instead, we used performance on a putative neurogenesis-dependent task to quantify hippocampal function as an indictor of neurogenesis and measured BDNF and IGF-1 serum concentrations.

Future directions

To expand on the current study, further investigations should focus on the effects of exercise and cognitive training on BDNF, IGF-1, and potentially other molecular markers to discover the mechanisms underlying the observed behavioural effects. Additionally, incorporating hippocampal imaging through MRI would provide more information as to how the training directly impacts the hippocampus.

An important next step for this research would be to conduct the current study in populations with less than optimal neurogenesis, such as older adults.

This would not only inform how simple lifestyle factors affect neurogenesis across the lifespan, but would provide an ideal stepping stone to eventually implement exercise and cognitive therapies into clinical practice to boost neurogenesis and cognitive function in clinical populations at risk for dementia and Alzheimer's disease.

Conclusion

In summary, combined exercise and cognitive training enhanced pattern separation processing, however BDNF and IGF-1 do not seem to be the mechanisms responsible for these effects. These findings are especially important, as memory benefits were found from a relatively short intervention in high functioning young adults. Thus, the combination of simple lifestyle factors such as exercise and cognitive training may be able to induce much larger improvements in memory for populations with reduced neurogenesis.

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