FUNCTIONAL ROLE OF ADULT NEUROGENESIS IN HUMANS

ASSESSING THE FUNCTIONAL ROLE OF ADULT HIPPOCAMPAL NEUROGENESIS IN HUMANS USING COGNITIVE AND NEUROBIOLOGICAL CORRELATES

By

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfilment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

DOCTOR OF PHILOSOPHY (2014)
(Neuroscience Graduate Program)
McMaster University
Hamilton, Ontario

TITLE: Assessing the functional role of adult hippocampal neurogenesis in humans using cognitive and neurobiological correlates
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SUPERVISOR: Professor Suzanna Becker
NUMBER OF PAGES: (xviii, 273)

Abstract

Adult hippocampal neurogenesis, the generation of new neurons in the adult hippocampus, represents the most drastic form of ongoing plasticity in the human brain. When these adult-born neurons are a few weeks old, they have developed enough connections with surrounding hippocampal neurons to evoke meaningful change in network dynamics, but still have different morphological and physiological properties compared to developmentally generated neurons that render them more plastic. As such, and due to their location in the hippocampus, many have theorized that these new neurons play an important role in certain forms of learning memory as well as emotion. This dissertation outlines the first attempt to answer the question "what are new neurons in the hippocampus good for?" using human participants.

Aerobic exercise is a lifestyle factor well-established from the animal literature to upregulate neurogenesis, while chronic stress is a known downregulator of neurogenesis. The second chapter of this thesis describes a study in which aerobic capacity and depression inventory scores demonstrated a significant positive correlation and a significant negative correlation with putative neurogenesis-dependent memory, respectively, in separate cohorts of healthy young adults. The third chapter outlines a study that expands on the one presented in the second by elucidating another potential role for neurogenesis in human cognition – remote memory. Finally, Chapter 4 describes a study investigating the utility of neurotrophins measured from peripheral blood as biomarkers for neurogenic activity in humans by examining how changes in their

expression following chronic exercise predict changes in putative neurogenesis-dependent memory performance.

These studies are the first to explicitly test and provide supporting evidence for the theoretical roles of adult hippocampal neurogenesis in humans. Taken together, these studies provide a strong foundation for how investigators and clinicians can indirectly quantify and test the function of adult-born neurons in the human brain.

Preface

The following thesis is comprised of five chapters. The first chapter provides general background and context for chapters two through four. Each of chapters two, three and four are written as primary research articles. The fifth and final chapter discusses the importance of these empirical research studies and explores limitations as well as future directions for the work.

The second chapter has been published in a special topics edition of the *Frontiers in Neuroscience* journal series[1]. Content from Chapter 2 was included in this thesis in accordance with the terms of the Creative Commons Attribution License (CC-BY 3,0). Chapters 3 and 4 have been drafted in preparation for submission to *Learning and Memory* and *Neurobiology of Learning and Memory*, respectively.

The original idea for the study described in Chapter 2 was conceived during a phone conversation between me, Dr. Suzanna Becker, Dr. Glenda MacQueen and Dr. Michael D. Noseworthy. I was responsible for designing the study, obtaining ethics approval, recruiting and running participants, data collection and analysis as well as writing the manuscript. The ideas for chapters 3 and 4 were conceived by me. I was responsible for all aspects of both studies presented in the third and fourth chapters. Coauthors for the studies in Chapters 3 and 4 were given authorship for helping with data collection or analysis. Dr. Becker edited the manuscripts prior to submission.

[1]Déry N, Pilgrim M, Gibala M, Gillen J, Wojtowicz JM, MacQueen G, Becker S (2013) Adult hippocampal neurogenesis reduces memory interference in humans: Opposing effects of aerobic exercise and depression. Front Neurosci 7:66.

Support for the study outlined in Chapter 2 was provided, in part, by a grant from Astra Zeneca awarded to Dr. Glenda MacQueen. Otherwise, the research in this thesis was supported by operating grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to Dr. Suzanna Becker. It was also supported by an Ontario Graduate Scholarship awarded to me in year four and a doctoral-level postgraduate scholarship from NSERC that was awarded to me in years five and six.

Acknowledgements

First and foremost, I would like to thank my supervisor Dr. Suzanna Becker for giving me the opportunity to carry out graduate studies in her lab. Without her, my thesis would not have been possible. Suzanna Becker's supervisory style is the perfect balance between guidance and independence, which fosters a scientific mind ready for any challenge. I would also like to thank my committee members Dr. Michael D. Noseworthy and Dr. Margaret McKinnon for their unwavering support, insightful comments and helpful ideas throughout my doctoral studies.

A big thank you must be given to Dr. Margaret Fahnestock who allowed me the honor of working in her lab. I am also very grateful to have had the privilege of working with the best lab technician I have ever met: Bernadeta Michalski. Her knowledge of scientific techniques and molecular biology, along with her kindness, are truly second to none.

Former lab mates Dr. Michal Chrostowski, Dr. Brandon Aubie and Malcolm Pilgrim deserve special thanks for putting up with me over the years and providing me with endless words of encouragement. I hope the years we shared together in the Becker Lab are merely the seeds that will sprout into lifelong relationships, both personal and professional. I would also like to acknowledge the numerous undergraduate students that I had the opportunity to supervise. I learned as much from each and every one of you as I hope that you learned from me. My long-time friends that were with me before and during my doctoral studies also deserve recognition. Thank you for keeping me grounded

and preventing me from becoming socially awkward (although you were not completely successful in the latter).

I wish to express my deepest gratitude to my significant other Dr. Amanda Ramdyal who is so smart, funny and always cheerful. She reminds me each and every day to be a better person, while at the same time enjoying all that life has to offer. I am forever grateful to McMaster University for providing me with the opportunity to meet such a beautiful girl and am reminded every day of how lucky I am to have met her. Her wonderful parents Jay and Bibi and her beautiful sisters Stacy and Alyssa have loved me as if I was part of the family and I thank them for that.

Many thanks to my mother Bonnie who often let me vent my frustrations, while asking so little in return. I would also like to thank my older sister Dominique who paved the way for me. She completed her undergraduate studies at McMaster University a year before me, then moved on to Duke University where she continues to inspire my thirst for education. I am grateful to my younger brother Greg, who motivated me to keep exercising and stay fit during my studies – my arteries thank you. Finally, I would like to dedicate this work to my late father Jean Déry who, through his sense of humor and tireless work ethic, has undoubtedly shaped me into the man I am today.

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List of Abbreviations

AHN	adult hippocampal neurogenesis
BBB	blood-brain barrier
BDI	beck depression inventory-II
BDNF	brain-derived neurotrophic factor
BPS-O	behavioural pattern separation - object task
BrdU	5-bromo-2-deoxyuridine
CA1	cornu ammonis 1
CA3	cornu ammonis 3
CFC	contextual fear conditioning
CNQX	6-cyano-7-nitroquinoxaline-2, 3-dione
CTT	competitive trace theory
DCX	doublecortin
DG	dentate gyrus
DT	diphtheria toxin
DTI	diffusion tensor imaging
DTRs	diphtheria toxin receptors
EC	entorhinal cortex
Flk-1	fetal liver kinase-1
Flt-1	FMS-like tyrosine kinase-1
fMRI	functional magnetic resonance imaging
GR	glucocorticoid receptor
HPA	hypothalamic-pituitary-adrenal

IEG	immediate early gene
IGF-1	insulin-like growth factor-1
IGFBP-1	IGF-1 binding protein-1
IGFBP-3	IGF-1 binding protein-3
IRR	irradiation
LID	liver IGF-1 deficient
LTP	long-term potentiation
MAM	methylazoxymethanol acetate
MAPK	mitogen-activated protein kinase
MCI	mild cognitive impairment
MR	mineralocorticoid receptor
MRI	magnetic resonance imaging
MRS	proton magnetic resonance spectroscopy
MTT	multiple trace theory
MWM	morris water maze
PGF	placental growth factor
PI3k	phosphatidylinositol-3-kinase
PLCγ1	phospholipase C
PSS	cohen's perceived stress scale
RAVLT	rey auditory verbal learning test
SGZ	subgranular zone
SVZ	subventricular zone

TAM	tamoxifen
TrkB	tropomyosin-related kinase B
TTX	tetrodotoxin
VEGF	vascular endothelial growth factor

Background and General Introduction

1.1 Background: The Hippocampus

The hippocampus plays a critical role in episodic memory (Scoville and Milner, 1957; Eichenbaum et al., 1999; Burgess et al., 2002; Squire et al., 2004). It is a structure in the medial temporal lobe resembling an inverted seahorse (belonging to genus *Hippocampus*), from which its name is derived. It is made up of the dentate gyrus (DG) and the hippocampus proper – cornu ammonis 1 and cornu ammonis 3 (CA1 and CA3). A defining characteristic of the hippocampus is its ability to efficiently encode new and potentially overlapping memories, while at the same time keeping them separate – a computational process known as pattern separation (Marr, 1971; McNaughton and Morris, 1987; Treves and Rolls, 1994; O'Reilly and McClelland, 1994; Hasselmo and Wyble, 1997; McClelland et al., 1995). Pattern separation can be achieved through global remapping, a change in the rate and spatial fields of neurons firing, or by rate remapping, a substantial change in the firing rate, with little to no change in place field locations (Piatti et al., 2013). When pattern separation fails, the many similar episodes that we experience on a daily basis could interfere with one another, reducing our ability to remember the distinct features of any one episode. In the worst cases, such overgeneralizations in memory could lead to catastrophic interference, whereby the original memory is bound together with or completely overwritten by another (Wiskott et al, 2006). David Marr (1971) was the first to propose that the CA3 region of the hippocampus was ideally suited for performing such a task, binding partial or degraded

information together with previously stored memories into coherent events – a computational process termed pattern completion. Pattern completion processes are needed to reactivate the patterns of neural activity present at the time of memory encoding, thereby retrieving the original memory trace. Pattern completion could be achieved through the recurrent collateral connections in the CA3, which allow the region to act as an auto-associative network. Since Marr's initial work, many modellers have followed up on the CA3s potential role in pattern completion processing (e.g. Amaral and Witter, 1989; Treves and Rolls, 1994; Eichenbaum et al., 2007). Though it could lead to errors in recall, it should be noted that pattern completion is nonetheless an important process in remembering complex events, since most episodes are not fully replicated or lack sufficient amounts of context to allow rapid recollection of the original event. On the other hand, overactive pattern separation could be maladaptive by leading to a fixation on minute details of an event and leading to pathological focusing on irrelevant information, which are symptoms commonly observed in those with obsessive compulsive disorder and autism spectrum disorder (Sahay et al., 2011; Kheirbek et al., 2012; Lissek, 2012). Thus, maintaining a balance between the two processes, pattern separation and pattern completion, would be ideal.

Ever since the CA3 was suggested to perform pattern completion, researchers advocated the importance of having a region upstream in order to decorrelate incoming information, thus avoiding convergence between episodes. Anatomically, the DG, which receives input from the entorhinal cortex (EC) and sends projections to the CA3 via the mossy fibre pathway, is an ideal candidate. In fact, the DG has many more cells than the

EC or the CA3 and activity levels within the DG region are sparse, with only a few granule cells (2-5%) active at any given time (Chawla et al., 2005; Leutgeb et al., 2007; Tashiro et al., 2007; Treves et al., 2008; Alme et al., 2010; Marrone et al., 2011). Evidence for this 'sparseness' has been provided largely in part by quantifying immediate early gene (IEG) expression. IEGs are expressed in neurons that have recently undergone firing, although quantifying activity in this way actually underestimates the percent of neurons firing because IEGs, such as *Arc*, are not always expressed following depolarization (Bramham et al., 2008). Relative to the DG, the EC is more active both in the proportion of neurons firing and in their rate of activity (Barnes et al., 1990). Although sparse, DG neurons have been proposed to act as powerful "detonators" of CA3 neurons downstream, because DG granule neurons can reliably activate 11–15 CA3 pyramidal neurons (McNaughton and Morris, 1987; Treves and Rolls, 1992; Treves and Rolls, 1994; Acsady et al., 1998; Henze et al., 2002). The anatomical connection between the DG and CA3 and cellular properties of dentate granule cells have long been the basis for theoretical models of how the DG makes functional contributions to memory, including pattern separation. Another, more recent finding that has been incorporated into contemporary models of hippocampal processing is adult hippocampal neurogenesis (AHN).

1.1.1 Adult Hippocampal Neurogenesis

The term AHN describes the generation of neurons tissue in the adult hippocampus. Neurogenesis actually occurs within two regions of the healthy brain: the subgranular zone (SGZ) of the DG and the subventricular zone (SVZ) of the lateral

ventricle. Those young cells generated in the SGZ migrate to the granular cell layer of the DG whereas those born in the SVZ migrate via the rostral migratory stream into the olfactory bulb. Data regarding the effects of stress on neural proliferation in the SVZ are mixed (Kaneko, Okano and Sawamoto, 2006; Silva et al., 2008) – stress is one of the measures I use to estimate neurogenesis in humans. Moreover, I am interested in hippocampal-dependent memory processes. Therefore, for the purposes of my thesis, I will strictly focus on neurogenesis in the hippocampus. AHN occurs in a wide variety of mammals, including humans, and persists throughout life (Eriksson et al., 1998; Knoth et al., 2010; Spalding et al., 2013). There are a number of developmental stages between newborn cell and functional neuron (Figure 1.1; Kempermann et al., 2004; Seri et al., 2004). New neurons in the SGZ begin as stem-like radial glia cells (Type-1), which differentiate to become neural progenitor cells (Type-2), while maintaining the parent Type-1 cell. It is at this point of the maturation cycle that the cell phenotype is determined (Seri et al., 2001; Fukuda et al., 2003; Suh et al., 2007). The intermediate progenitors generate post-mitotic immature neurons, known as neuroblasts (Type-3). Eventually, Type-3 cells differentiate into immature granule cells (Type-4) that may go on to make functional connections with neurons in the CA3 (Ge et al., 2006; Laplagne et al., 2006; Zhao et al., 2006; Toni et al., 2007, 2008). Notably, the majority of these new cells die off via apoptosis before making functional connections with the hippocampal network (Zhao, Deng and Gage, 2008). Finally, Type-4 cells become mature granule cells at four to six weeks of age. At each step of the maturation process newly generated cells can be labelled *in vivo* using a plethora of endogenous and exogenous markers. For

example, stem-like cells can be labelled using GFAP and nestin (Seri et al., 2001), early progenitors with 5-bromo-2-deoxyuridine (BrdU) or Ki67 (Kee et al., 2002), and neuroblasts with doublecortin (DCX; Encinas et al., 2006). In addition, immature neurons can be labelled using calretinin and NeuN (reviewed in Pan et al., 2013). Two to three weeks after exiting the cell cycle, immature neurons begin to resemble more mature neurons, and can be labelled using calbindin (Kempermann et al., 2004). Double or even triple-labelling with these various markers can provide a researcher with confidence in making conclusions about the age and phenotype of newborn cells. AHN in humans was well established in a study by Eriksson and colleagues (1998), who injected BrdU into cancer patients in order to better characterize their tumours and discovered the presence of neural stem cells in the post-mortem analysis of their hippocampal tissue.

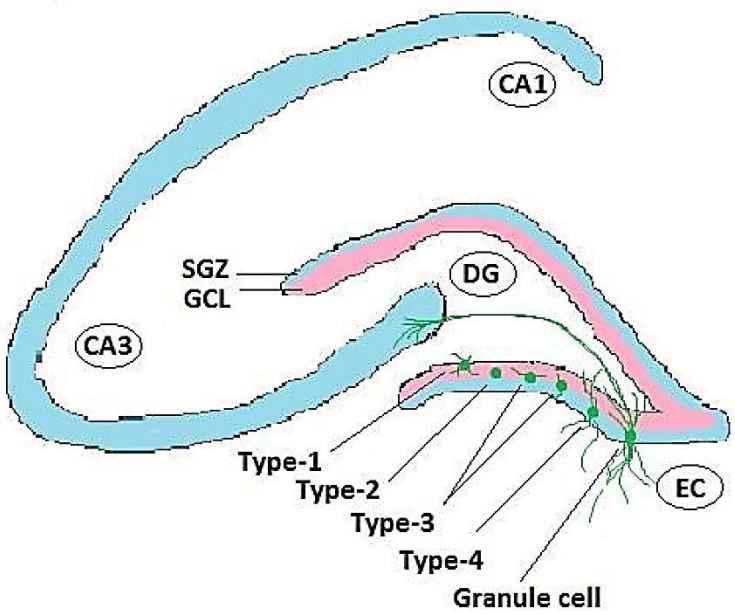

Figure 1.1. The distinct stages of granule cell maturation. Newborn granule cells start out as stem-like radial glia cells (Type-1) before maturing into neural progenitors (Type-2) and then neuroblasts (Type-3). Importantly, Type-1 -2 and -3 cells are proliferative, while Type-4 cells are not. Type-3 cells become immature granule cells (Type-4).

1.1.2 Properties of Adult-born Granule Cells

The first thought that jumps to mind when contemplating the addition of adult-born neurons into the hippocampal network is whether or not there are enough new cells being formed on a daily basis that they might actually influence learning and memory in a meaningful way. If we use rats as a model on which to base our assumptions, then we can say that there are approximately 10,000 new cells generated in the DG each day and about 4,000 of those cells become functional, mature neurons (McDonald and Wojtowicz, 2005). A more conservative estimate places that number at approximately 2,250 new neurons generated per day (Cameron and McKay, 2001). If each of those 2,250 neurons contacts approximately 11–15 pyramidal cells in downstream CA3 (Acsady et al., 1998; Zhao et al., 2006; Toni et al., 2008), then that would result in 24,750–33,750 CA3 neurons being activated by immature granule cells alone – approximately 8–11% of the total population. Indeed, 18% of the neurons in CA3 are active when a rat is exploring a novel environment (Vazdarjanova and Guzowski, 2004). Although this is almost certainly an overestimation of the actual contribution that AHN makes to CA3 activity, adult-born neurons in the DG are clearly able to affect network dynamics in a meaningful way (reviewed in Snyder and Cameron, 2012). Importantly, it is only those adult-born granule cells that are four to six weeks old at the time of learning (mature granule cells) that are preferentially recruited and thus might make unique contributions to learning and memory (Kee et al., 2007). Spalding and colleagues (2013) have recently reported that there is substantial neurogenesis in the human hippocampus

throughout life, thus supporting the notion that AHN may contribute to cognitive processes in the human brain.

1.2 Background: Environmental Influence on Adult Neurogenesis

Activation of the hypothalamic-pituitary-adrenal (HPA) axis leads to a release of glucocorticoids from the adrenal glands and into peripheral blood. These glucocorticoids, such as corticosterone (or cortisol in humans), cross the blood-brain barrier (BBB) and bind to their receptors in the hippocampus: the high-affinity mineralocorticoid receptor (MR) and the low-affinity glucocorticoid receptor (GR). The BBB is a tissue interface between the peripheral circulatory system and the brain, protecting against the entry of toxins, while facilitating the entry of essential proteins. Since the MR is higher-affinity, most of these receptors have ligands bound at baseline, which means that the effects of stress are likely mediated through activation of the lower-affinity GR. Under normal conditions, the binding of corticosterone to its receptors (i.e. those found on newborn neurons in the hippocampus) leads to negative feedback regulation of the HPA axis, thereby preventing further release of stress hormones (reviewed in Lopez, Akil and Watson, 1999). In the case of depression, it is believed that high levels of stress have impaired this negative feedback control over the HPA axis, leading to even higher levels of circulating corticosterone at baseline and in response to future adversity. Interestingly, stem-like cells and neural precursors in the DG contain only the GR and completely lack expression of the MR, while mature neurons contain both (Garcia et al., 2004). Thus, newborn neurons might play a particularly important role in HPA axis regulation. In fact, ablating neurogenesis results in elevated corticosterone release in response to a stressful

event (Schloesser et al., 2009). On the other hand, increasing neurogenesis by treatment with antidepressants demonstrated a direct correlation with restored feedback control over the HPA axis (Surget et al., 2011). Thus, new neurons may be critical for the negative feedback loop (Surget et al., 2011). Moreover, adult-born granule cells are highly sensitive to the effects of corticosterone. Stress is a potent downregulator of AHN. Corticosterone injection (Cameron and Gould, 1994), inescapable shock (Malberg and Duman, 2003; Dagyte et al., 2009), chronic restraint (Pham et al., 2003), chronic mild or unpredictable stressors (Alonso et al., 2004; Heine et al., 2004, 2005; Joëls et al., 2004; Lee et al., 2006; Oomen et al., 2007; Xu et al., 2007; Silva et al., 2008), forced swimming (Stranahan et al., 2006) and social stress (Yap et al., 2006; Czéh et al., 2007; Ferragud et al., 2010) all inhibit cell proliferation and/or survival in the DG. However, it should be noted that stress does not always decrease neurogenesis in the DG of rodents (e.g. Snyder et al., 2009b). Nonetheless, there exists a clear interaction between stress and adult-born neurons in the DG. For example, the age-associated decline in granule cell proliferation is paralleled by an increase in circulating stress hormones (Heine et al., 2004). Preventing the release of corticosterone into the periphery via adrenalectomy rescued this age-dependent decline in AHN and improved Morris Water Maze (MWM) learning (Cameron and McKay, 1999; Montaron et al., 2006). In fact, adrenalectomy in old animals increased the rate of proliferation beyond that found in young rats, an effect that was reversed by treatment with low-dose corticosterone replacement (Cameron and McKay, 1999).

The relationship between neurogenesis and depression is complicated by the observation that halting hippocampal neurogenesis does not result in a depressive-like phenotype (Snyder et al., 2011). However, Snyder and colleagues (2011) recently found that mice with a drug-induced suppression of neurogenesis displayed HPA axis dysregulation and a depressive-like phenotype following 30 minutes of restraint stress. Thus, it would appear that the missing link was stress: Whereas the ablation of neurogenesis alone did not cause behavioural symptoms of depression, ablation of neurogenesis combined with a stressful event did (Snyder et al., 2011). Snyder and colleagues (2011) posit that adult-born hippocampal neurons may buffer the negative effects of corticosterone by providing negative feedback control over the HPA axis. In contrast to the deleterious effects of stress, chronic treatment with various antidepressant pharmaceuticals upregulates neurogenesis in the hippocampus (Malberg et al., 2000; Huang and Herbert, 2006; Bjornebekk, Mathe and Brene, 2010; Castro et al., 2010). Further, newborn neurons in the DG are actually required for the behavioural efficacy of antidepressant drugs (Santarelli et al., 2003). Taken together, these results provide compelling evidence that dentate granule cells play a regulatory role in HPA axis activity and that newborn neurons are highly sensitive to corticosteroids.

Physical activity has been proposed as a preventative therapy for a great number of diseases, including those associated with poor cognition, and for good reason. Exercise can protect against the age-associated decline in memory (Erickson et al., 2011; Intlekofer and Cotman, 2013) and delay the onset of age-related dementias including Alzheimer's Disease (Larson et al., 2006). Despite its positive effects on brain health and

plasticity in humans, little is known about how exercise actually imparts its many benefits. In contrast to stress, aerobic exercise has been shown to reliably upregulate AHN and hippocampal-dependent learning in both mice and rats (van Praag et al., 1999a, 1999b, 2005; Trejo et al., 2001; Cotman and Berchtold, 2002; Fabel et al., 2003, 2009; Eadie et al., 2005; Ernst et al., 2006; Kronenberg et al., 2006; Olson et al., 2006; Wojtowicz et al., 2008; Snyder et al., 2009b; Creer et al., 2010; Josselyn and Frankland, 2012; Kohman et al., 2012; Winocur et al., 2012; Voss et al., 2013). For example, Creer and colleagues (2010) demonstrated that wheel running upregulated AHN in mice and improved spatial memory performance, but only for high interference, finer spatial discriminations. The exercise-induced upregulation of AHN coincides with newborn neurons generating novel connections alongside existing ones in the CA3 (Josselyn and Frankland, 2012). Thus, aerobic exercise does not merely increase a pool of new neurons which then die off before making functional connections, but rather it evokes structural plasticity and can alter the wiring of pre-existing hippocampal circuits (Josselyn and Frankland, 2012). Taken together, these findings suggest that physical activity may, at least in part, exert its therapeutic benefits through its pro-proliferative effects on AHN. Further, mice that were exposed to running wheels in old age experienced a significant increase in newborn cells that differentiated into mature neurons and a concomitant improvement on the MWM task (van Praag et al., 2005). These results and those of many others suggest that the therapeutic potential of aerobic exercise persists into advanced age (van Praag et al., 2005; Kronenberg et al., 2006; Wu et al., 2008; Marlatt et al., 2012).

Measuring neurogenesis directly in the human brain is not yet possible. However, several indirect magnetic resonance imaging (MRI)-based measures of potential correlates of neurogenesis have been put forward. Some of these measures make use of the well established link between exercise and neurogenesis. Higher levels of physical activity are associated with higher hippocampal volumes in aged participants (Erickson et al., 2009). Further, one year of exercise was associated with increased hippocampal volumes in older adults (Erickson et al., 2011). Herting and Nagel (2012) demonstrate that aerobic capacity in adolescents is associated with larger hippocampal volumes and improved performance on a virtual water maze task akin to the pool version used in rodents. Although aerobic exercise can affect plasticity in multiple ways in and outside of the DG, such as dendritic complexity in the DG (Eadie et al., 2005; Redila and Christie, 2006) and spine density in CA1 pyramidal cells (Stranahan, Khalil and Gould, 2007), an increase in adult-born granule cell proliferation and/or survival may underlie at least some of the changes in hippocampal volume. Unfortunately, volumetry does not have the resolution to accurately assess regional specificity or microscopic alterations to anatomy. Rather, it is only accurate to the size of a voxel, which is derived from averaged signal of all tissue types that reside within the voxel. In contrast, Pereira and colleagues (2007) measured blood volume changes in the hippocampus and describe a DG-specific increase in blood volume following 12 weeks of physical activity in older adults. The blood volume changes correlated with change in aerobic fitness and change in cognitive ability on the Rey Auditory Verbal Learning Test (RAVLT; Pereira et al., 2007). Exercise also has antidepressant-like effects across a variety of species (e.g. Blumenthal et al., 1999;

Ernst et al., 2006; Marlatt et al., 2010; Voss et al., 2013). It has been described as an effective therapy for several stress-related mood disorders such as major unipolar depression (Lawlor and Hopker, 2001; Greenwood et al., 2003), bipolar disorder and post-traumatic stress disorder (Newman and Motta, 2007; Diaz and Motta, 2008). Physical activity may also counteract the negative effects of stress by reducing inflammatory cytokines (Petersen and Pedersen, 2005) and restoring growth factor signaling cascades (reviewed in Cotman et al., 2007). Growth factors are proteins that support everything from the proliferation, development and survival to the function of neurons. The growth factors that have been most strongly tied to AHN are: brain-derived neurotrophic factor (BDNF); Insulin-like growth factor type-1 (IGF-1), and; vascular endothelial growth factor (VEGF).

1.2.1 Environmental Influence on Growth Factors

BDNF is a well-established regulator of neuronal plasticity including AHN (Duman and Monteggia, 2006; Lipsky and Marini, 2007; Cowansage et al., 2010). Although BDNF in the central nervous system cannot be measured non-invasively in humans, insight into central levels of BDNF can be gained from serum neurotrophin assays. It has been estimated that the source of 70-80% of serum neurotrophins is the brain (Rasmussen et al., 2009). Serum BDNF is reduced in patients suffering from major depression (Sen, Duman and Sanacora, 2008) and is even correlated with hippocampal volumes in a group of drug-free, first-episode depressed patients (Eker et al., 2010). Hippocampal BDNF is also downregulated by stress (Nibuya et al., 1995; Shi et al., 2010; First et al., 2011). However, it should be noted that the negative effects of stress on

BDNF may depend on the timing, duration or intensity of stress, as BDNF is sometimes elevated when measured shortly after exposure to an acute stressor (Larsen et al., 2010; Shi et al., 2010). In contrast to stress, antidepressant drugs can increase BDNF expression in serum (Sen et al., 2008; Brunoni, Lopes and Fregni, 2008) and in the hippocampus (Huang et al., 2008). Even peripheral administration of BDNF is able to exert antidepressant effects in mice (Schmidt and Duman, 2010), demonstrating that serum BDNF may play a role in recovery from depression. Indeed, the behavioural effects of antidepressant treatment have been shown to require availability of BDNF (Monteggia et al., 2004; Musazzi et al., 2009). Conditional deletion of the tropomyosin-related kinase B (TrkB) gene, the major receptor for BDNF, on neural progenitor cells resulted in reduced proliferation and survival of newborn cells, long-term potentiation (LTP) and prevented the behavioural efficacy of imipramine and fluoxetine (Sairanen et al., 2005; Bergami et al., 2008; Li et al., 2008). LTP is the observed enhancement in neural response to an electrical stimulus following previous stimulating current and is widely considered to be the cellular correlate of learning (Bliss and Collingridge, 1993). Importantly, the antidepressant effects of BDNF may rely on its binding to receptors specifically in the DG. Knockdown of BDNF in the DG, but not the CA3 or CA1, blocked the behavioural effects of desipramine and citalopram (Adachi et al., 2008; Taliaz et al., 2010). Further, the antidepressant effects of pharmaceuticals and aerobic exercise are specifically associated with enhanced expression of mature BDNF (as opposed to proBDNF, defined below), suggesting that these treatments act on posttranscriptional mechanisms and not just BDNF expression (Musazzi et al., 2009; Sartori et al., 2011). Finally, a genetic

mutation that lowers basal expression of BDNF, specifically a methionine substitution for valine at codon 66 of the BDNF gene (val66met), is associated with a higher incidence of major depression and anxiety disorder (Castren, 2004). Further, mice with the methionine substitution display lower basal rates of AHN (Bath et al., 2012).

Aging is associated with dysregulation of the HPA axis, enhanced release of circulating stress hormones in response to stress (Sapolsky, McEwen and Rainbow, 1983), and declining IGF-1 (Xu and Sonntag, 1996; Junnila et al., 2013). Depression is also associated with elevated cortisol at baseline (Halbreich et al., 1985) and impaired negative feedback of the HPA axis in response to challenge (Holsboer et al., 1987; Heuser et al., 1994; Snyder et al., 2011). Further, long-term suppression of serum IGF-1 results in a depressive phenotype (Mitschelen et al., 2011). In contrast, IGF-1 expression is enhanced following antidepressant treatment (Khawaja et al., 2004).

VEGF expression is also influenced by stress – although it is unclear in which direction, as findings are mixed. VEGF mRNA and protein are increased in the peripheral blood of drug-free depressed patients compared to healthy controls (Iga et al., 2007; Kahl et al., 2009). On the other hand, Heine et al (2005) demonstrate that chronic stress decreased VEGF as well as its high affinity fetal liver kinase-1 (Flk-1) receptor in the hippocampus. Finally, others have found that circulating VEGF was no different between patients with major depression and controls (Ventriglia et al., 2009; Kotan et al., 2012). Likewise, results are mixed when it comes to the effects of antidepressant treatment on VEGF expression, with some groups showing that antidepressant pharmaceuticals increase VEGF expression in the hippocampus (Newton et al., 2003; Warner-Schmidt

and Duman, 2007), while others show no change (Iga et al., 2007; Ventriglia et al., 2009). Importantly, VEGF is associated with increased proliferation and survival of adult-born granule cells as well as spatial memory (Jin et al., 2002; Cao et al., 2004). Further, inhibiting VEGF signaling through the Flk-1 receptor blocked the antidepressant-dependent upregulation of AHN and behavioural recovery (Warner-Schmidt and Duman, 2007).

In contrast to the AHN-suppressing effects of stress, exercise upregulates AHN. The mechanisms underlying the activity-dependent increase in AHN are not fully understood. However, many postulate that neurotrophins may be responsible for the pro-neurogenic effects of exercise (Trejo et al., 2001; Farmer et al., 2004; Wu et al., 2008; Marlatt et al., 2010, 2012; Hanson, Owens and Nemeroff, 2011). Aerobic exercise is a potent upregulator of BDNF (van Praag, 2008; Islam, Loo and Heese, 2009). BDNF mRNA and protein are increased in the hippocampus following exercise (Neeper et al., 1995; Cotman and Berchtold, 2002). Enhanced BDNF mRNA in the hippocampus might, in turn, underlie the exercise-induced upregulation of AHN. Indeed, infusing BDNF directly into the hippocampus is sufficient to increase AHN, even in the contralateral side (Scharfman et al., 2005). Further, the increase in hippocampal BDNF mRNA following physical activity might be driven mostly by changes in the DG, rather than CA1 (Vaynman, Ying and Gomez-Pinilla, 2004). Serum BDNF is increased following acute exercise in healthy adults (Gold et al., 2003; Rojas Vega et al., 2006; Ferris, Williams and Shen, 2007). The exercise-induced upregulation of serum BDNF has been proposed as

one mechanism for increasing hippocampal volume (or preventing age-dependent hippocampal volume decline) in older adults (Erickson et al., 2011).

IGF-1 is also an important activity-dependent regulator of AHN. Physical activity increases IGF-1 protein both in serum and in the hippocampus, and upregulates both the proliferation and survival of adult-born neurons (Carro et al., 2000; Trejo et al., 2001). IGF-1 also exerts effects in the absence of exercise: peripheral and intracerebroventricular injection of IGF-1 increases proliferation and survival of adult-born neurons in rats (Aberg et al., 2000, 2003; Lichtenwalner et al., 2001). On the other hand, peripheral infusion of IGF-1 antibodies reduces survival of newborn cells in sedentary animals and prevents the exercise-induced upregulation of AHN in both mice and rats (Trejo et al., 2001; Llorens-Martin et al., 2010). Mice that are genetically altered in such a way that they are no longer able to produce IGF-1 in the periphery have a lower basal rate of neurogenesis, which is unchanged in response to physical activity (Trejo et al., 2007; Trejo, Llorens-Martin and Torres-Aleman, 2008). However, when these mice were injected with IGF-1 subcutaneously, rates of AHN were returned to control levels and cognitive deficits reversed (Trejo et al., 2007, 2008). The uptake of IGF-1 into the brain is facilitated by exercise (reviewed in Trejo et al., 2002). IGF-1 gene expression is increased following exercise (Ding et al., 2006). Besides its effects on neurogenesis, peripherally-generated IGF-1 is also important for the exercise-dependent changes in vasculature (Lopez-Lopez et al., 2004). Since angiogenesis and neurogenesis are tightly linked (Louissaint et al., 2002) and neural precursors migrate in clusters along capillaries (Palmer et al., 2000), the effects of IGF-1 on vessel remodeling in the brain might also

indirectly impact neurogenesis by providing structure for the migration of new neurons and increasing metabolic support. Angiogenesis is the formation of new vessels from pre-existing vasculature. Further, the exercise-induced upregulation of IGF-1 is associated with increased levels of BDNF in the brain (Trejo et al., 2001; Berchtold et al., 2005). Infusion of IGF-1 into the carotid artery, in the absence of exercise, induced upregulation of BDNF in the hippocampus (Liste et al., 1997). Further, IGF-1 applied to hippocampal cultures *in vitro* increased the number of TrkB receptors on neurons (McCusker et al., 2006). Thus, IGF-1-induced plasticity might be mediated directly through IGF-1 binding to its tyrosine kinase receptor or indirectly by interfacing with BDNF signal transduction. Indeed, the exercise-induced upregulation of both hippocampal BDNF (Ding et al., 2006; Koopmans et al., 2006) and adult-born neurons requires peripheral IGF-1 (Trejo et al., 2001; Chen and Russo-Neustadt, 2007). Basal levels of BDNF mRNA in the hippocampus (Koopmans et al., 2006) and the exercise-induced increase in hippocampal BDNF protein (Ding et al., 2006) are abolished upon injection of an anti-IGF-1 antibody. Thus, BDNF is a potential downstream target of IGF-1 (Ding et al., 2006). Finally, there is a well-established link between BDNF and LTP in the hippocampus (Soule et al., 2006), but no established link between IGF-1 and LTP. Thus, BDNF and IGF-1 may converge on BDNF signaling to mediate the effects of physical activity on LTP (Cotman et al., 2007).

Peripheral VEGF is also necessary for the exercise-induced upregulation of adult neurogenesis in the hippocampus (Fabel et al., 2003). Exercise upregulates VEGF specifically in the hippocampus in addition to peripheral areas such as muscle (Tang et

al., 2010). Blocking peripheral VEGF from entering the brain during bouts of physical activity prevents the positive effects of exercise on proliferation of newborn cells in the hippocampus (Fabel et al., 2003). Likewise, VEGF knockout mice show impaired AHN (Sun et al., 2006). On the other hand, injecting VEGF into the ventricles is sufficient to increase AHN (Jin et al., 2002; Schanzer et al., 2004). Mice overexpressing VEGF display enhanced proliferation and survival of adult-born neurons and demonstrate improved hippocampal-dependent learning (Cao et al., 2004). The IGF-1-dependent vessel remodeling following exercise might be mediated, at least in part, by interfacing with VEGF (Lopez-Lopez et al., 2004). Indeed, VEGF mRNA and protein in the brain are required for exercise-induced vessel remodeling (Ding et al., 2006). Interestingly, the activity-dependent upregulation of AHN is coupled with increased hippocampal vasculature (Fabel et al., 2003; Heine et al., 2005; van Praag et al., 2005; van der Borght et al., 2009). Cotman and colleagues (2007) suggest that while BDNF and IGF-1 seem to mediate the effects of physical activity on learning, memory and emotion, the exercise-induced upregulation of neurogenesis seems to rely more heavily on the actions of IGF-1 and VEGF. While there is marked controversy surrounding VEGF as an influential factor in the onset and recovery from depression, differences in methodologies between studies prevent a definitive conclusion from being drawn (reviewed in Clark-Raymond and Halaris, 2013). Further, when considered together with the effects of aerobic exercise on VEGF, the protein remains a potential biomarker for neurogenesis, especially if measured alongside other growth factors (e.g. BDNF, IGF-1).

1.2.2 Growth factors

Adult-born cells in the hippocampus are regulated by a variety of growth factors at each stage of morphological development. Such factors include neurotrophins, such as BDNF (Cotman et al., 2007; Schmidt and Duman, 2007), as well as other proteins including IGF-1 (Llorens-Martin et al., 2009) and VEGF (During and Cao, 2006). At the molecular level, BDNF, IGF-1 and VEGF appear to be related members of an activity-dependent signalling cascade (reviewed in Cotman et al., 2007; Llorens-Martin et al., 2009). Peripheral BDNF and IGF-1 can cross the BBB (Reinhardt and Bondy, 1994; Poduslo and Curran, 1996; Pan et al., 1998; Pardridge et al., 1998; Armstrong et al., 2000, Carro et al., 2000, Pan and Kastin, 2000; Zhang and Pardridge, 2001). It is more controversial as to whether peripheral VEGF can cross the BBB (Fabel et al., 2003; Lopez-Lopez, LeRoith and Torres-Aleman, 2004; Storkebaum et al., 2005), but it can nonetheless influence BBB permeability by interfacing with its two receptors, FMS-like tyrosine kinase-1 (Flt-1) and Flk-1 (Mayhan, 1999; Zhang et al., 2000; Schreurs et al., 2012). Importantly, BDNF, IGF-1 and VEGF are all growth factors that are established mediators of exercise-induced plasticity, including neurogenesis (reviewed in Cotman et al., 2007).

1.2.2.1 Brain-Derived Neurotrophic Factor

BDNF is a member of the neurotrophin gene family, which includes nerve growth factor, neurotrophin-3 and -4/5, and is important for the maintenance and survival of neurons (Acheson et al., 1995). It is synthesised and released into the blood from vascular endothelial cells, smooth muscle and leukocytes (Gielen et al. 2003; Nakahashi et al.

2000). BDNF is also synthesized in the brain and can be transported out through the BBB and into the periphery (Pan et al., 1998). It is synthesized as a 32 kDa precursor, called proBDNF, which is cleaved into its mature 14 kDa isoform or a truncated 28 kDa version (Mowla et al., 2001). Mature BDNF preferentially binds to the TrkB receptor (Cowansage et al., 2010), but also binds with low affinity to the p75 receptor (Chao, 2003). On the other hand, proBDNF preferentially binds to the p75 neurotrophin receptor (Woo et al., 2005). While TrkB receptor binding mediates cell survival, p75 receptor binding initiates cell death (Lu et al., 2005; Reichardt, 2006). Cleavage of proBDNF to mature BDNF is accomplished by plasmin (Pang et al., 2004; Lu et al., 2005). BDNF facilitates LTP by promoting cytoskeleton changes (Rex et al., 2007) and by increasing neurotransmitter release (Bekinschtein et al., 2007) through the mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol-3-kinase (PI3k) pathway and the phospholipase C (PLCγ1) pathway (Chao, 2003; Mei et al., 2011). BDNF is strongly expressed within neurons of the DG and is anterogradely transported to their axons (Conner et al., 1997; Yan et al., 1997). BDNF promotes growth and survival of dentate granule cells in vitro (Holtzman and Lowenstein, 1995; Patel and McNamara, 1995). Exposure to BDNF leads to excitation in the DG (Messaoudi et al., 1998; Asztely et al., 2000) and CA3 (Scharfman, 1997) as well as the CA1 (reviewed in Lu, 2004). It can also affect local inhibitory neurons within the DG (Marty et al., 2000; Olofsdotter et al., 2000).

1.2.2.2 Insulin-Like Growth Factor Type – 1

IGF-1 is a basic 7.5 kDa peptide that is part of the insulin-like hormone family that includes insulin and relaxin (Russo et al., 2005). The source for the vast majority of peripheral IGF-1 is the liver (Yakar et al., 1999), where its production is stimulated by growth hormone (Balu and Lucki, 2009). IGF-1 is also synthesized in a variety of other tissues including centrally in neurons, glia and vascular cells (Bondy et al., 1992; Donahue et al., 2006). However, the importance of serum IGF-1 is made clear by the fact that mice lacking peripheral IGF-1 display marked brain abnormalities and behavioural deficits (Lopez-Lopez et al., 2004; Ding et al., 2006; Svensson et al., 2006; Trejo et al., 2007, 2008; Llorens-Martin et al., 2009), whereas those lacking central IGF-1 display relatively minor brain pathology (Davila et al., 2007). The IGF-1 receptor is a member of the tyrosine kinase receptor family and, just like BDNF, initiates the MAPK and PI3k signalling cascades (Shen et al., 2001). Neural progenitor cells in the hippocampus express the IGF-1 receptor (Anderson et al., 2002; Aberg et al., 2003). Approximately 99% of circulating IGF-1 is bound to binding proteins, namely IGF-1 binding protein-1 (IGFBP-1) and IGF-1 binding protein-3 (IGFBP-3; Pollak, 2008). IGF-1 binding proteins modulate the bioavailability of free IGF-1.

1.2.2.3 Vascular Endothelial Growth Factor

VEGF, also referred to as VEGF-A, is one member of the VEGF family that also includes VEGF-B, VEGF-C. VEGF-D, VEGF-E, VEGF-F and placental growth factor (PGF). VEGF is a 34 to 42 kDa protein (Hoeben et al., 2004). It is synthesized throughout the body, in both central and peripheral locations (Tang et al., 2010).

Peripherally, it is most detectable in lung, kidney, heart and the adrenal glands (Hoeben et al., 2004). VEGF is a cytokine critical for angiogenesis (Millauer et al., 1993), able to initiate endothelial cell proliferation and migration (Ferrara et al., 2003). It promotes angiogenesis by binding to its receptor tyrosine kinases on endothelial cells (Krum et al., 2002). It is also involved in the proliferation of adult-born granule neurons (Jin et al., 2002; Newton et al., 2003). The Flk-1 receptor is found on neural progenitor cells in the DG (Yang et al., 2003). Binding of VEGF to Flk-1 results in binding of PLCγ1, activating protein kinase C, which in turn activates Ras (Hoeben et al., 2004). This pathway activates MAPK. Binding of VEGF to Flk-1 also activates the PI3k pathway and promotes cell survival (Gerber et al., 1998).

1.3 General Introduction

1.3.1 Pattern Separation

It was first suggested that new neurons in the DG might play an essential role in clearing older memories (Feng et al., 2001). At the same time, AHN could provide a fresh substrate for newer memories to be formed. Kempermann (2002) took this idea one step further and claimed that new neurons may be required to deal with novelty. Indeed, when adult-born granule cells were added to a model of the hippocampal network, catastrophic interference was prevented, such that novel input did not overwrite pre-existing memories (Wiskott et al, 2006). Becker (2005) further suggested that AHN facilitates the formation of distinct memory traces, specifically for highly overlapping information. Her model shows that neuronal turnover aids in the correct recall of multiple overlapping patterns, while not affecting the model's ability to correctly select between unrelated items or

paired associates (Becker, 2005). Later, the involvement of adult-born neurons in the encoding of time was hypothesized by Aimone and colleagues (2006, 2009). They modeled time via successive bouts of neurogenesis and found that new neurons may actually enhance the similarity between patterns presented close together in time (a process termed pattern integration), while reducing the similarity between events farther apart in time (Aimone et al., 2009). Thus, when stimuli are very similar, but their presentation temporally distinct, pattern separation occurs to a greater extent when new neurons are available.

While the CA3 alone could rapidly encode incoming stimuli, this representation of the external environment would likely lack precision as compared to the decorrelated information arriving from immature granule cells (or by the fine-tuning action of adult-born neurons on local inhibitory networks). Accordingly, under conditions of low neurogenesis the direct perforant path connection between the EC and CA3 may dominate processing and the hippocampus may favour pattern completion (Figure 1.2). Conversely, under conditions where AHN is high, the pathway from DG to CA3 might dominate encoding and bias the system towards pattern separation. Indeed, such a trade-off between the two processes has already been suggested (O'Reilly and McClelland, 1994). A constant balancing between pattern separation and pattern completion makes sense in order to keep memories distinct enough to avoid constant mistakes during recall, while maintaining completion processes to such a degree as to keep processing efficient. Adult-born granule cells may themselves be the active population of the DG affecting activation rates in CA3 neurons, thus carrying the message (Figure 1.3; Piatti et al.,

2013). There are many factors that render newborn neurons in the DG especially suited for performing such a task. Adult-born neurons in the DG are transiently hyperexcitable when they are still relatively immature, owing largely to a lower threshold for calcium spikes and weaker perisomatic inhibition (Schmidt-Hieber et al., 2004; Marin-Burgin et al., 2012) and so they are more likely to depolarize given excitatory stimulation. For a brief period of time when they are still hyperexcitable (approximately three to four weeks of age), young DG neurons are becoming functionally connected to existing hippocampal circuitry and thus may provide functional contributions to learning and memory (Toni et al., 2008; Gu et al., 2012). Indeed, immature granule cells demonstrate a greater likelihood for LTP than do more mature DG neurons (Snyder et al., 2001; Ge et al., 2007). Younger cells are also preferentially activated by hippocampal-dependent tasks, further suggesting that they play a meaningful role in cognition (Gould et al., 1999; Ramirez-Amaya et al., 2006; Dupret et al., 2007; Epp et al., 2007; Snyder et al., 2009a, 2009b; Trouche et al., 2009). Besides a direct role in cognition, the contribution of AHN to learning and memory may also include, or instead be the result of, indirect mechanisms. For instance, newborn cells may preferentially recruit local inhibitory neurons to control the level of inhibition imposed on more mature granule cells, thus fine-tuning the signal being transferred from the DG to CA3 (Piatti et al., 2013). With an ever-changing ratio of young to mature granule neurons, there would also be an ever-changing gradient of excitability in the DG – thus altering the pattern of granule cells activating downstream CA3 neurons. Electrophysiological and behavioural evidence from rodent studies has largely supported the theoretical role for AHN in pattern separation.

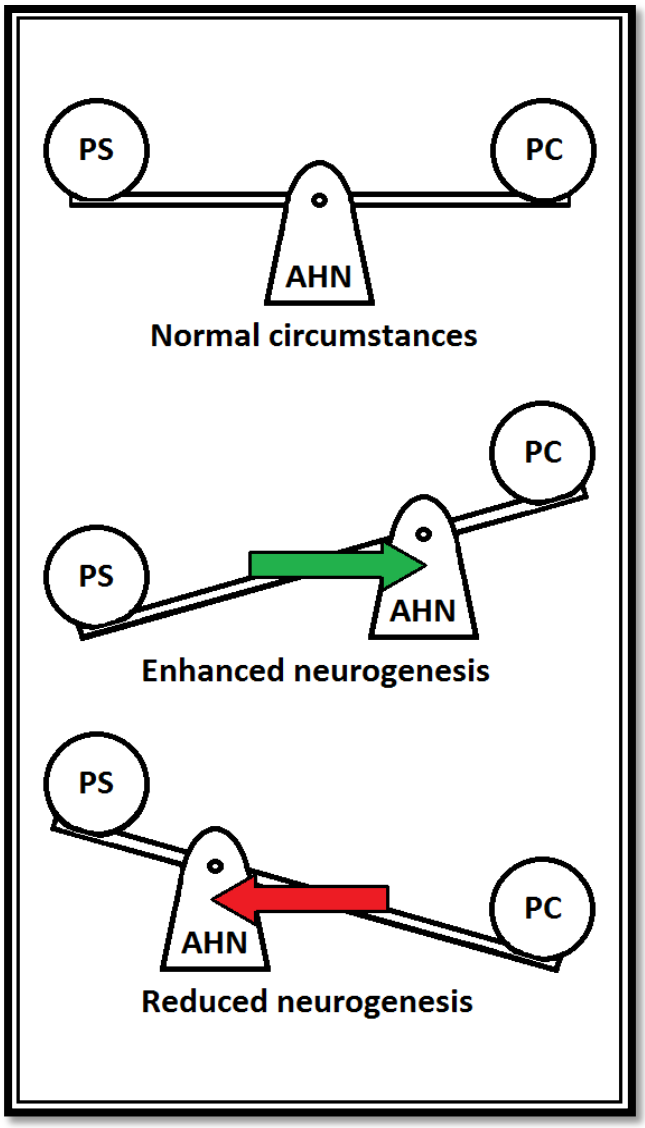

Figure 1.2. The proverbial balancing act between pattern separation and pattern completion processing in the hippocampus. Enhancing the rate of proliferation or survival of new cells by aerobic exercise, for example, may adjust the balance of processing in the hippocampus in such a way as to favour pattern separation (PS) processing. On the other hand, chronic stress, for instance, may downregulate adult neurogenesis and shift the balance of processing towards pattern completion (PC). A bias towards pattern completion may lead to overgeneralization, the inability to assign the appropriate context to separate events.

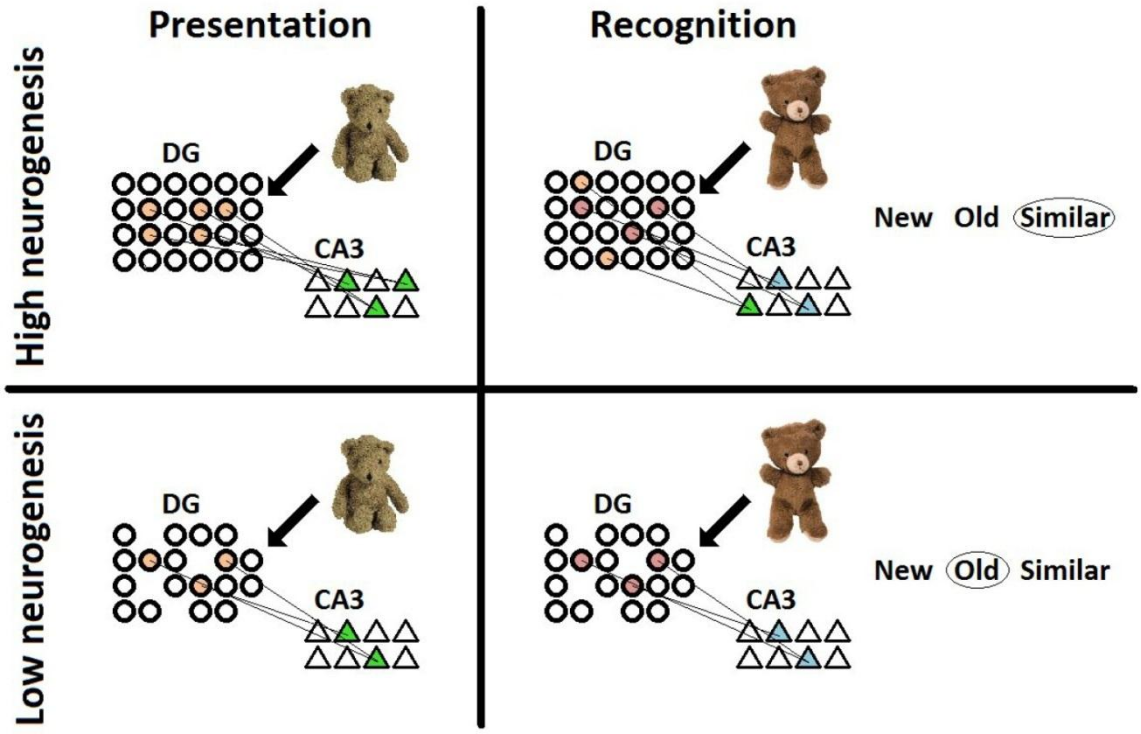

Figure 1.3. Simplified representation of global remapping in the hippocampus and its effects on behavioural pattern separation performance. Global remapping is said to have occurred when very different sets of neurons are activated in response to small variations in input. Upon exposure to the first bear (on the left), a certain population of neurons fire in the Dentate Gyrus after receiving input from the Entorhinal Cortex. These Dentate Gyrus neurons then pass the signal to a certain population of neurons in the CA3, which act to bind the individual features of the event, such as the color brown or the furry texture, into a coherent memory – a teddy bear. Upon exposure to the second bear (on the right), a completely different set of neurons fires in the Dentate Gyrus following input from the Entorhinal Cortex. As a result of differential firing in the Dentate Gyrus, a unique population of neurons is now activated in the downstream CA3, so the memories are non-overlapping – there is no interference between memories. In contrast, when the Dentate Gyrus is compromised or neurogenesis is reduced, global remapping is less likely to occur and the memories for Bear #1 and Bear #2 are more likely to overlap. Such overgeneralizations in memories could lead to an individual misidentifying the second bear as an "old" image, even though they have never seen it before. Additionally, neurons in the Dentate Gyrus might also affect population firing in the CA3 by rate remapping (no change in neurons firing, but change in rate of firing) or by affecting local inhibitory networks.

1.3.1.1 Empirical Evidence Supporting Pattern Separation

Leutgeb and colleagues (2007) used multi-unit recording to provide evidence that the DG orthogonalizes information before it is sent to the CA3. Specifically, they showed that the greatest degree of decorrelation between the two regions' firing patterns occurs when animals are re-exposed to an environment that is highly similar to one they have recently experienced (Leutgeb et al., 2007). Further, as rats are exposed to environments that are increasingly more distinct from those previously experienced, activity between the DG and CA3 becomes more correlated until, eventually, there is no difference (Leutgeb et al., 2007). Leutgeb et al (2007) conclude from these data that the DG contributes to pattern separation when there are very small differences between inputs, by using the same small sub-population of DG neurons that exhibit spatially and temporally decorrelated firing patterns to signal these fine differences. On the other hand, the CA3 contributes to pattern separation when there are large differences between inputs, by using non-overlapping populations of neurons to encode these environments. In terms of behaviour, therefore, we would expect that in cases where overlap is minimal between stimuli, there will be little to no need for adult-born granule cells and pattern separation[1].

[1] While pattern separation in neurocomputational terms represents the orthogonalization of inputs, such that information leaving the DG is more decorrelated than information entering the DG, pattern separation in behavioural terms simply refers to the process of discriminating between highly similar stimuli (such as objects, spatial locations or even contexts). Thus, it has been argued that the often-used term 'behavioural pattern separation' should no longer be used because it may be confused with the decorrelation-type processes occurring in the DG, and so the term 'behavioural discrimination' should be used instead (Santoro, 2013). However, I prefer to use the term behavioural pattern separation or just pattern separation when referring to behavioural discrimination tasks containing a high-interference component because I am assuming that orthogonalization in the DG is occurring during the high-interference discrimination trials. Nonetheless, I

However, in cases of high interference between events, we would expect that AHN is needed for reducing overlap between stimuli. This hypothesis can be tested in animals using a variety of strategies. For instance, Clelland and colleagues (2009) use two different tasks, the radial 8-arm maze and a touchscreen apparatus to assess the requirement of AHN in spatial pattern separation. They found that animals with dentate granule cells ablated, either by via focal irradiation (IRR) or inhibition of *Wnt* signaling specifically in the DG, exhibited marked deficits in spatial discrimination on both tasks, but only when the stimuli were presented in similar but not distinct spatial locations (Clelland et al., 2009). *Wnt* signaling is critically involved in the generation of newborn neurons in the DG (Lie et al., 2005; Clelland et al., 2009; Jessberger et al., 2009). This study provides perhaps the most compelling evidence for the role of AHN in pattern separation thus far, because the researchers used two different methods to knock-down neurogenesis. Moreover, they also employed two different pattern separation tasks that varied in their behavioural requirements, whereas most studies use only one. Notably, a plethora of other rodent studies have described similar results, for example, using the MWM to assess spatial memory (Snyder et al., 2005), a contextual fear conditioning (CFC) apparatus to assess context-fear associations (Saxe et al., 2006; Winocur et al., 2006; Imayoshi et al., 2008; Warner-Schmidt et al., 2008; Wojtowicz et al., 2008; Hernandez-Rabaza et al., 2009; Ko et al., 2009; Guo et al., 2011; Nakashiba et al., 2012; Pan et al., 2012a, 2012b, 2013) and a variety of spatial, odour as well as contextual

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urge the reader to keep in mind that when I use the term behavioural pattern separation, I am only *assuming* and not stating that orthogonalization processes are actually taking place in the DG.

discrimination tasks specifically designed to test pattern separation processes (McHugh et al., 2007; Guo et al., 2011; Luu et al., 2012; Pan et al., 2012a; Tronel et al., 2012; Winocur et al., 2012). Another way to knock down neurogenesis is by deleting ERK5. ERK5 is a mitogen-activated protein kinase that is expressed in both neurogenic regions of the brain, the SVZ and SGZ, and is a vital regulator of AHN (Pan et al., 2012a, 2012b, 2013). Specifically, adult male nestin-creER™/ERK5 loxP/loxP mice are treated with tamoxifen (TAM) to delete ERK5. These mice are termed ERK5 icKO mice. Pan and colleagues (2012a) describe a spatial pattern separation deficit in ERK5 icKO mice at both short and long delays. Thus, the pattern separation function of adult-born granule cells seems to extend across a variety of time points. Interestingly, rodents with reduced AHN have also been shown to display deficits in tasks that require cognitive flexibility, such as forgetting previous task demands to respond effectively to new task demands (e.g. Saxe et al., 2007; Burghardt et al., 2012, Pan et al., 2012a). While it seems that there is a wide variety of tasks in which AHN has been implicated, it could be argued that each of these tasks falls into one of two broad categories: either they require overcoming interference or memory persistence. Learning a new platform location in the MWM, distinguishing between overlapping odour pairs, adjacent spatial locations, highly similar contexts or learning a new shock location in the same environment as one previously learned (in the case of fear avoidance) all require overcoming interference generated by highly similar and previously learned environments, spatial location or stimuli. Pattern separation processes could be also required for tasks that demand cognitive flexibility, whereby animals must overcome the proactive interference from previously learned

experiences in order to generate appropriate responses to new task demands. It is possible that rodents lacking AHN are not experiencing a deficit in memory extinction, but rather interference between a new experience and an overlapping old memory of an experience taking place in a similar environment. Manipulations that upregulate neurogenesis have the opposite effect, enhancement of performance on tasks that require pattern separation. For example, an exercise-induced upregulation of AHN results in fewer errors on tasks that presumably require pattern separation (Creer et al., 2010; Kohman et al., 2012). Moreover, Sahay and colleagues (2011) reported that increasing AHN via targeted genetic manipulation also increased performance on a CFC task with a high interference component. These data suggest that the benefits of running on behavioural pattern separation were not afforded by the pleiotropic effects of exercise on cognition, but rather by the more specific running-induced enhancement of AHN. Thus, it would seem that performance on tasks requiring adult-born granule cells can be enhanced or rescued using interventions targeted at increasing neurogenesis in animals (and perhaps humans) that have normal or down-regulated levels of AHN, respectively. However, it is unclear if the amount of neurogenesis is linearly related to pattern separation performance, or if there is a steep drop-off in performance at a certain threshold. It should be stated that there has been some debate as to which tasks are neurogenesis-dependent and which tasks are not. Data from various labs have been contradictory and so the precise role that AHN plays in behaviour remains controversial. For example, CFC was shown to be impaired in animals lacking AHN, as mentioned above (Saxe et al., 2006; Winocur et al., 2006; Imayoshi et al., 2008; Warner-Schmidt et al., 2008; Wojtowicz et al., 2008; Hernandez-Rabaza et

al., 2009; Ko et al., 2009; Guo et al., 2011; Nakashiba et al., 2012; Pan et al., 2012a, 2012b, 2013), however, others have reported that there was no difference in CFC performance between animals with reduced neurogenesis and controls (e.g. Shors et al., 2002; Clark et al., 2008; Dupret et al., 2008; Pollak et al., 2008; Zhang et al., 2008; Deng et al., 2009). Inconsistency likely stems from the differences in conditioning protocols used or the differences in approaches used to ablate neurogenesis. For instance, IRR, neurotoxic drugs, a variety of genetic manipulations (such as *Wnt*, *ERK5* etc.) and blocking neural transmission specifically in dentate granule cells have all been used to reduce or completely knock-out neurogenesis in the hippocampus. All of these methods decrease neurogenesis to various degrees and all have been criticized as having unwanted effects outside of the DG, which may also contribute to changes in cognition. Further, it was recently found that it was only when rodents were given a brief training exposure or relatively weak shock (which are more demanding training protocols) that AHN became required to form specific context-shock associations (Drew et al., 2010; Pan et al., 2012a, 2013). Finally, Snyder et al (2009a) found no CFC deficit in mice that were exposed to IRR, but did find a CFC deficit in irradiated rats. Thus, inter-species variability may also contribute to disparate results. Nonetheless, a majority of the data from rodent studies support a role for AHN in pattern separation. Despite a great deal of evidence implicating AHN in cognitive processes, there have been relatively few attempts to investigate the functional role of AHN directly in humans (reviewed in the section *Previous Attempts to Measure Adult Neurogenesis in Humans*). This lack of evidence from human participants largely stems from the fact that measuring AHN non-invasively in humans *in vivo* is

currently unachievable. Nonetheless, methods used in the animal literature, such as manipulating perceptually similar objects, have been modified for use as computer-based tasks that can be used to assess behavioural pattern separation in human participants. With a behavioural pattern separation task designed for use in humans, one could then test how activation in the DG, as measured by high resolution functional magnetic resonance imaging (fMRI), or lifestyle factors associated with upregulated or downregulated neurogenesis corresponds with task performance. One such putative neurogenesis-dependent task that has gained much attention in recent years is the Behavioural Pattern Separation – Object task (BPS-O; Kirwan and Stark, 2007).

The BPS-O was originally developed by Kirwan and Stark (2007). Since its first implementation, several variations have been developed. There are now explicit and implicit versions that have been used in both study–test and continuous recognition paradigms both in and outside of an MRI machine. The intentional version is usually completed in front of a computer screen (in or outside of an MRI scanner) and has participants explicitly judge whether each item observed is "old", "similar" or "new" to one previously seen (Figure 1.4; Kirwan and Stark, 2007; Toner et al., 2009; Yassa et al., 2010a, 2010b, 2011; Bakker et al., 2012; Kirwan et al., 2012; Motley and Kirwan, 2012; Déry et al., 2013; Shelton and Kirwan, 2013; Stark et al., 2013). The incidental (implicit) version requires participants to passively view a series of objects or categorize them as "indoor" or "outdoor" and is traditionally completed during an fMRI scan (Bakker et al., 2008; Yassa et al., 2010c, 2011; Lacy et al., 2011; Motley and Kirwan, 2012). Specifically, the BPS-O involves having participants carefully observe a series of

everyday objects, which are displayed one at a time. Some objects are exact copies of those already seen (repetitions), some objects are highly similar, but not identical, to ones previously observed (lures) and the remaining objects are completely unique and unlike from any of those previously seen (foils). In the study–test version, participants simply observe the items one at a time or they are asked to judge whether each item is more of an "indoor" item or more of an "outdoor" item during the study (presentation) phase and then participants are asked to determine whether each object is "old", "similar" or "new" during a separate test (recognition) phase. The highly similar objects, termed lures, are thought to generate interference between stimuli, based on their overlapping object features, and thus tax the pattern separation function attributed to the DG and AHN in particular. Accordingly, the correct identification of a lure as "similar" is said to require pattern separation, whereas misidentifying it as "old" is considered to be evidence of pattern completion.

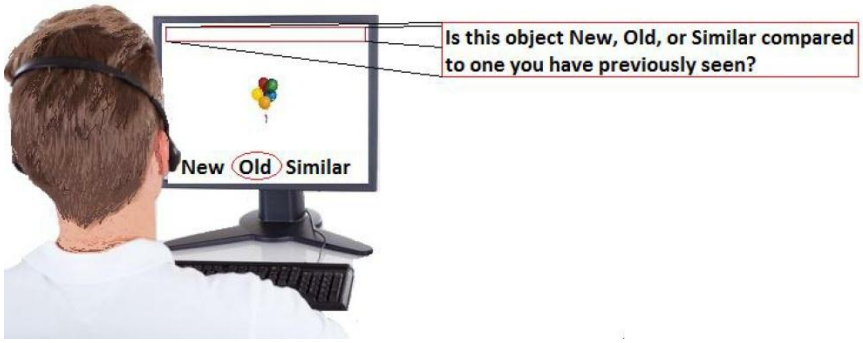

Figure 1.4. A participant seated in front of a computer screen, completing the BPS-O. After viewing a series of everyday objects, the participant enters into a recognition phase. In the recogntion phase he is presented with another series of objects, one at a time, and must classify them as "New", "old" or "Similar" compared to an object he has previously seen.

Kirwan and Stark (2007) demonstrated that activity in the hippocampus changed depending on whether a participant correctly identified a repetition as "old", a lure as "similar" or falsely identified a lure as "old", implicating the hippocampus in BPS-O performance. Unfortunately this study did not differentiate between hippocampal subregions. Later, it was found that the DG/CA3 subregion (combined due to insufficient spatial sensitivity to confidently separate the two) was equally strongly activated by both novel items and highly similar lures, but less so for repeated items – due to the repetition suppression effect (Bakker et al., 2008). Thus, it was concluded that the DG region treats similar items just like novel ones (Bakker et al., 2008; Lacy et al., 2011). Interestingly, this subfield-level activity changes with old age and so does BPS-O performance. Specifically, high-resolution fMRI and diffusion tensor imaging (DTI) studies have demonstrated that hyperactivity in the DG/CA3 region and perforant path degradation manifest with old age and mild cognitive impairment (MCI; Yassa et al., 2010b, 2010c, 2011; Yassa and Stark, 2011). Importantly, both DG/CA3 hyperactivity and perforant path degradation negatively correlate with pattern separation performance (Yassa et al., 2010b, 2010c, 2011; Yassa and Stark, 2011). Toner and colleagues (2009) support the finding that aged individuals are impaired at visual pattern separation. By subtracting the proportion of pattern completion errors (i.e. calling a lure "old") from the number of correct responses to lure trials (i.e. calling a lure "similar"), one can determine a participant's bias towards pattern separation or pattern completion. In this way, Toner and colleagues (2009) found that younger adults were biased towards pattern separation, whereas older adults were biased towards pattern completion, further suggesting that

under normal circumstances the hippocampus should favour pattern separation. Indeed, there is a gradual negative transition from pattern separation towards pattern completion over the lifetime of healthy individuals (Stark et al., 2013). Perhaps there is good reason for why older adults would not require pattern separation processing to the same extent that younger adults do.

Motley and Kirwan (2012) manipulated objects by rotating them by various degrees from the original target, thereby parametrically adjusting their level of similarity. The participants' behavioural pattern separation performance increased as items became more rotated (less similar), while simultaneously making fewer pattern completion errors. Furthermore, activity in the hippocampus followed a power function with decreasing slope as items decreased in similarity. Interestingly, both incidental and intentional encoding strategies led to a pattern separation-like function in the hippocampus. Though methods such as fMRI do not permit one to discern the individual contributions of each hippocampal subregion, it is clear that the hippocampus plays a critical role in visual pattern separation. Lacy and colleagues (2011) found that it was the DG/CA3 region's response function in particular that was altered in a discontinuous manner (again, a power function with decreasing slope) by varying the degree of target–lure similarity. In contrast, a more linear response function was observed in the CA1 (Lacy et al., 2011). The authors suggest that these different response functions are due to the DG/CA3 subregion being more sensitive to small changes in input, whereas the CA1 subregion is more resistant to change (Lacy et al., 2011). Together, the data of Lacy and colleagues (2011) and Motley and Kirwan (2012) suggest that pattern separating regions have large

differences in fMRI-measured activity in response to small changes in stimulus input (e.g. object rotations), whereas pattern-completing regions demonstrate large changes in activity, but only when the level of similarity exceeds a certain threshold. Not surprisingly, the discontinuous response function in the DG/CA3 is also altered in older individuals, whereby more dissimilarity is required before activity in the region shows evidence of pattern separation (Yassa et al., 2011). Further, this change in activity is linked to behavioural performance, whereby older adults are better able to correctly identify lures as "similar" the more dissimilar they are from the original target (Yassa et al., 2011). To summarize, old age is associated with perforant path degradation, hyperactivity and an altered response function in the DG/CA3 region that are all associated with impaired lure trial performance on the BPS-O. Hyperactivity in the CA3 might reflect a lack of inhibition due to deterioration of the perforant path (Barnes, 1979; Barnes et al., 2000), in turn biasing the hippocampal network towards pattern completion (Wilson et al., 2006). A hallmark of the DG, key to its ability to orthogonalize incoming information, is sparse firing (Treves and Rolls, 1994). Therefore, reduced inhibitory control in that region could certainly underlie the pattern separation deficit observed in old age. Nakashiba and colleagues (2012) used transgenic mice that had older dentate granule cells silenced, while leaving the more immature granule cells intact. Interestingly, they found that younger neurons in the DG supported pattern separation, whereas more mature neurons in the DG were required for pattern completion (Nakashiba et al., 2012). It remains to be determined if ablating young cells fundamentally changes how mature cells function, or vice versa. Nonetheless, perhaps a reduced number of newborn cells in

the aged DG might also underlie a network shift towards pattern completion – explaining the pattern separation deficits observed in older adults.

Importantly, healthy older adults perform worse on the BPS-O than younger adults, while older adults with MCI perform worse than healthy aged-matched controls, suggesting a divergence in performance as people age (Yassa et al., 2010b, 2011). In a different study, older adults were separated into age-impaired or age-unimpaired groups based on standardized word learning performance on the RAVLT (Stark et al., 2010). The age-impaired group was worse at identifying lures in the BPS-O than both the age-unimpaired and young adult groups, regardless of the amount of separation between stimuli (Stark et al., 2010). Their findings further suggest that behavioural pattern separation is only impaired in a subset of older adults, even when excluding those diagnosed with MCI (Stark et al., 2010). More recently, Stark and colleagues (2013) showed that age-unimpaired adults outperformed both age-impaired adults (non-clinical) and those with MCI, specifically on lure trials. On the other hand, adults with MCI also demonstrated worse performance than both non-clinical groups at identifying repetitions (Stark et al., 2013). Thus, while the MCI group was impaired on both traditional measures of recognition (identifying repetitions) and behavioural pattern separation (identifying lures), the age-impaired group was selectively impaired on lure trials, with traditional recognition being spared (Stark et al., 2013). These data suggest that while behavioural pattern separation deteriorates with aging, there is a divergence between adults in the extent of that deterioration. Perhaps deficits in pattern separation ability with increasing age reflect early stage MCI or a predisposition for developing MCI or another

form of dementia. Further investigation is required to determine what makes the difference between age-impaired and -unimpaired adults, such as participation in physical activity versus history of stress or alcohol abuse, and what can be done to prevent the age-related declines in pattern separation (e.g. pro-neurogenic activities).

Deficits in pattern separation seen in aging may not only be limited to the purely visual domain. A paper by Stark and colleagues (2010) described that spatial pattern separation was also impaired in older adults. A subsequent study by Holden and colleagues (2012) examined the ability of young adults and old adults without MCI to perform another spatial pattern separation task involving varying degrees of interference. During the study phase of each trial, a circle briefly appeared on a computer screen and during the test phase, two circles were displayed simultaneously and the participant was asked to select which circle was in the same location as one previously presented. The two circles in the test phase were separated by one of four possible separations: 0, 0.5, 1.0, and 1.5 cm. Young adults outperformed older adults at every separation, further suggesting that pattern separation is less efficient in old age (Holden et al., 2012). When the aged group was split into age-unimpaired and -impaired groups, it was found that only the age-impaired group displayed a deficit in spatial pattern separation compared to younger adults (Holden et al., 2012). Thus, just as it was for visual pattern separation, only a subset of older adults accounted for the deficit in spatial pattern separation performance associated with aging (Holden et al., 2012). These data suggest that age-related deficits in pattern separation are not limited to a particular stimulus type (e.g. visual or spatial) and further underscore the divergence in behavioural pattern separation

performance as people age. The above-mentioned studies provide compelling evidence that the human hippocampus, and specifically the DG and CA3 subregions, play a critical role in behavioural pattern separation performance. These studies also lend indirect evidence to the hypothesis that AHN is required for pattern separation. Specifically, as humans age, they lose their ability to orthogonalize highly similar information, and at the same time experience a concomitant decline in neuronal turnover (Imayoshi et al., 2009; Knoth et al., 2010; Spalding et al., 2013).

Notably, there have been several reports of a correlation between BPS-O performance and RAVLT delayed word list recall score (Stark et al., 2010; Stark et al., 2013). Further, the integrity of the perforant path has also been shown to correlate with RAVLT performance (Yassa et al., 2010c). By stressing rapid learning of a new "List B" while overcoming proactive interference from the previously studied "List A", the RAVLT has the hallmarks of a task that should require neurogenesis, namely, a high interference component. One study used RAVLT to assess cognitive performance following 12 weeks of exercise in older adults (Pereira et al., 2007). In this study, they used a steady-state MRI perfusion measure to assess blood volume in the following hippocampal subregions: EC, DG/CA3, CA1 and subiculum. Given the tight coupling between angiogenesis and neurogenesis (Palmer et al., 2000; Louissaint et al., 2002), Pereira and colleagues (2007) hypothesized that increased blood volume is an indicator of neurogenic activity and thus, exercise-induced enhancement of blood volume should be limited to the DG/CA3 subregion. Indeed, a significant increase in blood volume was limited to the DG/CA3 subregion and this change in blood volume correlated with

changes in aerobic capacity, measured by peak oxygen uptake (VO_2*peak*; Pereira et al., 2007). Further, the change in blood volume within the DG/CA3 subregion also correlated with performance on RAVLT trial 1 learning, but not delayed word list recall, the putative measure of neurogenesis-dependent cognition (Pereira et al., 2007). The reason that exercise (and the putative exercise-induced increase in neurogenesis) did not improve delayed word list recall might be due to the relatively small sample size, or the fact that Pereira and colleagues did not separate the study population into impaired and unimpaired groups based on standardized word learning performance. Any difference in RAVLT performance at baseline could reduce the chance of finding a correlation post-exercise. Nonetheless, this study was the first to demonstrate that a putative biological correlate of neurogenesis in humans correlated with memory performance (Pereira et al., 2007).

1.3.2 Memory Persistence

A growing body of literature has implicated AHN in memory persistence (Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009; Kitamura et al., 2009; Inokuchi, 2011; Pan et al., 2012a, 2012b, 2013). AHN has been central to many theories of learning and memory, most notably at the time of encoding. However, few researchers have incorporated the addition of adult-born neurons into their models of long-term memory retention or, in contrast, memory clearance. Indeed, AHN may be one mechanism in which a new substrate for learning is provided, while older memories are simultaneously cleared, forgotten (Feng et al. 2001; Deisseroth et al. 2004; Inokuchi, 2011). Presumably, if newborn neurons in the adult hippocampus are required for reducing interference

during encoding of new memories, then they may also influence how older memories are transformed, overwritten by new events or completely lost. If the hippocampus acts as a gateway for memory transformation (as postulated in Teyler and DiScenna's Memory Indexing Theory; Teyler and DiScenna, 1986) then perhaps adult-born neurons act as the gatekeeper, helping to decide what aspects of a memory are strengthened and when. How does AHN fit into some of the more contemporary theories of learning and memory? One of the more popular models, the Multiple Trace Theory (MTT; Nadel and Moscovitch, 1997; Winocur et al., 2010) is summarized below, along with some recent expansions: the Transformation Hypothesis (Winocur et al., 2010), Indexing theory (Teyler and DiScenna, 1986; Teyler and Rudy, 2007) and the Competitive Trace Theory (CTT, Yassa and Reagh, 2013). MTT holds that the hippocampus remains important for the retrieval of all episodic memories, whether recent or remote. Further, each reactivation of the memory, during recollection, results in reconsolidation, creating a new trace within the hippocampus. An extension of MTT, the Transformation hypothesis (Winocur et al., 2010), posits that memories mediated by the hippocampus are fundamentally different from those that are consolidated in extra-hippocampal structures in that they are rich with contextual detail, whereas consolidated memories mediated by the cortex are more schematic and less detailed. Thus, memories are eventually transformed from episodic to semantic as they age and become consolidated in the cortex. Importantly, an optogenetic study in mice has recently provided evidence that the hippocampal CA1 region is necessary for the retrieval of remote memories (Goshen et al., 2011). However, Goshen and colleagues (2011) also described that, following a longer period of inhibition

sufficient to allow for compensation from other regions, this dependence on the CA1 was abolished. At first glance this finding would seem to argue against MTT as the hippocampus was no longer required for the retrieval of remote memories as other regions, namely the anterior cingulate cortex, were able to compensate. However, as the authors state, this finding does not rule out the possibility that the hippocampus still acts to provide some aspects of remote memories, such as context (Goshen et al., 2011). Indexing theory (Teyler and DiScenna, 1986) treats the hippocampus as a gateway. Specifically, when a remote memory trace in the cortex is partially cued via incomplete or degraded input, the cortex then activates a small population of neurons in the hippocampus. In turn, the hippocampus, through back-projections to the neocortex activates the full memory. As the memory is consolidated, connections between the hippocampus and neocortex are strengthened, generating a physical representation of the memory trace. Importantly, the hippocampus, though not necessary for memory retrieval, may always play an integral role (regardless of the age of a memory). Interestingly, Indexing theory suggests that hippocampal-neocortical interactions play a part in pattern completion – a function traditionally ascribed to the CA3 (Marr, 1971). Building on previous theories, Yassa and Reagh (2013) proposed the Competitive Trace Theory (CTT) of learning and memory, which includes a description of how the balance between pattern separation and pattern completion could influence how memories are stored. According to CTT the hippocampus is not the site of storage, but rather links the individual components of a neocortically stored memory together, much like as described in Indexing theory. In this way, the memory trace can be partially retrieved by the

neocortex alone or fully retrieved by the hippocampus in conjunction with the neocortex. As previously mentioned, the CA3 is hypothesized to be the location where pattern separation and pattern completion processing is balanced. Incoming information could be orthogonalized by passing through the DG and mossy fiber pathway before arriving in CA3. As long as there is ample neurogenesis, memories will be non-overlapping due to the pattern separation processes afforded by input from adult-born granule cells. On the other hand, a remote memory that is reactivated by partial or degraded cues via recurrent collateral connectivity within the CA3 or by direct perforant path connections between the CA3 and neocortex may be susceptible to interference. Specifically, when the full representation of a neocortical trace is retrieved by the hippocampus, the non-overlapping aspects of the memory (now subject to reconsolidation) are susceptible to interference, while the overlapping aspects of the memory are strengthened. The reinstated trace contains some overlapping features (the attractor state) along with some unique features from the novel event. Non-overlapping information between the new and old events would generate conflict and lead to a slightly altered version of the original trace. The altered memory is then able to be consolidated into a new trace, going through the same storage process as the original. Indeed, a key principle of CTT is that once a memory trace is activated then it can undergo re-consolidation. Thus, interference caused by non-overlapping environmental input would result in the decontextualization of the neocortical trace and, hence, a more semantic-like memory. Accordingly, while a recent memory (residing in the hippocampus) is rich in contextual detail, a remote memory contains stronger and more resilient semantic information that is less susceptible to

interference or degradation by the intrusions of non-overlapping information during re-activations. It has previously been suggested that the quality of memories transform from precise (i.e. detailed) to less precise (i.e. more schematic or generic) with a similar time course to that of consolidation (Nadel and Moscovitch, 1997; Moscovitch et al., 2006; McKenzie and Eichenbaum, 2011). In other words, the amount of detail recalled would decline with the age of a memory. McKenzie and Eichenbaum (2011) propose that even consolidation of the original memory itself (before any reactivation) is susceptible to interference because there is no such thing as forming a 'new' memory. Rather, novel input is constantly reorganizing pre-existing memory representations. Thus, it may be important to consider that even completely novel information could be in competition with previously stored memories. Nonetheless, according to Yassa and Reagh (2013) the competition between recent and remote memories may be adaptive in the sense that it would help to maintain and strengthen remote memories by preserving the repeated and important elements of a trace while at the same time degrading the novel and competing elements. In summary, according to CTT pattern separation in the DG is the most important factor in re-encoding a slightly different version of the experience, which causes subsequent competition among overlapping traces in hippocampal- neocortical stores (Yassa and Reagh, 2013). When the dissimilarity between memories is enhanced by pattern separation, the amount of non-overlapping information would increase as a by-product. In turn, this would enhance the amount of interference between incoming information and previously stored memories, leading them to be altered in such a way that they are no longer retrievable. The idea that pattern separation can increase

competitive consolidation between new and old memories could be one mechanism by which neocortical interference is facilitated, while hippocampal interference is minimized. Indeed, Weisz and Argibay (2009) have shown, using a biologically plausible model of the entire hippocampal network, that the addition of neurogenesis would increase the hippocampus' ability to store new information, while simultaneously helping to clear older memories. More recently, they expanded on their model and concluded that, much like the theoretical work of Aimone and colleagues (2006, 2009), neurogenesis is important for integrating information presented close together in time and orthogonalizing information presented far apart in time (Weisz and Argibay, 2012). Moreover, AHN may actually contribute to interference between new and old memories, thus creating a potential mechanism for forgetting. Specifically, their model was presented with cues composed of 5%, 10%, 15% or 100% of the original patterns. The network was then trained with a variable number of patterns in order to assess the model's storage capacity. When neurogenesis was added to the model its ability to retrieve memories formed after neurogenesis was enhanced, as measured by the mean output correlation – that is, the difference between input and output signals. The neurogenic model also had a superior storage load compared to another model lacking the addition of new neurons. On the other hand, neurogenesis interfered with the retrieval of remote memories (those formed before neurogenesis had taken place). Therefore, according to their model, it is memories that are several weeks old that are the ones most likely to suffer from interference. However, AHN is an ongoing process, so a one-time addition of immature neurons may not be capturing the true reality of network dynamics

in vivo. Another drawback of the model is that neurogenesis does not seem to have an effect on model performance until it is heavily trained i.e. the model is reaching its saturation limit. Rather, it is likely that AHN is required under any circumstance whereby there is a high potential for interference, although a high memory load may enhance the requirement of AHN. Another limitation of the model is that new neurons are included only after previous learning has taken place. Therefore, it is possible that the old memories are preferably forgotten because they did not have the advantage of recruiting a population of hyperexcitable young neurons to encode more specific features, like the more recent memories did. In reality, both recent and remote memories would have had the benefit of being encoded in a neurogenic environment. Finally, it should be mentioned that these findings are in contrast to a number of other abstract models, as well as more biologically inspired models, that use ongoing neurogenesis or additive neuronal turnover. In these models neurogenesis is consistently shown to prevent the degradation of older memories (e.g. Becker, 2005; Wiskott et al., 2006; Appleby and Wiskott, 2009; Appleby et al., 2011). For example, Becker's model does not support this view (Becker, 2005). Her model was trained on a full stimulus set, and tested on its ability to retrieve each of the training stimuli when presented with a partial or degraded version. In other words, the model was tested on its ability to perform pattern completion. Between each of the ten training sessions, a turnover event occurred on a controlled amount of units in order to simulate ongoing neurogenesis. The results demonstrated a beneficial effect of unit turnover, but only under conditions of high interference (Becker, 2005). Nonetheless, the model of Weisz and Argibay (2009, 2012) provides compelling evidence for the role

of newborn neurons in memory clearance and offers one potential mechanism for doing so. It provides computational support for the notion that AHN can, at least in some circumstances, create interference associated with forgetting. Another interesting result of this work is that it suggests that memories will be most affected by interference when they are several weeks old, approximately the same age that it takes for memories to become more dependent on the neocortex (Frankland and Bontempi, 2005). It might be that the original representation for older memories fundamentally changes over time, including a number of intrusions from newer events, rather than being completely forgotten. Josselyn and Frankland (2012) have also recently proposed that increased rates of AHN may serve to clear memories from the hippocampus. According to their neurogenic hypothesis for infantile amnesia, the higher rates of AHN observed in early post-natal life account for the inability to remember anything that happened during that time (< 2–3 years of age; Josselyn and Frankland, 2012). Although infants and young children are able to acquire new memories, higher rates of AHN lead to proportionally higher rates of forgetting (Josselyn and Frankland, 2012). Interestingly, the levels of neurogenesis and memory stability are inversely related (Josselyn and Frankland, 2012). Thus, just as aging downregulates neurogenesis in humans (e.g. Imayoshi et al., 2009; Knoth et al., 2010; Spalding et al., 2013), and observation that led to our earlier hypothesis that age-related changes in neurogenesis underlie the observed behavioural pattern separation deficit in older adults (Toner et al., 2009; Yassa et al., 2010b, 2010c, 2011; Stark et al., 2010, 2013; Yassa and Stark, 2011; Holden et al., 2012), increased neurogenesis during early postnatal life is thought to underlie the destabilization and

increased forgetting (or clearance) of memories in infants and young children (Josselyn and Frankland, 2012). Indeed, neurogenesis in humans is highest following birth and declines rapidly in the first few months of life until it levels off and continues to decline more steadily (Knoth et al., 2010; Spalding et al., 2013). Josselyn and Frankland (2012) further suggest that the integration of adult-born neurons can degrade older memories in at least two different ways: 1) by increasing excitability within the hippocampus, thus destabilizing memory networks, or 2) by taking over or adding to the synaptic connections of pre-existing memory networks. Both of these mechanisms could result in the failure to retrieve old memories (reviewed in Josselyn and Frankland, 2012; Frankland et al., 2013). On the other hand, Josselyn and Frankland (2012) predict that more remote memories, which are not as dependent (or no longer dependent) on the hippocampus will be less sensitive to fluctuating rates of AHN and synaptic remodelling. If true, then it would be important to keep in mind that more neurogenesis does not necessarily mean better memory, further underscoring the need for a balance between pattern separation and pattern completion, in turn, leading to a balance between memory formation and clearance.

1.3.2.1 Empirical Evidence Supporting Memory Persistence

It has been shown in a number of behavioural studies in animals that AHN plays an important role in remote memory (e.g. Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009; Kitamura et al., 2009; Pan et al., 2012a; 2012b, 2013). The ablation of immature granule cells that were one to four weeks old at the time of learning resulted in a remote memory deficit for spatial location (Deng et al., 2009). In contrast,

when older cells (four to eight weeks old at the time of learning) were ablated instead, there was no impairment (Deng et al., 2009). Snyder and colleagues (2005) provide additional evidence for a deficit in remote memory in rodents lacking neurogenesis. Specifically, rats subjected to IRR, thus having neurogenesis in the hippocampus largely abolished, were not impaired at recalling the target quadrant during a MWM probe trial when testing took place one week after initial training (Snyder et al., 2005). This result is consistent with many other reports, whereby memory for the platform location in MWM was no different between animals with ablated neurogenesis and controls when they were tested shortly after learning (e.g. Shors et al., 2002; Raber et al., 2004; Meshi et al., 2006; Wojtowicz et al., 2008). However, rats that underwent IRR exhibited a long-term retention deficit: they were impaired at recalling the hidden platform location when they were tested two or four weeks post-training (Snyder et al., 2005). IRR might also affect post-mitotic neurons and cause a substantial inflammatory response (Monje et al. 2003), thus it has been argued that such memory impairments may be the result of extrahippocampal damage. Importantly, similar results have been described by Jessberger and colleagues (2009), in animals that had neurogenesis in the DG selectively inhibited by reducing *Wnt* signalling. Rats with the highest knockdown of AHN performed normally on acquisition of the MWM, but were deficient at finding the hidden platform location when the probe trials were administered two or more weeks after training (Jessberger et al., 2009). Further, the highest knockdown group was able to identify novel objects in an object recognition task when tested one hour after learning, but was unable to distinguish between novel and repeated objects when tested three hours or four weeks

post-training (Jessberger et al., 2009). Goodman and colleagues (2010) used a common neurogenesis-inhibitor, the antimitotic agent methylazoxymethanol acetate (MAM), to reduce the number of adult-born neurons in the DG before training. They found that animals pre-treated with MAM displayed deficits at recent and remote (30 days later) spatial memory (Goodman et al., 2010). MAM treated animals also displayed a marked deficit in spatial memory and their ability to distinguish between novel and familiar spatial locations when tested one day post-training (Goodman et al., 2010). It should be noted that object recognition can be hippocampal-dependent or -independent contingent on the task design (Barker and Warburton, 2011). Though the one day results in MWM are inconsistent with some previous reports (e.g. Shors et al., 2002; Raber et al., 2004; Meshi et al., 2006; Wojtowicz et al., 2008), this discrepancy may be due to compensation by existing, developmentally generated granule cells. Thus, Goodman and colleagues (2010) provide additional evidence that new neurons recruited at the time of hippocampal-dependent learning are essential for memory persistence. Interestingly, liver IGF-1 deficient (LID) mice who lack peripheral IGF-1, a protein previously shown to be critical for increasing AHN in response to its infusion (Aberg et al., 2000) or wheel running (Trejo et al., 2001), also demonstrate a remote memory deficit (e.g. Ding et al., 2006; Llorens-Martin et al., 2010). Further, ERK5 icKO mice lacking neurogenesis, while not impaired in fear memory when tested 24 hours post-learning, were impaired when tested 21 days after training (Pan et al., 2012a). Pan and colleagues (2012b) trained mice in a CFC paradigm and a cued version of the task. Freezing in response to the cue or the context was measured one day, six days or five weeks post-training. Mice that had

ERK5 knocked-out pre-training were just as good as control mice at acquiring the task and when tested two minutes post-training (recent memory), but demonstrated significant impairment when tested six days or five weeks after training (remote memory). The deficit was context-specific because there was no difference in freezing between ERK5 icKO and control mice when they were tested in a novel environment. These data suggest that mice lacking AHN are able to form the initial context-dependent memory, but do not maintain that memory for extended periods of time (Pan et al., 2012b). Mice that had ERK5 knocked-out post-training had contextual fear memory tested at 5, 9, 12, and 16 weeks after acquisition. The mice with ERK5 knock-out post-training froze significantly less than control mice at every time point, most notably at 12 and 16 weeks post-training. Finally, a cohort of mice had ERK5 knocked-out 5 weeks after training and then memory was tested 15 weeks post-acquisition. The ERK5 icKO mice froze less than saline injected controls at this time, suggesting that these mice also had a marked deficit in the maintenance of remote memories, even when AHN was disrupted as late as five weeks after training. These data suggest that new neurons, once recruited, play a persistent role in the maintenance and reactivation of remote memories (Pan et al., 2012b). These data, taken together with the results described above, strongly implicate adult-born neurons, and ablation thereof, in a type of retrograde amnesia. Thus, new neurons are required for the persistence of remote memories. These studies provide support for the hypothesis that the hippocampus, and AHN in particular, is required for the accessibility or persistence of remote memories, regardless of their physical location: the hippocampus and/or neocortex (Frankland and Bontempi, 2005; Nakashiba et al., 2008, 2009). Importantly, it

would seem that a variety of researchers from unaffiliated labs and who used vastly different methods for downregulating or completely abolishing adult neurogenesis have consistently described a remote memory deficit in neurogenesis-deficient animals.

Although it is apparent that neurogenesis plays a critical role in the retrieval of remote memories, it is still unclear how strong a role it plays. It has been shown that new neurons in the DG are incorporated into hippocampal-neocortical networks that support remote memory (Trouche et al., 2009). Trouche and colleagues (2009) found high survival rates of immature neurons in mice that underwent MWM training 1 day or 30 days earlier, compared to mice that remained in their home cage and mice that swam, but did not undergo training. Consistent with previous reports (Gould et al., 1999; Dupret et al., 2007; Epp et al., 2007), their finding suggests that long-term survival of immature neurons in the DG is linked to the learning component of a spatial memory task. Approximately 1% of newborn neurons expressed the activation marker Zif268 upon re-exposure to the water maze for a probe trial occurring 30 days post-training, whereas caged and swim controls did not express a single new neuron positive for Zif268. These data suggest there was enhanced activation among those adult-born neurons recruited during MWM training (Trouche et al., 2009). Interestingly, around 4% of newborn neurons expressed Zif268 when mice were exposed to a hidden platform test 30 days after training (same condition as training) versus 1% in the mismatch condition with no platform. This finding indicates that re-activation of immature neurons during re-exposure is situation-specific (Trouche et al., 2009). Further, 11% of immature DG neurons expressed Zif268 when mice were subjected to nine re-exposure trials (as

opposed to one) one month after initial acquisition. On the other hand, only 4% of recently born neurons in the DG expressed Zif268 in partial mice. Partial mice are those who received nine days of exposure to the MWM, but who never received training. Thus, being exposed to the same training environment multiple times activated a greater number of adult-born granule cells than did a single re-exposure and, further, a greater proportion of cells were activated when mice were exposed to an environment they experienced in the distant past (> 4 weeks) versus one experienced more recently (on the order of days). Therefore, the authors suggest that the recruitment of new neurons is determined at the time of training and those newly recruited neurons are later required for updating and strengthening of remote memories (Trouche et al., 2009). Importantly, similar numbers of activated mature neurons were found in all groups, indicating that the differences in Zif268 expression among younger neurons was not due to a general increase in DG activation, but rather reflected the differential contribution of immature neurons to the specific memory conditions. A question that remains is how these immature neurons are recruited during training and preferentially re-activated during re-exposure to a similar task or environment. Nonetheless, this study provides compelling evidence that new neurons of a certain age (~9 days old) at the time of training are re-activated when a similar event is encountered, thereby providing a mechanism to update or strengthen remote memory traces (Trouche et al., 2009). Arruda-Carvalho and colleagues (2011) developed a "tag and ablate" transgenic strategy that allowed them to identify adult-generated neurons and then ablate them prior to or following training. In these transgenic mice who express simian diphtheria toxin receptors (DTRs), the

administration of TAM induces the expression of DTRs on adult-born cells and associated daughter cells, and the subsequent administration of diphtheria toxin (DT) then serves to ablate this tagged population. When transgenic and control mice received DT injection one day after fear conditioning, control mice exhibited longer freezing behaviour in the original training environment than they did in a similar environment. On the other hand, transgenic mice with ablated neurogenesis exhibited similar levels of freezing in both environments, suggesting they lost the ability to discriminate between similar contexts (Arruda-Carvalho et al., 2011). In a completely novel environment, both transgenic and control mice exhibited similar levels of freezing, thus mice lacking AHN only lost the ability to discriminate between similar, but not dissimilar, contexts. Interestingly, ablating new neurons before training had no effect on memory for context. As the authors suggest, this discrepancy is likely because when neurons are ablated prior to training, the remaining population of dentate granule cells is able to compensate (Arruda-Carvalho et al., 2011). The potential for compensation by the older developmentally generated granule cell pool may account for the variable effects of pre-training ablation on memory performance. Indeed, the post-training ablation of young neurons in the DG impaired performance in the MWM, whereas pre-training ablation neurogenesis had no effect. Consistent with other reports described above, post-training ablation of tagged neurons one month (as opposed to one day) after training led to a marked deficit in remote memory for platform location in the MWM. Thus, memory for the platform location depends on adult-born neurons in the hippocampus for at least one month after training, suggesting that AHN plays a critical and long-lasting role in

preserving the memory trace (Arruda-Carvalho et al., 2011). Importantly, the 'tag and ablate' protocol used by Arruda-Carvalho and colleagues (2011) served to ablate a population of adult-born granule cells that were several weeks old at the time of training (representing the hyperexcitable and functional population), without disrupting ongoing proliferation in the region. Taken together, these results indicate that ablating dentate granule cells after training leads to degradation of the memory, such that some features of the memory remain, whereas others are lost – impairing the animal's ability to differentiate between highly similar environments. Further, adult-born cells were activated upon re-exposure to a similar environment, even one month post-training, suggesting that they are required for retrieval of remote memories.

Kitamura and colleagues (2009) recently demonstrated that AHN can also determine the speed at which a memory becomes independent of the hippocampus. Specifically, when immature dentate granule cells were ablated via IRR, they found the duration of LTP in the hippocampus of rats was prolonged from around two weeks to three or four (Kitamura et al., 2009). Further, when mice had neurogenesis genetically knocked-down or ablated via IRR, they did not experience any deficits in acquiring a context-fear association, nor did they display any deficits when tested one day post-training (Kitamura et al., 2009). On the other hand, mice lacking neurogenesis were impaired at recalling the original context when tested four weeks after training and when LTP in the hippocampus was blocked via intrahippocampal administration of tetrodotoxin (TTX, a sodium channel blocker) and 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX, a selective antagonist of AMPA receptors) 30 minutes prior to retrieval

(Kitamura et al., 2009). In contrast, control mice that had intrahippocampal infusion of TTX and CNQX did not display the same deficit in recall when tested four weeks after training, suggesting that the memory was no longer dependent upon the hippocampus (Kitamura et al., 2009). Therefore, suppressed neurogenesis prolonged the time it took for the memory to become independent from the hippocampus, thus extending the time dependence of fear memories on the hippocampus (Kitamura et al., 2009). In contrast, wheel-running accelerated the transfer of memories from the hippocampus to neocortex (Kitamura et al., 2009). When the hippocampus was inactivated in control mice just one week after training, they were much worse at remembering the original context than were mice given access to running wheels that displayed increased AHN (Kitamura et al., 2009). Importantly, in a retrieval test of one day-old memory, intrahippocampal infusion of TTX and CNQX significantly reduced the freezing response of both non-irradiated and irradiated mice, suggesting that one day post-training the memory still resided in hippocampus in both groups. Just like Feng and colleagues (2001), Kitamura and colleagues (2009) propose that AHN can promote the formation of new memories by preventing the hippocampus from filling up with old memories. However, rather than promoting clearance, they propose that the hippocampus achieves this by transferring the dependence of memories from the hippocampus to neocortex (Kitamura et al., 2009). If memories are more susceptible to interference when they are dependent on the hippocampus, then faster transfer to the neocortex should result in remote memories that are more factual, with fewer intrusions from non-overlapping events. However, what happens after the memory becomes independent of the hippocampus is still not well

understood. It should be noted that real-time inhibition of the CA1 region, while sparing other hippocampal subregions, is also sufficient to impair remote memory (Goshen et al., 2011). On the other hand, inhibition of the CA1 does not cause impaired short-term memory, presumably because hippocampal-neocortical interactions (working through the CA1) are not required for retrieval. Thus, while the CA1 may be one region required for the recall of remote memories, the CA3 along with input from the DG is required for encoding and reconsolidation.

Although convergent evidence from multiple groups seems to implicate AHN in memory persistence, the preservation of memories over extended periods of time, conflicting evidence from other groups points in the opposite direction. Data coming out of Paul Frankland and Sheena Josselyn's labs, in particular, provide compelling evidence for a role of neuronal turnover in destabilizing memories and, hence, memory clearance (reviewed in Frankland, Kohler and Josselyn, 2013). They take the theory one step further by suggesting that relatively high rates of AHN immediately after birth is what causes the phenomenon known as infantile amnesia, that is the inability to remember anything from early post-natal life (reviewed in Josselyn and Frankland, 2012). The supporting evidence for their assertions is relatively less extensive than the evidence for AHN in assisting remote memory formation, but it is quite convincing nonetheless. Campbell and Campbell (1962) trained rats of various ages in a place discrimination task and then tested memory retention following a delay of 0, 7, 21 or 42 days. They found that young rats (of a corresponding age to human infants) displayed memory deficits, whereas adult rats were unimpaired at all time-points (Campbell and Campbell, 1962).

Interestingly, in guinea pigs, which are born with a more fully developed hippocampus as compared to rats, there was no difference in memory retention between infants and adults (Campbell et al., 1974). In fact, guinea pigs were able to retrieve memories that were formed several months earlier, even when they were only five days old at the time of training (Campbell et al., 1974). Similar to rats, memory persistence in monkeys gradually increases with age (Zeamer et al. 2010). Evidence from Frankland and colleagues (2001, 2004) has also lent evidence to the hypothesis that AHN is important for memory clearance. Mice with genetically enhanced levels of AHN (deficient for the synaptic protein α-calcium/calmodulin kinase II) similar to that observed in infancy are able to acquire spatial and context-dependent memories, but are unable to retain those memories for an extended period of time. However, these mice also have impaired cortical plasticity, so their inability to form remote memories may be due to impaired hippocampal-neocortical interactions. Finally, a set of elegant studies described in Akers and colleagues (2014) provides perhaps the most compelling and comprehensive set of data implicating neurogenesis in forgetting and not only in new-born rodents, but also in adults. Briefly, infant mice, which have exponentially higher rates of AHN than do adult mice, were impaired at remembering the shock-context association, but only when tested more than one day after training (Akers et al., 2012). Adult mice that underwent physical activity (had free access to running wheels) post-training had significantly more adult-born neurons in the DG than non-runners, but performed significantly worse on a probe test of associative fear memory six weeks after learning (Akers et al., 2014). Similarly, mice treated with memantine or fluoxetine, common antidepressant pharmaceuticals that

upregulate neurogenesis, also experienced more forgetting (Akers et al., 2014). On the other hand, when running- or drug-induced increases in neurogenesis were blocked, forgetting was prevented (Akers et al., 2014). Together, these data strongly suggest that neurogenesis is tied to memory extinction, rather than perseverance. However, the results of these data are in contrast to those described above. Difference in the type of memory being tested – contextual fear versus spatial learning or timing of pro- or anti-neurogenic interventions could underlie some of these discrepancies. For now, the precise role of neurogenesis in memory persistence remains open for debate, but nonetheless, it seems that adult-born granule cells play some sort of role in the balance between forgetting (destroying old connections) and memory formation (making new connections).

1.3.3 Previous Attempts to Measure Adult Neurogenesis in Humans

We will not be able to fully understand the functional role of newborn neurons in the adult human brain unless we can quantify those neurons *in vivo*. Unfortunately, we cannot yet measure neurogenesis in the living human brain, owing to the invasiveness and technological limitations associated with currently available methods. However, there have been a number of recent studies that were designed with the intention of elucidating putative biological markers of neurogenesis in humans. The majority of these studies used MRI-based techniques to indirectly quantify neurons in the human brain. Proton magnetic resonance spectroscopy (MRS) has been used to evaluate the concentration of various brain metabolites, in an attempt to quantitatively measure neural progenitor cells in the human hippocampus (Manganas et al., 2007). Manganas and colleagues (2007) identified a peak at 1.28 ppm on the proton spectrum using a 3 Tesla MRI scanner that

they claim represents neural progenitor cells. Major strengths of this method are that it is non-invasive and would allow for quantification of AHN. However the specificity of the peak at 1.28 ppm identified as a marker of neural progenitor cells has been called into question (Romer et al., 2008; Dong et al., 2009; Ramm et al., 2009). Spatial resolution is also an issue, as MRS typically results in larger voxels than other MRI techniques, such as structural imaging, which would prevent researchers from achieving subfield specificity. Another way to assess changes in neurogenesis using MRI technology is by measuring regional blood volume, a putative correlate of neurogenesis. There is a well-established link between increased neurogenesis and the growth of new vasculature, or angiogenesis (Palmer et al., 2000; Louissaint et al., 2002). For example, endothelial cells and neural progenitors divide together in a vascular niche (Palmer et al., 2000). The vascular niche is a proposed microenvironment of capillaries that allows for the passage of nutrients and trophic support to the proliferative clusters of endothelial, neuronal and glial cells (Palmer et al., 2000). This metabolic and neurotrophic support offered by endothelial precursors and adjacent blood vessels is essential for the proliferation, maintenance and survival of neural progenitor cells (reviewed in Yang et al., 2011). In turn, angiogenesis can be accurately quantified by certain MRI techniques (Dennie et al., 1998; Sugahara et al., 1998; Aronen et al., 2000). Pereira and colleagues (2007) were the first to use a gadolinium enhanced, steady-state, MRI technique to indirectly measure neurogenesis in mice via DG blood volume. They showed that the exercise-induced changes in DG blood volume corresponded with immunohistochemical cell counts of neural progenitors in the DG (Pereira et al., 2007). Further, irradiation abolished the

activity-dependent increase in DG blood volume (Pereira et al., 2007). Pereira and colleagues (2007) also demonstrated that 3 months of exercise in humans aged 21–45 years old resulted in the same DG-specific increase in blood volume, which correlated with aerobic capacity and memory function (Pereira et al., 2007). Research from our group also demonstrated a correlation between change in blood volume in the right DG in a small pilot group of healthy young adults and change in aerobic capacity following exercise (Déry et al., 2010). Further, the change in right DG blood volume demonstrated a near-significant correlation with change in accuracy on a behavioural pattern separation task. In contrast to the static contrast-enhanced method used by Pereira et al (2007), we used a dynamic contrast enhanced MRI acquisition technique. A major advantage of dynamic perfusion imaging is that it measures the wash-in, plateau and washout of the bolus, allowing researchers to measure contrast kinetics and perform absolute as opposed to relative quantification (Essig et al., 2013). The chief drawback with using hippocampal blood volume as a correlate of neurogenesis is the location of the hippocampus itself. The air-tissue interface between the hippocampus and ear canal can result in distortions and a drop in signal (Olman, Davachi and Inati, 2009). Injecting a contrast agent, such as chelated gadolinium, is required to achieve the signal needed for accurate post-processing. Gadolinium remains in the vascular space and its paramagnetic properties increase contrast between this and surrounding tissue. Besides being mildly invasive, people with renal disease should not be injected with the tracer. While those with vascular disease and diabetes mellitus are commonly injected with gadolinium-based tracers in clinical settings, it would likely be difficult to rationalize injecting them with

such a tracer for research purposes. This severely limits the sample demographics of any study interested in indirectly measuring neurogenesis in humans. Notably, some common comorbidities in those with major depression, a disorder characterized by downregulated neurogenesis (see above for review), are cardiovascular disease and diabetes (Rudisch and Nemeroff, 2003; Katon, 2008). Thus, it would be quite cumbersome to recruit the desired sample sizes for at least two populations that would benefit most from our understanding the functional significance of AHN, those with major depression and those of advanced age. Others who have measured hippocampal volume changes associated with chronic stress, antidepressant treatment, or lifestyle changes have suggested that volume change might be associated with change in neurogenesis (Maguire et al., 2003; Draganski et al., 2006; Yucel et al., 2008). Some studies have even evaluated grey matter volume of individual hippocampal subfields and concluded that volumetric changes in the DG/CA3 region (which cannot be differentiated due to a lack of resolution) more specifically reflect changes in neurogenesis (Neylan et al., 2010; Shing et al., 2011; Teicher et al., 2012). Erickson and colleagues (2011) measured grey matter changes in the brain in response to aerobic exercise in older adults. They found that long-term aerobic exercise increased anterior hippocampal volumes in this population (Erickson et al., 2011). Moreover, the increased hippocampal volumes were associated with greater BDNF and improved spatial memory (Erickson et al., 2011). Herting and Nagel (2012) demonstrated that running can also increase hippocampal volumes and improve spatial memory in adolescents. Further, a recent study described an association between antidepressant treatments, associated with increased neurogenesis in animals, and

increased hippocampal volume (Boldrini et al., 2012). Interestingly, this study also looked at post-mortem tissue and found that there was a positive correlation between DG volume and angiogenesis (Boldrini et al., 2012). In contrast to exercise and antidepressant treatment, duration and number of depressive episodes or the number of reported stressful life events both predict reduced hippocampal volume (MacQueen et al., 2003; McKinnon et al., 2009; Papagni et al., 2011). Interestingly, lifelong engagement in physical activity can mitigate against the negative effects of lifetime stress on hippocampal volume (Head et al., 2012). Finally, aging is also associated with decline in hippocampal volume (Kramer et al., 2007). There are a number of factors that influence hippocampal volume as humans age (Fotuhi et al., 2012). The factors that prevent the age-related decline in hippocampal volume may be working, in part, by promoting higher rates of AHN. For instance, it has been proposed that the amount of physical activity undertaken earlier in life might have an impact on how their mind ages and how responsive they will be to neurogenic-promoting therapies later in life (Kempermann, 2008). Thus, it is quite encouraging that a number of lifestyle factors known to upregulate or downregulate neurogenesis from the animal literature are also associated with hippocampal volume changes in the same direction. Unfortunately, without post-mortem analysis of tissue there is no way to tell what anatomical changes actually underlie the volumetric changes. An additional way to indirectly measure neurogenesis using MRI is to use DTI to assess integrity of the perforant pathway combined with high resolution fMRI in order to assess how perforant path integrity can affect subfield level activity. For example, it has been found that perforant path degradation and hyperactivity in the DG/CA3 region manifests

in old age as well as in those with mild cognitive impairment (MCI; Yassa et al., 2010b, 2010c, 2011; Yassa and Stark, 2011). Importantly, both perforant path degradation and hyperactivity in the DG/CA3 negatively correlate with behavioural pattern separation performance (Yassa et al., 2010b, 2010c, 2011; Yassa and Stark, 2011). Functional imaging cannot be used to measure anatomy, but can be used to provide insight into how small-scale changes affect large-scale activity. An advantage of functional imaging techniques is that they can simultaneously measure activity across different brain regions or subregions within the hippocampus, allowing researchers to, for example, calculate the amount of decorrelation between the DG and CA1 when using 3 Tesla MRI scanners (e.g. Yassa et al., 2010b). However, at this time it is unknown how changes in AHN would affect population level activity in the human hippocampus. Further, it is not clear how precise neuronal activity maps onto haemodynamic changes. Since behavioural pattern separation is improved in rodents that have undergone aerobic exercise (Creer et al., 2010; Kohman et al., 2012) or genetic manipulation to increase neurogenesis (Sahay et al., 2011), and impaired in mice with neurogenesis ablated (Clelland et al., 2009) it seems likely that behavioural pattern separation performance itself may be an indicator of neurogenesis. Accuracy on neurogenesis-dependent memory tests would provide the highest degree of functional information compared to any other correlate available. The caveat is that we would have to assume mechanisms underlying both animal and human cognition are similar. Nonetheless, as mentioned above, we do find that lifestyle factors associated with decreased neurogenesis are also associated with impaired pattern separation performance in humans, most notably aging (Toner et al., 2009; Stark et al.,

2010, 2013; Yassa et al., 2010b, 2010c, 2011; Yassa and Stark, 2011; Holden et al., 2012). It should be noted that the major limitation of all of these methods is that they are indirect measures that rely on correlations with putative neurogenesis-dependent interventions or lifestyle factors. Nonetheless, it is promising that most of the data gained from human studies using such a vast array of techniques to indirectly assess AHN support the theories put forward by theoretical and computational models as well as the preclinical data obtained using animals.

1.3.4 Summary of Studies

The studies described in this thesis embody the first attempt to test some of the proposed roles of AHN directly in humans. These studies largely support what has been found in rodents, and identify two lifestyle factors that can influence putative neurogenesis-dependent memory performance in opposing ways: aerobic exercise and stress. The results outlined in Chapter 4 provide new insight into the mechanisms underlying the improvement in putative neurogenesis-dependent memory following exercise in humans. Taken together, the studies described in this dissertation ultimately provide a foundation for future work in the field by providing important evidence with regards to the functional role of adult neurogenesis in human memory.

The second chapter is comprised of two studies. In the first study we utilize a long-term high-intensity interval training program in an attempt to stimulate neurogenesis in the hippocampus. Prior to and following the exercise program we administered the gold standard test of aerobic capacity, a ramp test to exhaustion, as well as a battery of cognitive tests comprised of a putative neurogenesis-dependent behavioural pattern

separation task and a hippocampal-dependent, but putatively non-neurogenesis-dependent control task. In the second study described in Chapter 2 we had a distinct cohort of participants complete the Beck Depression Inventory-II (BDI) as well as the same cognitive battery as in the first study. Our results demonstrated that two lifestyle factors known to increase and decrease neurogenesis, aerobic exercise and stress respectively, predict cognitive performance on a high interference visual object pattern separation task in the same manner.

The third chapter builds on the findings reported in the second chapter in three important ways. First, we replicate our previous findings and show a significant negative relationship between depression scores and pattern separation performance. Second, we extend these findings by introducing an added measure of stress, Cohen's Perceived Stress Scale (PSS), which better indicates more recent (as opposed to more distant) levels of stress compared to the BDI. We found a negative correlation between PSS scores and accuracy on the same behavioural pattern separation task. Lastly, we tested another putative role for neurogenesis in human cognition – memory persistence. Specifically, we introduced a two week delay between study and test. We found that those with lower stress and depression scores, hypothesized to have higher levels of neurogenesis, outperformed those with relatively high scores at identifying exact repetitions of images they had seen two weeks earlier as "old", as opposed to "new" or "similar".

In the final study we followed participants over the course of a highly controlled six week-long exercise program. Once again, we measured aerobic capacity and putative neurogenesis-dependent and -independent cognition before and after exercise. In an

attempt to identify putative biomarkers of neurogenesis, we also took peripheral blood samples from each participant prior to and following the exercise program. There is a tight coupling between the bioavailability of certain growth factors and rate of neurogenesis in preclinical animal studies. Therefore, we assayed the blood for expression levels of these various growth factors. We found that change in aerobic capacity pre- versus post-exercise was a strong positive predictor of change in behavioural pattern separation. Further, change in serum IGF-1 following exercise was a significant positive predictor of change in performance on the behavioural pattern separation task, specifically on high interference trials.

Taken together, these studies provide support for the proposed role of AHN in humans: interference reduction. It also outlines several potential ways to indirectly assess neurogenesis in the human brain. First, lifestyle factors known to regulate neurogenesis from the animal literature may be used predict neurogenic activity in humans. Correlating these various lifestyle factors with putative neurogenesis-dependent cognition could provide an indirect means for assessing the function of neurogenesis in the living human brain. Further, growth factors found in peripheral blood may serve as biomarkers of AHN, improving predictive power over that of lifestyle factors alone. These studies provide an important contribution to the scientific literature. Being able to indirectly assess neurogenesis in the human brain would allow policy makers to develop preventative strategies and clinicians to improve current treatments for disorders associated with downregulated neurogenesis, such as major depression and pathological aging.

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Chapter Two

Preamble

Pattern separation deficits observed in humans of advanced age (Toner et al., 2009; Yassa et al., 2010a, 2010b, 2011; Stark et al., 2010, 2013; Yassa and Stark, 2011; Holden et al., 2012) nicely parallel the type of deficits typically observed in rodents who lack AHN (McHugh et al., 2007; Clelland et al., 2009; Guo et al., 2011; Luu et al., 2012; Pan et al., 2012a, 2012b, 2013; Tronel et al., 2012; Winocur et al., 2012). Further, neurogenesis declines exponentially with age in humans (Imayoshi et al., 2009; Knoth et al., 2010; Spalding et al., 2013). Therefore, it seems plausible that the age-associated decline in behavioural pattern separation performance is linked to the age-related decline in AHN. If true, then we would expect that other populations who might be at risk for downregulated neurogenesis would demonstrate analogous impairments in behavioural pattern separation performance to those experienced in old age. On the other hand, we would expect that participating in pro-neurogenic activities, such as long-term aerobic exercise, would improve accuracy on tasks that potentially assay pattern separation.

Chapter 2 outlines two studies. In the first study, we recruited sedentary, but otherwise healthy, young participants to take part in a six week-long high-intensity interval training program. Our intention was to increase the available pool of immature neurons in the hippocampus and track how performance on putative neurogenesis-dependent memory, lure trials in the BPS-O, changed from pre- to post-exercise. In the second study, we recruited another group of healthy young undergraduate students at

McMaster University, and asked them to fill out a self-report questionnaire that assessed levels of depression (the BDI). Our reasoning was that if aerobic exercise, a pro-neurogenic activity, increased behavioural pattern separation performance, then chronic stress, a potent downregulator of AHN, should impair performance.

frontiers in NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 30 April 2013
doi: 10.3389/fnins.2013.00066

Adult hippocampal neurogenesis reduces memory interference in humans: opposing effects of aerobic exercise and depression

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Since the remarkable discovery of adult neurogenesis in the mammalian hippocampus, considerable effort has been devoted to unraveling the functional significance of these new neurons. Our group has proposed that a continual turnover of neurons in the DG could contribute to the development of event-unique memory traces that act to reduce interference between highly similar inputs. To test this theory, we implemented a recognition task containing some objects that were repeated across trials as well as some objects that were highly similar, but not identical, to ones previously observed. The similar objects, termed lures, overlap substantially with previously viewed stimuli, and thus, may require hippocampal neurogenesis in order to avoid catastrophic interference. Lifestyle factors such as aerobic exercise and stress have been shown to impact the local neurogenic microenvironment, leading to enhanced and reduced levels of DG neurogenesis, respectively. Accordingly, we hypothesized that healthy young adults who take part in a long-term aerobic exercise regime would demonstrate enhanced performance on the visual pattern separation task, specifically at correctly categorizing lures as "similar." Indeed, those who experienced a proportionally large change in fitness demonstrated a significantly greater improvement in their ability to correctly identify lure stimuli as "similar." Conversely, we expected that those who score high on depression scales, an indicator of chronic stress, would exhibit selective deficits at appropriately categorizing lures. As expected, those who scored high on the Beck Depression Inventory (BDI) were significantly worse than those with relatively lower BDI scores at correctly identifying lures as "similar," while performance on novel and repeated stimuli was identical. Taken together, our results support the hypothesis that adult-born neurons in the DG contribute to the orthogonalization of incoming information.

Keywords: hippocampus, neurogenesis, exercise, depression, interference, pattern separation

INTRODUCTION

The hippocampus, a structure in the medial temporal lobe, has long been implicated in the encoding and retrieval of contextual and associative memories. More specifically, the hippocampus is thought to be required for laying down distinctive memory traces for highly similar events, a process known as pattern separation. Marr's (1971) computational model attributed this orthogonalizing function specifically to the dentate gyrus (DG) sub-region of the hippocampus (Marr, 1971). The CA3, on the other hand, via its extensive recurrent collateral connections, was hypothesized to be ideally suited for binding incomplete or degraded components of an event into a coherent memory (pattern completion) (Marr, 1971). This pattern completion capability is important for memory retrieval, but may also lead to errors in recognition.

There are several unique electrophysiological and anatomical features of the DG that led Marr to hypothesize its crucial role in pattern separation. There are many more principle neurons in the DG than in layer II of the entorhinal cortex (EC), its major source of input (a 5 to 10-fold increase in the rat, for example, Amaral et al., 1990). Furthermore, DG granule cells have much lower activity levels and higher spatial selectivity than other hippocampal regions (Barnes et al., 1990; Jung and McNaughton, 1993). This highly divergent, ultra-sparse neural code in the DG is thought to be crucial for its ability to perform pattern separation. Unit recordings from DG cells lend further support to the hypothesized role of the DG in pattern separation, although the exact mechanism of this process at the cellular level remains unclear (Leutgeb et al., 2007).

Another unique property of the DG is that it undergoes neuronal turnover throughout the lifespan. This ongoing process of neurogenesis may contribute further to the proposed function of keeping similar memories distinct, thereby minimizing

interference (Becker, 2005). There is mounting empirical support from rodent studies for the role of DG neurogenesis in minimizing interference between overlapping memories. For example, animals with reduced neurogenesis are impaired at distinguishing between highly similar spatial cues (Clelland et al., 2009), learning overlapping odor pair discriminations (Luu et al., 2012) and long-term retention of a learned visual discrimination when an interfering visual task is performed subsequently (Winocur et al., 2012). Conversely, exercise upregulates neurogenesis and has been shown to enhance performance on memory tasks that have a high interference component (Creer et al., 2010; Sahay et al., 2011; Winocur et al., 2012). Moreover, DG neurogenesis is not only critical for memory, but may play an important role in stress and mood regulation. For example, neurogenesis seems to be important for buffering the stress response (Snyder et al., 2011) and mediating the effects of antidepressants (e.g., Santarelli et al., 2008).

Given the critical role that DG neurogenesis seems to play in memory and mood regulation in rodents, it would be of tremendous interest to confirm the functional role of neurogenesis in the human brain. Unfortunately, there is currently no way to directly and non-invasively assay neurogenesis *in vivo*. A few studies have employed magnetic resonance imaging (MRI)-based methods to assay putative correlates of neurogenesis. For example, Manganas et al. (2007) used MR spectroscopy to measure a 1.28 ppm peak that they claim represents neural progenitor cells. Pereira et al. (2007) used contrast enhanced MRI to track DG blood volume changes induced by exercise. Importantly, 12 weeks of exercise lead to increased DG blood volume in both mice and humans, and in mice the DG (blood) volume increase was further shown to correlate with increased neurogenesis. Such MRI-based methods hold promise and merit further research. Another approach is to examine lifestyle factors known to correlate with neurogenesis. For example, aerobic exercise has been shown to upregulate neurogenesis (van Praag et al., 1999a,b, 2005; Olson et al., 2006; Pereira et al., 2007; Fabel et al., 2009), whereas high levels of stress, alcohol bingeing and BDNF polymorphisms are associated with reduced DG cell proliferation and/or survival (Cameron and Gould, 1994; Jang et al., 2002; Mirescu and Gould, 2006; Warner-Schmidt and Duman, 2006; Morris et al., 2010; Bath et al., 2012).

The approach we have taken is to examine performance on human analogues of tasks found to be neurogenesis-dependent in rodents, and assay lifestyle factors known to correlate with neurogenesis. We can thus test hypotheses concerning the functional role of neurogenesis in the human brain. As a first step in this direction, we previously examined the association between stress, depression and performance on several subscales of the Cambridge neuropsychological test automated battery (CANTAB®) battery. Based on the well-accepted role of stress in the pathogenesis of human depression (Brown et al., 1999), and the observation that chronically stressed animals have suppressed neurogenesis (Cameron and Gould, 1994; McEwen, 2001), it was predicted that individuals who have elevated scores on the Beck Depression Inventory (BDI) would have suppressed neurogenesis, and exhibit selective memory deficits on neurogenesis-dependent memory tasks. The CANTAB delayed match to sample (DMS) task at long delays was predicted to be sensitive to neurogenesis because it has many of the characteristics of neurogenesis-dependent tasks identified in rodent studies. It requires participants to encode complex configural visual patterns, and later discriminate between the studied patterns and highly similar lures. Rodents with reduced neurogenesis are impaired at visual recognition memory on DNMS task at long delays (Winocur et al., 2006), and when there is high visual similarity or spatial similarity between the targets and lures (Clelland et al., 2009; Creer et al., 2010); all of these tasks have a high interference component, and place high demands on the pattern separation capabilities attributed to the DG. We found that young adults with elevated BDI scores were selectively impaired on the DMS task at long delays, in spite of intact performance on DMS at shorter delays as well as on a large battery of other memory tests (Becker et al., 2009). Here, we sought to extend these findings to another test that may require adult neurogenesis, the visual pattern separation task developed by Kirwan and Stark (2007). This task has been used in several human fMRI studies (e.g., Kirwan and Stark, 2007; Bakker et al., 2008; Yassa et al., 2011) with results that are consistent with the notion of the DG being responsible for pattern separation. Specifically, the DG/CA3 subregion is strongly activated by both novel items and highly similar lures, but much less active for repeated items (Kirwan and Stark, 2007).

In the first experiment, we tracked performance on the visual pattern separation task as a function of a 6-week exercise training intervention in previously sedentary but otherwise healthy young adults. As noted above, exercise causes chronic elevations in DG blood volume in both mice and humans (Pereira et al., 2007) and is a well-established up-regulator of neurogenesis in rodents (van Praag et al., 1999a,b, 2005; Olson et al., 2006; Fabel et al., 2009). In the second experiment, we examined the relationship between performance on this task and depression scores. We predicted that a participant's ability to identify "similar lures," that is, images of objects that are highly similar to previously studied ones, would require hippocampal neurogenesis for optimal performance. Therefore, pattern separation performance was predicted to be enhanced in exercisers, particularly in those who exhibited large changes in fitness, and impaired in those with elevated depression scores.

EXPERIMENT 1

METHODS

Participants

All aspects of our aerobic exercise training study were approved by the Hamilton Integrated Research Ethics Board (HIREB). We recruited 13 healthy but sedentary young adult participants from the McMaster University student population using ethics board approved advertisements that were posted across campus. All participants provided written informed consent and met the inclusion criteria for our study: a healthy body mass index ($\leq$25), the requirements for beginning physical activity: a sedentary lifestyle (no more than 1 h of physical activity per week) and no prior history of psychiatric illness. Two of the 13 participants did not take part in the high intensity interval training (HIT) aspect of the program, but participated in all other aspects of the study, pre- and

post-training. A week prior to the commencement, and a week following the completion of a 6 week HIT program, an intervention previously shown to be effective for improving aerobic fitness (Hickson et al., 1977), each participant performed a battery of cognitive tests and a VO_{2peak} test of fitness.

Cognitive testing

Putative neurogenesis-dependent task. A cognitive task that generates high levels of interference between previously learned and subsequently tested stimuli is Kirwan and Stark's (2007) "pattern separation task," a three alternative forced choice visual recognition memory task that includes some objects that are repeated across trials, which are termed "repetitions," some images that are highly similar, yet not identical to ones previously viewed that are called "lures" and some completely novel objects, which are termed "foils." The version of the task that we used here was adapted from Kirwan and Stark's (2007) task, whereby participants are shown a series of 88 images of familiar everyday objects (32 first presentations, 16 old "repetitions," 16 similar "lures," and 24 unrelated "foils"). Images are presented one at a time in pseudorandom order for 2500 ms each with a 500 ms inter-trial interval, with the constraint that there is an average distance of 30 items separating any first presentation from either a subsequent repetition or from a subsequent similar object. Following a brief delay, a second series of 112 images (48 repetitions, 48 lures, 16 foils) is presented and the participant is asked to judge whether each object is "old," "similar" to an object presented in the previous set, or "new." Correct responses are made when repetitions are identified as "old," when lures are correctly classified as being "similar" but not identical to the target stimulus, and when unrelated foils are classified as "new." Thus, the correct identification of a lure stimulus constitutes a correct rejection, whereas incorrectly classifying a lure stimulus as "old" constitutes a false positive (i.e., falsely identifying a lure stimulus as being identical to the target stimulus). The presentation and recognition phases are then repeated in a second block of trials, using an entirely different set of stimuli. As mentioned, Kirwan and Stark's object recognition task involves discriminating between previously learned patterns (targets) and highly similar lures, a potential source of interference, and may thus benefit from the so-called "pattern separation" capability attributed to the DG (Marr, 1971; McClelland et al., 1995; Kesner, 2007), and more specifically to neurogenesis (Becker, 2005; Aimone et al., 2006; Appleby and Wiskott, 2009; Becker et al., 2009). We therefore posit that participants with putatively higher rates of neurogenesis in the DG will be able to better overcome interference and, consequently, outperform those with relatively lower levels of neurogenesis at correctly identifying lure stimuli as "similar." On the other hand, we do not expect to see any measurable difference in performance between groups on conditions that do not likely require adult-born neurons in the DG. Specifically, both high and low neurogenesis groups should perform the same when it comes to classifying exact repetitions of previously viewed object as "old" or when categorizing novel stimuli as "new." If one possesses a lower level of newborn cells in the DG than is required for optimal task performance, we would expect that the CA3, a region of the hippocampus thought to be important for binding information, will dominate processing and lead to an increased number of pattern completion errors. In terms of the pattern separation task, the misidentification of a lure stimulus as "old"—a false positive—would constitute a pattern completion error.

Putative neurogenesis-independent task. As a control task, we sought a memory test that is known to be hippocampal dependent but was not predicted to be neurogenesis-dependent. We chose the paired associate learning (PAL) task from CANTAB®, a visuospatial associative learning task which is well-established to be sensitive to hippocampal pathology, but lacks a high-interference component that may rely heavily on DG neurogenesis. We previously showed that young adults with elevated depression scores performed identically to non-depressed participants on the PAL task (Becker et al., 2009). In this task, in each trial, 6 white squares are displayed on the computer screen. One at a time, each square momentarily disappears to reveal what is hiding underneath—either nothing or an abstract object. Once the content of each square is known, the participant is presented with another series of abstract objects, one at a time, and now must appropriately select the white square that is occluding the target object being shown. With each series, the task becomes progressively more difficult as the number of abstract objects hidden underneath the squares increases from 1 to 6. In the final iteration of the task two more white squares are added and the participant must try to remember the location of 8 unique objects.

Aerobic exercise

VO_{2peak} ***testing.*** Peak oxygen uptake (VO_{2peak}) is the gold-standard measurement of aerobic fitness and is obtained by measuring oxygen uptake continuously during an incremental test to exhaustion. A baseline VO_{2peak} score was obtained from each participant during the week prior to the start of exercise. A second VO_{2peak} score was then obtained from each participant in the week following our chronic exercise intervention (described below).

High-intensity interval training intervention. Gormley and colleagues (2008) found that with the volume of exercise controlled, it was those who exercised at near-maximal intensity that achieved the greatest increase in aerobic VO_{2peak}. Volume of exercise was controlled by multiplying the intensity (% of heart rate reserve or % of VO_2 reserve) of the exercise program by the duration, in minutes, and the frequency, in days (Gormley et al., 2008). Accordingly, we used a 6 week HIT program comparable to the near-maximal intensity condition used by Gormley et al. (2008) except that our exercisers ran on an outdoor track rather than using stationary cycles. In each week of the program participants ran in three sessions that varied in both duration and intensity as outlined below. Before and after each session, subjects completed 5 min of running at 50% of heart rate reserve (HRR) as well as sport-specific stretching in order to warm-up and -down. Each session was also separated from the next one by at least 24 h in order to promote recovery. In week 1, participants ran for 15 min sessions at 65% of HRR, while in week 2 they ran in 20 min sessions at 65% of HRR. In weeks 3–6 we had runners

start at 75% of HRR for 5 min, followed by alternating sprint and rest phases of 95% HRR and 50% HRR, respectively. The number of sprints in each session started at 3 on week 3, and increased by 1 in each subsequent week. Participant heart rate information was displayed on Timex Ironman® Road Trainer™ watches and was monitored periodically throughout each training session by one of the experimenters.

Statistical analyses

For all correlative analyses Pearson's r was used. The participants in our exercise study were split into two groups based on their median change in VO_{2peak}. Accordingly, those who experienced a change in VO_{2peak} below the median were considered low responders to exercise, while those with a change in VO_{2peak} greater than the median were considered high responders. Low and high responders to exercise were compared using the non-parametric Mann–Whitney U-test (which does not require the assumption of equal variances, because there were two non-exercisers in the former group), while paired samples t-tests were used for within group repeated measures comparisons. Results are presented as mean (M) ± standard deviation (SD). For each statistical test a p value (two-tailed) ≤ 0.05 was considered significant, except where otherwise stated.

RESULTS

Aerobic fitness and pattern separation performance

Correctly identifying unrelated foils as "new" in our visual pattern recognition task is relatively easy, with participants typically scoring near ceiling ($M = 89\%$, $SD = 11.1\%$). We therefore considered any participant who scored more than two standard deviations away from the mean on these items to have not properly understood or disregarded the task instructions, or to have implemented a deviant response strategy (e.g., answering only "old" or "similar" for every object displayed in the recognition phase, completely ignoring the "new" response option). On this basis, 1 participant's data was discarded, leaving 12 participants' data that were included in subsequent analyses (4 male, 8 female; mean age = 21.83, $SD = 2.25$). Six weeks of highly controlled physical activity had a highly significant impact on fitness, as revealed by a paired samples t-test of VO_{2peak} pre- vs. post-exercise [$t_{(9)} = 4.10$, $p = 0.003$]. However, a high degree of variability in changes to VO_{2peak} following chronic exercise is common among previously sedentary participants (Hickson et al., 1977). Indeed, individuals in our study varied widely in their response to the fitness intervention, ranging from no change to a 29% improvement in fitness. Therefore, we split the group into exercise responders ($N = 6$, $M = 14\%$, range = 9–29%) and non-responders ($N = 6$, $M = 3\%$, range = −3 to 8%) based on the median change in VO_{2peak}, and then analyzed differences in cognitive task performance between these two groups. Accordingly, those who experienced a change in VO_{2peak} of 8% or less were considered low responders to exercise, while those with a change of 9% or greater were considered high responders. We included in the non-responder group two individuals who did not complete the exercise program, but completed all pre- and post-six week cognitive and fitness testing. Prior to making any comparisons between the change in fitness and the change in performance on the visual object recognition task pre- vs. post-exercise, we applied a correction (as described in Yassa et al., 2011) in order to control for response bias across groups. For example, the (uncorrected) probability of judging an old item as "old" could be inflated if one judged every stimulus as "old." Therefore, we subtracted the proportion of "old" responses given the presentation of an unrelated foil (new item) from the proportion of correct "old" responses given a repetition (old item):

Formula 1. $[p(\text{"Old"}|\text{Target}) - p(\text{"Old"}|\text{Foil})]$

Similarly, we subtracted the proportion of "similar" responses given the presentation of a foil (new item) from the proportion of correct "similar" responses given the presentation of a lure (similar item):

Formula 2. $[p(\text{"Similar"}|\text{Lure}) - p(\text{"Similar"}|\text{Foil})]$

The application of Formulae 1 and 2 resulted in the correction for a participant's bias to select "old" or "similar," respectively. Thus, it was these bias-corrected scores that were used for the following analysis. As previously mentioned, it was expected that exercise would cause increased levels of hippocampal neurogenesis, leading to an enhanced ability to correctly classify lure objects as "similar." Indeed, when comparing the average difference scores of responders and non-responders on the pattern separation task post- minus pre-exercise, we found that it was specifically those with a higher change in fitness (and putatively a larger increase in hippocampal neurogenesis) who demonstrated an improved ability to correctly identify lure stimuli as "similar," going from 60% ($SD = 18.77$) correct before exercise to 71% ($SD = 18.73$) correct following exercise [$t_{(5)} = 2.62$, $p = 0.05$]. The exercise responders' improved accuracy at appropriately classifying lures as "similar" (evidence of pattern separation) was mirrored by a concurrent reduction in the number of misidentifications of lures as "old" (evidence of pattern completion) post- vs. pre-exercise [$t_{(5)} = 3.16$, $p = 0.03$]. On the other hand, non-responders to exercise began by correctly identifying lures as "similar" 69% ($SD = 13.76$) of the time and post-exercise remained at 69% ($SD = 12.63$) accuracy. Thus, there was a significant difference between exercise responders and non-responders in their post-training improvement at correctly classifying lures as "similar," with a change of 11% ($SD = 10.26$) in high responders and 0% ($SD = 5.19$) in non-responders ($z = 2.08$, $p = 0.04$, **Figure 1A**). Since non-responders were slightly more accurate than responders at baseline (not significant), it could be that they also had inherently higher levels of fitness and neurogenesis at the outset. Indeed, although not significant, the non-responders were marginally more fit than responders at the outset of training, as their mean VO_{2peak} score was 114% ($SD = 9.00$) of their expected values, while responders' mean VO_{2peak} score was 105% ($SD = 15.63$) of their expected values prior to commencing our 6 week exercise regime [$t_{(10)} = 1.29$, $p = 0.23$]. This variability in baseline fitness could explain why non-responders failed to show any post-exercise improvement in either VO_{2peak} or in pattern separation performance.

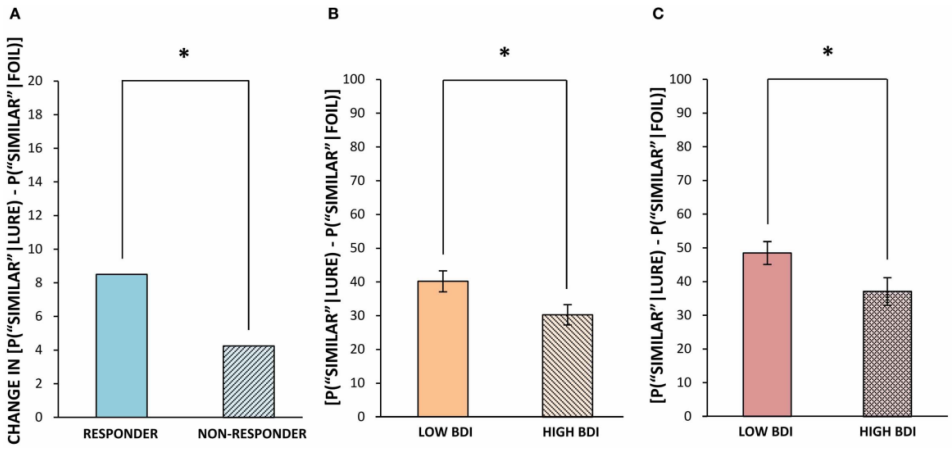

FIGURE 1 | Correct classification of similar lures pre- vs. post-exercise in Experiment 1 (A), and in those with below median and above median BDI scores in Experiment 2 (B,C). (A) Median change in % correct identification of lures as "similar" (bias corrected ranked difference scores) following exercise for responders vs. non-responders. **(B)** Mean % correct identification of lures as "similar" (bias corrected, ± standard error) when the original target appeared in the same block. **(C)** Mean % correct identification of lures as "similar" (bias corrected, ± standard error) when the target appeared in a previous block. $^*p \leq 0.05$.

Yassa and colleagues (2011) have reported that aging, which is associated with lower rates of hippocampal neurogenesis in many species (e.g., Klempin and Kempermann, 2007; Johnson et al., 2010; Knoth et al., 2010), is accompanied by a shift in the pattern separation vs. pattern completion tuning curve in CA3, such that larger changes in input (greater dissimilarity) are required for pattern separation to occur. Since lures may vary in their degree of similarity to the original targets, those that are most similar to the original target stimulus should generate maximal interference and thus present a higher opportunity for pattern completion to dominate processing and lead to additional false positives. If this shift in pattern separation vs. pattern completion behavior is dependent on the rate of adult hippocampal neurogenesis, then it would stand to reason that responders and non-responders to chronic exercise should also vary in their relative change in position on the tuning curve following exercise, such that responders demonstrate an increased bias toward pattern separation, whereas non-responders demonstrate a bias toward pattern completion. Furthermore, their ability to orthogonalize overlapping stimulus inputs should also depend on the degree of similarity between targets and lures. Therefore, we had a separate cohort of participants rate the similarity of each pair of target and lure stimuli from our pattern separation task on a 5 point likert scale, with 5 being most similar and 1 being least similar. Taking a median split of their average similarity ratings for each pair of target and lure objects allowed us to classify each set as "more similar" or "less similar." In order to examine if the discrepancy in pattern separation performance between the exercise responders and non-responders was dependent on the degree of similarity between the target stimulus and the lure, we performed separate *post-hoc* analyses of performance on these two categories of items ("more similar" or "less similar"). For less similar lures, exercise responders showed a significant 12% ($SD = 9.58$) improvement in performance [$t_{(5)} = 2.94$, $p = 0.02$ (one-tailed)] at appropriately classifying "less similar" items as "similar" following exercise, going from 62% ($SD = 15.93$) to 74% ($SD = 22.73$) correct, while non-responders did not experience any change, remaining at 71% correct. Exercise responders showed a trend toward improved performance at correctly identifying "more similar" items as "similar" post-exercise a 10% ($SD = 13.98$) improvement, [$t_{(5)} = 1.77$, $p = 0.07$ (one-tailed)] going from 58% ($SD = 18.56$) to 68% ($SD = 15.60$) correct, which was mirrored by a significant decrease in the number of misidentifications of "more similar" lures as "old" [$t_{(5)} = 3.80$, $p = 0.01$ (one-tailed)]. On the other hand, the non-responder group experienced a mere 1% ($SD = 8.82$) change in the negative direction [$t_{(5)} = 0.33$ $p = 0.38$ (one-tailed)] by going from 67% ($SD = 13.97$) to 66% ($SD = 11.48$) correct. Overall, our data suggest that: (1) 6 weeks of aerobic exercise is sufficient to normalize and/or improve pattern separation performance in those who experience a relatively large change in fitness, perhaps by increasing the availability of young neurons in the DG that, in turn, act to reduce interference between highly similar objects; (2) the exercise-dependent increase in pattern separation performance is coupled with fewer pattern completion errors (misidentifying similar items as "old"); and finally (3) the superior ability to correctly reject a lure stimulus as "similar" following exercise holds true regardless of how similar the two stimuli are. Importantly,

there were no significant changes in performance within or between high and low responder groups at correctly identifying repetitions as "old," or in correctly identifying foil objects as "new." There was also no significant difference between groups in their performance on the CANTAB® PAL task. Therefore, the improved ability of exercise responders to correctly recognize a lure item as being similar to, but not the same as, the target stimulus is likely due to a change in their ability to overcome interference, rather than a generalized improvement in memory functions.

Correlations between fitness and neurogenesis-dependent memory

In addition to analyzing between-group differences in participants who responded most vs. least to the exercise program, we examined their change in fitness levels (VO_{2peak} post- minus pre-exercise) as a continuous function of pattern separation performance. The change in fitness was positively correlated with the percent change in correctly identifying lures as "similar" [$r_{(10)} = 0.74$, $p < 0.01$, **Figure 2**]. In contrast, the change in VO_{2peak} negatively correlated with the percent change in "old" false positive responses to lure trials [$r_{(10)} = -0.79$, $p < 0.01$]. There was no correlation between change in VO_{2peak} and the percent change in miscategorizing lure objects as "new" following 6 weeks of physical activity. In other words, the more responsive that someone was to long-term aerobic training, represented by a relatively large change in fitness, the greater their improvement in response accuracy was on lure trials (i.e., correctly calling a similar object "similar"), while concurrently performing fewer pattern completion errors (i.e., incorrectly calling a lure stimulus "old"). Consistent with the comparisons between low and high responder groups, there was no significant correlation between change in VO_{2peak} and the change in ability to properly identify an unrelated foil as "new," or between the change in VO_{2peak} and the change in ability to correctly identify a repetition as "old." There was also no correlation between the change in VO_{2peak} following chronic exercise and the change in performance on PAL.

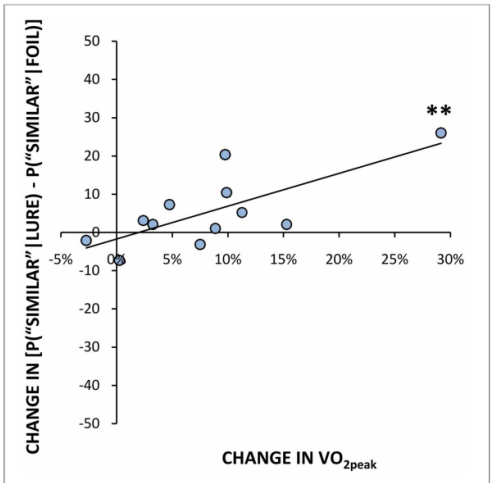

FIGURE 2 | Percent change in fitness scores (VO_{2peak}) vs. change in mean % correct identification of lures as "similar" following exercise ($r = 0.74$, $p < 0.01$). $^{**}p \leq 0.01$.

EXPERIMENT 2

METHODS

Participants

The study protocol for our investigation of depression scores vs. learning and memory performance on our battery of cognitive tasks was approved by the HIREB. We recruited 57 healthy young adults from the McMaster University student population using an online experiment sign-up tool. All participants gave informed consent and met the inclusion criterion for our study, having no history or previous diagnosis of any psychiatric disorder. Participant data was made anonymous to the experimenters; however, a code linking their personal information to their BDI scores was given to a third party for assessment. If any student was flagged to be at risk for suicide or major depression based on their responses on the BDI, their contact information was forwarded to a psychological counselor at the McMaster Student Wellness Centre who would then contact them for voluntary counseling or to discuss alternative treatment options.

Cognitive and neuropsychological testing

Each participant first completed the BDI in a quiet, private testing room. Next, they completed a putatively neurogenesis-dependent task, which was a modified version of the pattern separation task used in Experiment 1, and the CANTAB® PAL task described above. In this experiment, we augmented the visual object pattern separation task used in Experiment 1 in order to assess the effects of learning across longer time delays and contextual changes. Specifically, we used 8 blocks of presentation and recognition phases, as opposed to the 2 blocks used in our chronic exercise study. Following each presentation and recognition phase (i.e., each block), there was a change in visual context, whereby the background display changed from one outdoor environment to another (see **Figure 3**). In each presentation phase, there were only 16 images of everyday objects and there were no repetitions or lures. The 2500 ms display time and 500 ms inter-trial interval remained unchanged, however, we introduced a white visual noise mask after the presentation of each stimulus. Each recognition phase had a different number of images being presented, depending on the block number. The first recognition phase included 8 repetitions of target stimuli, 8 similar lures and 22 unrelated foils. The recognition phases occurring in blocks 2 through 8 contained the same number of within block repetitions and lures; however, they also contained 1 repetition and 1 lure whose targets were originally presented in the previous block(s), as well as 2 additional novel objects. Therefore, as an example, the recognition phase in block 2 contained 8 repetitions and 8 lures whose target stimuli were originally shown in the immediately preceding presentation phase, just as in block 1; however, the second recognition phase also contained 1 repetition and 1 lure whose target images were first seen in the presentation

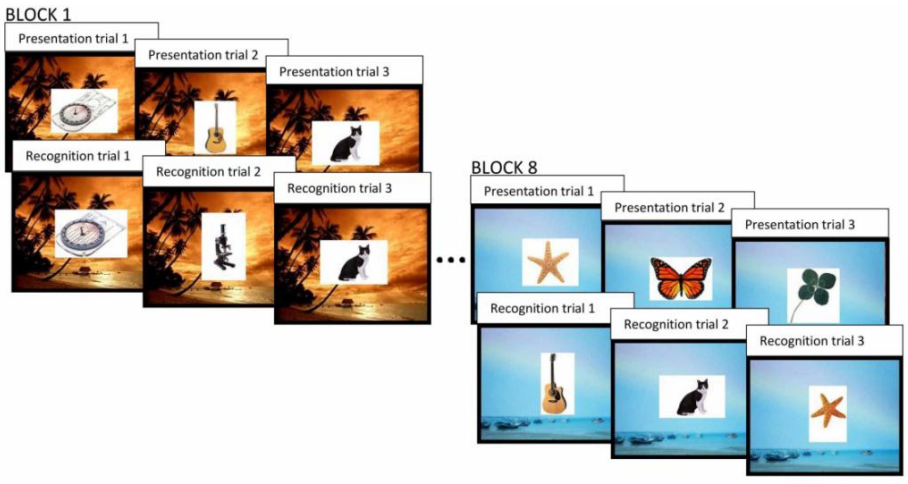

FIGURE 3 | The behavioral pattern separation task used in Experiment 2. In each block, everyday objects are displayed 1 at a time in a sequence of presentation trials followed by a sequence of recognition trials. In recognition trials, the object can either be old (an exact repetition of a previously presented object), similar, but not identical, to a previous target, or a new, completely unrelated foil. Each block has a distinct visual context (background image). In recognition trials, for repetitions and similar items, the associated target may have been seen within the same block (same temporal and visual context) or within a previous block (different temporal and visual context).

phase of block 1, as well as 24 novel objects, as opposed to 22. In contrast, the recognition phase within the eighth block contained 8 repetitions and 8 lures of target stimuli that were shown in the eighth presentation phase, as well as 7 repetitions and 7 lures whose target stimuli were originally presented across each of the 7 preceding blocks, and 36 unrelated foils. Constructing the task in this way allowed us to vary the amount of interference between target stimuli and lures. Each target object was only seen once during a presentation phase, but an identical or similar item could appear either within the same block and in the same context (as in Experiment 1), or a varying number of blocks later, within a different context. This afforded us the opportunity to analyze differences in performance as a function of depression score and as a function of the degree of spatio-temporal context change between the presentation of the item and its preceding target.

Statistical analyses

For all correlative analyses Pearson's r was used. For all comparisons between participants scoring in the lower and upper range on the BDI questionnaire, we used the student's t-test, with equal variance assumed. For each statistical test a p value (two-tailed) ≤ 0.05 was considered significant, unless otherwise stated.

RESULTS

Depression and putative neurogenesis-specific cognition

Performance within blocks. Performance on repetitions and lures that appeared within the same block and hence the same visual context as the original target (as was the case for all targets in Experiment 1) was analyzed separately, for ease of comparison with the results of Experiment 1. The same outlier screening method used in "Experiment 1" was applied here, resulting in the exclusion of 5 participants' data, leaving 52 participants who met criteria for inclusion in the analysis (13 male, 39 female; mean age = 19.48, $SD = 2.05$). In terms of self-reported levels of depression on the BDI in these participants, none scored in the severely depressed range (>29) and only 8 of 52 scored in the moderately depressed range (19–29). Therefore, we performed a median split based on the BDI scores to create two groups, low BDI scorers (BDI below 9; $N = 27$, mean BDI = 4.04, range = 0–8) and high BDI scorers (BDI 9 or greater; $N = 25$, mean BDI = 15.68, range = 9–29). These groups differed significantly in BDI score [$t_{(50)} = 9.71$, $p < 0.001$], but not significantly in age or gender. Next, we analyzed differences in cognitive task performance between individuals with lower vs. higher BDI scores. The same response bias corrections used in our first experiment were applied (see Formulae 1 and 2) prior to any further analyses. The low BDI group was superior to the high BDI group at correctly identifying lure items as "similar" in the visual pattern separation task, with mean scores of 40% ($SD = 16.53$) correct vs. 30% ($SD = 15.45$) correct, respectively [$t_{(50)} = 2.23$, $p = 0.03$, **Figure 1B**]. In order to compare our results to Toner and colleagues' (2009) findings in young vs. older adults, we calculated a measure of participants' bias toward pattern separation vs. pattern completion by subtracting the proportion of incorrect "old" responses to lure stimuli from the mean number of correct

"similar" responses to lure trials. A more positive score would indicate that the participant made a higher proportion of correct "similar" responses to lure trials and thus pattern separation had dominated processing. On the other hand, a more negative score would indicate that more false positives, or incorrect, responses to lure trials had occurred and thus pattern completion had dominated processing. The low BDI group's scores were more biased toward pattern separation (as opposed to pattern completion) given the presentation of a lure stimulus, and were significantly higher than those of the high BDI group [$t_{(50)} = 2.18$, $p = 0.03$, **Figure 4**]. Importantly, there were no differences between the low and high BDI groups in their ability to recognize novel objects or repetitions.

As in Experiment 1, the similar lures were then divided, based on their subjective similarity ratings, into "more similar" and "less similar" lures. In doing so, it became apparent that the source of the low BDI group's superior performance on lure trials was primarily driven by their performance at correctly identifying "less similar" lures. Specifically, those with lower BDI scores correctly selected 45% ($SD = 17.28$) of "less similar" lures as "similar," while those with higher BDI scores only identified 31% [$SD = 18.50$; $t_{(50)} = 2.85$, $p < 0.01$ (one-tailed)]. Those with lower BDI scores also made correspondingly fewer errors in calling "less similar" objects "old" as compared to those with higher BDI scores [$t_{(50)} = 2.34$, $p = 0.01$ (one-tailed)], whereas there was no difference between groups in the number of errors they made in miscategorizing "less similar" items as "new." These data suggest that the low BDI group's superiority in correctly identifying the "less similar" lures as "similar" within blocks was rooted in fewer pattern completion errors. In contrast to the "less similar" items, the low BDI group only held a slight, non-significant advantage over those with high BDI scores at correctly identifying "more similar" lures as "similar" [$t_{(50)} = 1.26$, $p = 0.11$, (one-tailed)]. These results can be explained, in part, by the fact that both low and high BDI scorers performed relatively poorly at identifying the highly similar lures. Thus, the low BDI group performed better on "less similar" lure trials (45%, $SD = 17.28$) than they did on "more similar" lure trials [36%, $SD = 16.84$, $t_{(26)} = 5.38$, $P < 0.001$]. On the other hand, the high BDI group performed equally poorly at correctly identifying both "less similar" lures (30%, $SD = 18.50$) and "more similar" lures as "similar" [30%, $SD = 15.80$, $t_{(24)} = 0.12$, $p = 0.90$]. With "more similar" objects generating inherently higher levels of interference, these particularly difficult items may have been beyond the threshold for correct pattern separation, even in the low BDI group. On the other hand, those with high BDI scores (and putatively less neurogenesis) demonstrate a comparable deficit at identifying lures regardless of their similarity rating.

Performance across blocks. The version of our visual pattern separation task used in Experiment 2 also contained some repetitions and lures that appeared across different blocks from the original presentation. Interestingly, both low and high BDI groups benefited from the increased temporal spacing between these items, and made significantly fewer errors in calling a lure stimulus "old" when it appeared in a different block, as opposed to the same block, from the target presentation. This effect did not depend on the similarity rating of the lure, as both "less similar" lures [Low BDI = −12%, $SD = 15.35$, $t_{(26)} = 4.01$, $p < 0.001$; High BDI = −12%, $SD = 14.42$, $t_{(24)} = 4.31$, $p < 0.001$] and "more similar" lures [Low BDI = −15%, $SD = 16.16$, $t_{(26)} = 4.71$, $p < 0.001$; High BDI = −12%, $SD = 16.89$, $t_{(24)} = 3.47$, $p < 0.01$] were less often misclassified as "old" (a repeat) when they were presented across blocks, as shown in **Figures 5A–D**. Thus, contextual changes occurring between the presentation and recognition trials improved the ability of both low and high

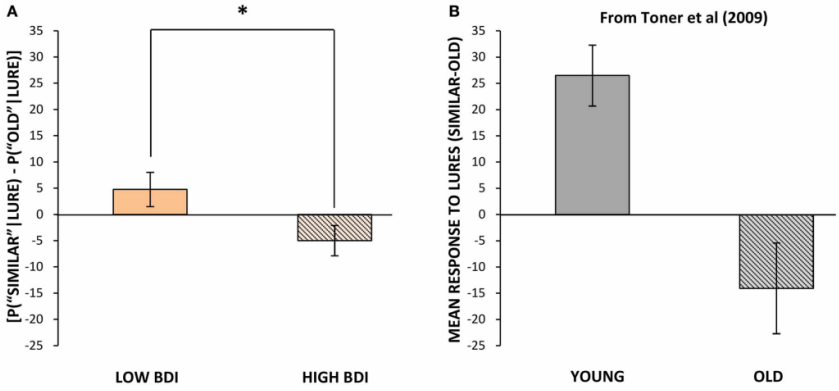

FIGURE 4 | Mean bias toward pattern separation vs. pattern completion (mean probability of correctly identifying lures as "similar" minus probability of misidentifying lures as "old," ± standard error) vs. BDI score in Experiment 2 (A) and vs. age in Toner et al. (B). (A) Mean bias scores when related target appeared in the same block as lure, for those with below median vs. above median BDI scores. **(B)** Mean bias scores for younger vs. older adults. Adapted from Figure 2 in Toner et al. (2009) and reproduced with permission from Cold Spring Harbor Laboratory Press. *$p \leq 0.05$.

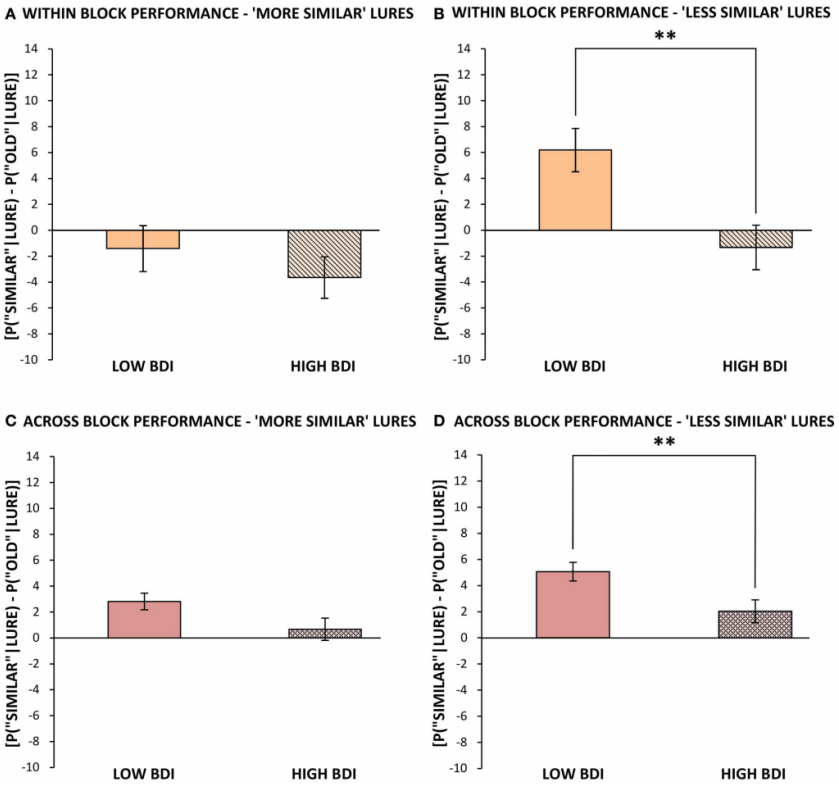

FIGURE 5 | Mean bias toward pattern separation vs. pattern completion (mean probability of correctly identifying lures as "similar" minus probability of misidentifying lures as "old," ± standard error) in Experiment 2 for those with below median vs. above median BDI scores, (A) for "more similar" lures, when target was within the same block, (B) for "less similar" lures, when target was within the same block, (C) for "more similar" lures, when target appeared in a different block, and (D) for "less similar" lures, when target appeared in a different block. $^{**}p \leq 0.01$.

BDI groups (although the difference was not significant in the higher BDI group) to correctly select lure images as "similar" [Low BDI = 8% increase, $SD = 19.08$, $t_{(26)} = 2.25$, $p = 0.03$; High BDI = 7% increase, $SD = 17.96$, $t_{(24)} = 1.91$, $p = 0.07$]. Evidently, the low BDI group had a resulting 11% advantage over the high BDI group at correctly calling a lure "similar" across blocks [$t_{(50)} = 2.08$, $p = 0.04$, **Figure 1C**]. Just as was the case within blocks, the high BDI group made significantly more pattern completion errors on lure trials than did the low BDI group, or conversely, the low BDI group displayed superior pattern separation [$t_{(50)} = 2.87$, $p = 0.01$].

As before, trials on which the lure appeared across blocks from the original target presentation were subdivided into two categories based on each lure's similarity rating into "less similar" and "more similar." There was no difference between the low and high BDI groups in the number of errors they made in calling a similar object "new," regardless of how similar the object was. In contrast, and comparable to the relationship described within blocks, those with lower BDI scores outperformed those with higher BDI scores at identifying "less similar" lures that appeared across blocks [$t_{(50)} = 2.14$ $p = 0.02$ (one-tailed)]. Interestingly, those with low BDI scores also exhibited superior performance at correctly identifying the "more similar" lures as "similar" when they appeared in a later block [$t_{(50)} = 1.82$, $p = 0.04$ (one-tailed)]. The greater ability of low BDI scorers to accurately categorize the "less similar" lures within a different block from its original target presentation was mirrored by significantly fewer pattern completion errors, or false positives, compared to higher BDI scorers [$t_{(50)} = 1.76$, $p = 0.04$ (one-tailed)]. In contrast, the low BDI group's improved ability to correctly distinguish "more similar"

lures from the original targets, when presented in a contextually distinct recognition phase, was not solely the result of a reduction in pattern completion errors; they showed a slight reduction in the number of errors in calling the "more similar" lures "old" as well as fewer errors in calling the "more similar" lures "new."

Taken together, the data within and across blocks suggest that when targets and lures appear within the same visual and temporal context (**Figures 5A,B**), if pattern overlap is too high (**Figure 5A**), neurogenesis levels are not sufficiently high in either group to successfully separate the patterns. On the other hand, provided that targets and lures do not overlap too strongly (**Figure 5B**), depression levels (putatively reflecting neurogenesis levels) cause a shift in performance from successful pattern separation to erroneous pattern completion. In contrast, when there is a longer temporal spacing between similar items, coupled with a change in visual context (**Figures 5C,D**), participants' performance shifts more toward pattern separation, regardless of BDI score or target-lure similarity. One possible explanation for this finding is that the change in time and context led to forgetting of the original image, thereby promoting a general bias toward selecting "similar" or "new" as opposed to "old" when the lure appeared. Another possibility is that the contextual changes occurring between the presentation and recognition trials led to reduced interference between the original image and the lure by adding sufficient contrast to the lure stimulus for it not to be encoded as an "old" image. If the former explanation was true, then we might expect that incomplete or low resolution memories for the original stimulus would lead to more errors in calling a similar object "new," but this was not the case. On the contrary, we found that the number of "new" misidentifications of lures when the target occurred in a different block was not significantly different in either group from their performance when the target occurred within blocks. Further, if there was a greater bias to select the "similar" response option when lures are presented in a different block from the original image, then there would be no significant effect after bias correction. Again, this was not the case. The second scenario, therefore, seems to be more strongly supported. We would expect that if time and contextual changes provide additional separation between the target stimulus and subsequent lure object, then performance should improve where pattern separation processes are needed. Indeed, we found that contextual changes occurring between the presentation and recognition trials improved the pattern separation abilities of both low and high BDI groups.

In sum, these data suggest that both within and across blocks: (1) those with lower BDI scores demonstrate superior performance at recognizing a lure as "similar," especially when there is less overlap in salient features (i.e., when the objects are "less similar" to one another); (2) the low BDI group's advantage in distinguishing between "old" and "similar" items reflects enhanced pattern separation processes and fewer pattern completion errors; and (3) a longer amount of time in combination with a contextual shift between encoding and recognition may benefit not only those with putatively greater neurogenesis, but also those with a deficit in pattern separation and putatively less adult-born cells available in the DG (i.e., high BDI scorers) by adding contrast between stimuli, consequently aiding them to correctly identify a lure as "similar." There was no difference between groups on the CANTAB® PAL task, further suggesting that the lower BDI group's superior performance on the pattern separation task is not due to a generalized advantage in memory.

Correlational analysis of depression and neurogenesis-dependent memory

In addition to the analyses described above based on a median split of BDI scores, we also considered BDI scores as a continuous measure of depression levels, and performed correlational analyses between BDI scores and our memory measures. BDI scores negatively correlated with the proportion of correct identifications of lure objects as "similar" when they were presented within the same block as the original target [$r_{(50)} = -0.272$, $p = 0.05$, **Figure 6A**] as well as when they were presented across blocks [$r_{(50)} = -0.297$, $p = 0.03$, **Figure 6B**], further suggesting that lower BDI scores (and presumably higher levels of neurogenesis) are associated with improved pattern separation ability, regardless of whether the similar items are closely or widely spaced in time. We also examined the correlation between BDI scores and our measure of bias toward pattern separation vs. pattern completion described above (number of correct "similar" responses to lure trials minus number of "old" false positives). BDI scores had a significant negative relationship with this measure both within blocks [$r_{(50)} = -0.284$, $p = 0.04$] and across blocks [$r_{(50)} = -0.408$, $p < 0.01$]. In other words, the lower the BDI score, the stronger the tendency is toward pattern separation and the weaker the tendency toward pattern completion; this holds whether the similar items are closely or widely spaced in time. BDI scores did not correlate with the correct identification of novel stimuli or repeated objects within or across blocks. Looking more specifically at performance on the lures that were rated as "less similar" vs. "more similar," we found that it was performance on the "less similar" items that was most strongly predicted by BDI scores. Within blocks, BDI scores were negatively correlated with the percent of correct classifications of "less similar" lures as "similar" [$r_{(50)} = -0.334$, $p = 0.02$], while being positively correlated with the number of "old" misidentifications of "less similar" lures [$r_{(50)} = 0.319$, $p = 0.02$]. Likewise, across blocks, there was a trend toward a negative correlation between BDI scores and the number of correct selections of "less similar" lures as "similar" [$r_{(50)} = -0.264$, $p = 0.06$], and, conversely, a positive correlation between BDI scores and the proportion of "old" false positive responses to "less similar" lure trials [$r_{(50)} = 0.284$, $p = 0.04$]. Additionally, for "more similar" lures, there was a significant negative correlation between BDI scores and the correct identification of lures appearing across blocks [$r_{(50)} = -0.299$, $p = 0.03$], but no significant correlation for lures occurring within blocks. In sum, those with higher BDI scores made more pattern completion errors (i.e., false positives) at the cost of pattern separation both within and across blocks. This effect was most pronounced when the lure stimuli were considered "less similar" to the target image, due to the fact that more similar lures were rather difficult for both high and low BDI scorers. Importantly, BDI scores did not correlate with performance on paired associates learning, which further argues against the

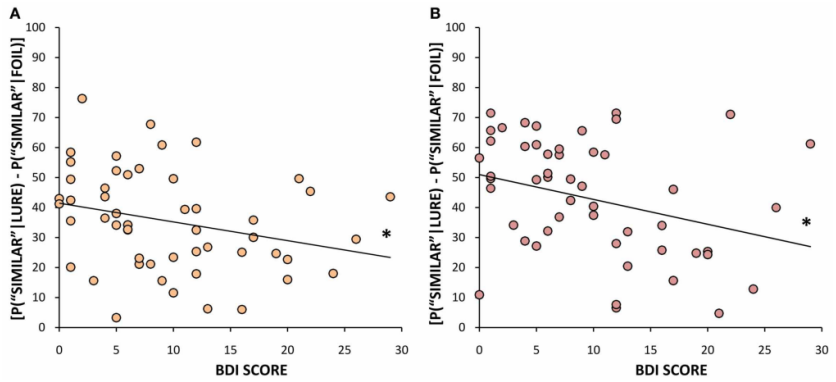

FIGURE 6 | Mean % correct identification of similar lures (bias corrected) as a function of BDI scores, (A) when they appear in the same block as the original target ($r = -0.272$, $p = 0.05$), (B) when they are presented in a different block from the original target ($r = -0.297$, $p = 0.03$). $^{*}p \leq 0.05$.

notion that impaired pattern separation in the high BDI group is the result of more generalized deficits in hippocampal-dependent processing.

DISCUSSION

In Experiment 1, we found that exercise enhanced both fitness levels and performance on a putatively neurogenesis-dependent visual pattern separation task. Specifically, it was those who exhibited the greatest improvement in VO_{2peak}, the gold-standard measurement of aerobic fitness, who showed a significant post-exercise enhancement in the ability to distinguish similar lures from previously studied targets. Consistent with these between-group differences, change in fitness taken as a continuous measure significantly correlated with improved pattern separation performance. Mirroring the effects of exercise reported in Experiment 1, we found in Experiment 2 that elevated depression scores negatively predicted visual pattern separation abilities. On the other hand, neither exercise response nor depression scores predicted performance on either the correct classification of repeated items and novel items, or the visuo-spatial PAL task (CANTAB® PAL). Importantly, the CANTAB® PAL task is well-established to be sensitive to hippocampal damage. Thus, both exercise and subclinical depression seem to selectively affect pattern separation, rather than having more generalized effects on hippocampal-dependent memory.

As mentioned previously, the non-responder group actually had a somewhat greater level of aerobic fitness as well as pattern separation performance at the onset of our investigation. Accordingly, it may be more accurate to describe the effects of our 6 week exercise intervention as normalizing, rather than enhancing, both fitness and putative neurogenesis-dependent memory function in the responder group. We plan to investigate the relationship between baseline VO_{2peak} and pattern separation performance in future work and expect to find that those with a superior VO_{2peak} score, previously shown to correlate with subjective measures of physical activity (e.g., Herting and Nagel, 2012), will outperform those with lower fitness levels at correctly identifying lures as "similar," while not differing in their respective ability to detect repetitions or novel objects. This raises the important point that attempts to increase neurogenesis and, therefore, its functional capacity might prove unsuccessful unless there is a deficit to begin with. More generally, the baseline level of neurogenesis must be taken into account. Indeed, in our study it seems that only those with lower levels of fitness, and putatively lower levels of neurogenesis before our exercise training program commenced were the ones who experienced the greatest benefit to putative neurogenesis-dependent cognition following exercise. On the other hand, our attempt to increase neurogenesis in those that had marginally higher levels of fitness and cognitive performance at baseline (non-responders), although no doubt still experiencing some neurogenesis-independent benefits from exercise, was likely redundant with respect to neurogenesis-dependent cognition, as they were already near or beyond the threshold for optimal performance at the onset of our training regime. Future work with the goal of elucidating the relationship between basal neurogenesis and the capacity for change is certainly warranted.

The neural benefits of aerobic exercise, including enhancements in neurogenesis and other physiological and behavioral outcomes, have long been realized (e.g., Cotman et al., 2007; van Praag, 2009). Conversely, it is well-established that high levels of stress contribute to reductions in adult hippocampal neurogenesis (Cameron and Gould, 1994; McEwen, 2001), and to the pathogenesis of major depression (Brown et al., 1999). However, one potential confound in attempting to link depression-related memory deficits to neurogenesis is that stress and depression may lead to broader hippocampal pathology. Conversely, exercise may have widespread effects such as the up-regulation of vasculature and neurotrophins both centrally and peripherally, leading to improved delivery of oxygen and other nutrients to brain tissues

etc., and generalized cognitive benefits. Thus, it is important to assess performance on both putatively neurogenesis-dependent memory tasks and other hippocampal-dependent tests of memory, to determine whether lifestyle correlates such as depression and exercise have selective or more generalized cognitive effects. Importantly, meta-analyses suggest that the number of lifetime episodes of major depression predicts the degree of hippocampal atrophy, with first episode sufferers typically showing no signs of hippocampal volume loss (MacQueen et al., 2003; Campbell et al., 2004). We therefore recruited participants in the current study and in our previous study (Becker et al., 2009) who had no prior or current diagnosis of a psychiatric illness and were therefore hypothesized to have reduced neurogenesis as a function of depression scores, in the absence of broader hippocampal pathology. Consistent with this prediction, across the two studies, those with higher depression scores exhibited selective deficits on two different memory tests postulated to be neurogenesis-dependent, in spite of intact performance on a range of other hippocampal- and non-hippocampal-dependent control tasks. Moreover, to our knowledge, the current study is the first to demonstrate opposite effects of chronic exercise and depression levels on performance on a putative neurogenesis-dependent learning task in humans. These results lend evidence to the hypothesis that adult-generated granule cells (or lack thereof) contribute to the cognitive enhancing effects of exercise and the stress-induced impairments in cognition, specifically on a high interference memory task: visual pattern separation.

With lower depression levels, and increased fitness levels, we observed a behavioral shift from pattern completion (erroneous classification of similar items as "old") to pattern separation (correct classification of similar items as "similar"), presumably due, at least in part, to the increased pool of available newborn cells in the adult DG associated with increased fitness and lower depression scores. This shift can be understood at a neural level, given that young neurons are more plastic and hyperexcitable relative to mature DG neurons (Snyder et al., 2001; Ge et al., 2006; Markwardt et al., 2009), making them more readily available to respond to subtle changes in their input. Furthermore, the young neurons functionally "turnover" by modifying these hyperplastic properties as they grow into maturity (e.g., Becker and Wojtowicz, 2007). In the absence of a pool of young neurons, there would be a shift in bias toward a less excitable and less plastic and static population of DG cells, decreasing the chance of evoking a novel response to a similar, overlapping stimulus in downstream CA3 neurons. Evidence from computational models suggests that when CA3 neurons are only weakly activated by DG inputs, they will respond more strongly and rapidly to their direct EC inputs, shifting their bias toward treating their input as familiar rather than novel, and engaging associative retrieval mechanisms (pattern completion) via their recurrent collateral connections (Nolan et al., 2011). Our findings are consistent with the prediction that exercise shifts the CA3 toward pattern separation, whereas depression levels shift processing toward pattern completion. These findings are also consistent with the well-established effects of ageing on neurogenesis and pattern separation. Age-related declines in neurogenesis have been documented in many species (Heine et al., 2004; Barker et al., 2005; Klempin and Kempermann, 2007; Johnson et al., 2010), and experimental ablation of neurogenesis has been accompanied by a shift from pattern separation to pattern completion in rodents (Clelland et al., 2009). Further, aged humans, who display an age-related decline in neurogenesis similar to that seen in rodents (Knoth et al., 2010), also show deficits on tasks that require pattern separation (Toner et al., 2009; Stark et al., 2010; Yassa et al., 2011; Holden et al., 2012; Stark et al., 2013).

Taken together with the well-established effects of exercise and stress on neurogenesis, and mounting evidence from rodent studies of a critical role for neurogenesis in high interference memory tests (see Introduction), our results are consistent with the hypothesis that neurogenesis is the underlying cause of the exercise-induced enhancement, and the depression-related deficit, in pattern separation that we observed in human participants. As previously alluded to, there was no direct measure of neurogenesis used in our study, therefore, we cannot rule out the possibility that one or more additional variables were affected by exercise and stress, which themselves could have caused or contributed to the observed effects on putative neurogenesis-dependent cognition that are described here. Indeed, both aerobic exercise and stress are known to act on multiple physiological targets (e.g., Brown et al., 1999; McEwen, 2001; Cotman et al., 2007; van Praag, 2009), which include the production of neurotrophins such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor type-I (IGF-I). Neurotrophins are activity-dependent regulators of brain plasticity. BDNF is increased by aerobic exercise and, while being a well-established positive regulator of neurogenesis in the DG (Sairanen et al., 2005; Scharfman et al., 2005; Henry et al., 2007; Young et al., 2007; Cunha et al., 2010), has also been shown to affect multiple other factors besides the number of adult-born neurons in the subgranular zone. For example, BDNF can influence the release of glutamate and GABA as well as the post-synaptic activation of their receptors which, in turn, can induce calcium influx and influence the cell's basal excitatory postsynaptic potential (Jovanovic et al., 2000; Cunha et al., 2010). BDNF can also activate signal transduction pathways that are important for LTP through binding to its TrkB receptor [reviewed in Cunha et al. (2010)]. All of these changes could contribute to overall brain plasticity and enhanced cognitive performance following exercise. In contrast, a decrease in synaptic plasticity via inhibition of BDNF signaling following chronic stress could contribute to the cognitive deficits observed in those who score high on depression scales. It has been observed that those within a depressed episode show decreased levels of peripheral BDNF in serum (Shimizu et al., 2003). On the other hand, antidepressant pharmaceuticals seem to require TrkB signaling in order to confer behavioral recovery (Li et al., 2008). Peripheral IGF-I is also elevated by aerobic exercise and is required for the running-induced increase in DG neurogenesis (Trejo et al., 2001) and spine density on basal dendrites in the hippocampal CA1 subregion (Glasper et al., 2010). IGF-1 has also been shown to converge on similar pathways as BDNF and has been described as a regulator of exercise-induced BDNF signaling (Ding et al., 2006; Cotman et al., 2007). The increased number of spines on the dendrites of pyramidal cells in the CA1 or IGF-1's effect on BDNF-dependent signaling cascades that lead

to increased plasticity in the hippocampus and abroad could be partly responsible for improved hippocampal processing in the absence of changes to hippocampal neurogenesis. Therefore, the putative exercise-induced upregulation of BDNF and/or IGF-1 and depression-associated reduction of BDNF availability could be affecting cellular plasticity in general and cognitive performance in the absence of changes to adult-born neurons in the DG that could, in theory, underlie the changes to learning and memory performance reported here. However, if BDNF and IGF-1 had more pleiotropic effects on cognition, then we would expect to find measureable differences in performance on hippocampal-dependent tasks that are not likely to require neurogenesis, such as CANTAB® PAL. Importantly, we did not observe any significant difference between groups on this particular task. Further, although BDNF and IGF-I can affect factors besides neurogenesis in the subgranular zone, they are nonetheless potent regulators of adult hippocampal neurogenesis. It would thereby be extremely difficult, at least in humans, to parse out or dissociate between the neurogenesis-dependent and -independent contributions of these neurotrophins on cognition. Again, since there was no measure of neurogenesis used here, it is also possible that normalization of pattern separation performance in exercisers and deficits in those with high BDI scores were observed in the absence of changes to neurogenesis. This possibility cannot be completely ruled out without a direct measure of neurogenesis in humans, however, based on the vast number of studies consistently demonstrating an increase in hippocampal neurogenesis following exercise and decrease following chronic stress or stress hormone administration in a variety of non-human species, we can be relatively confident in our assumption that neurogenesis varies between the groups of young adults described here. Breakthroughs in technology and a number of elegant studies would be needed before we can come close to dissociating between the neurogenesis-dependent and -independent effects on cognition in humans and how they might be regulated by, for instance, exercise and stress. In the interim, we have relied on established correlates of neurogenesis from the animal literature. In future work, we plan to elucidate additional biomarkers of neurogenesis in both rodents and humans. Despite the fact that exercise imparts a wide range of benefits on the brain and cognition, this does not mean that DG neurogenesis is not intimately linked to many of these cognitive benefits, and perhaps even its antidepressant effects.

The high intensity interval training manipulation employed here could be more stressful than traditional aerobic exercise training. In general, the intensity and duration of exercise may result in a cost-benefit trade-off. For example, in rodents, a single bout of high intensity exercise sensitizes the organism to the effects of an acute stressor (a radiation challenge), causing elevated markers of oxidative stress, whereas chronic exercise has the opposite effect, inducing protective mechanisms against oxidative stress (de Lisio et al., 2011). Hence, there is a need for more human studies examining the effect of variable durations and intensities of aerobic training in sedentary participants vs., for instance, trained athletes. Specifically, the trade-off in the effects of exercise on positive endpoints such as increased neurotrophins, vs. negative endpoints such as elevated cortisol, inflammatory and oxidative stress markers, as well as on cognitive endpoints, should be investigated. We plan to use salivary cortisol in follow-up experiments to control for stress levels in a non-subjective way.

A limitation of our exercise study is the relatively small sample size, accordingly, the results reported in study 1 should be considered preliminary. While significant effects of exercise on pattern separation were observed, it is possible that with a larger sample exercise might have affected performance on our control tasks as well (correct classification of repetitions and new items, and performance in the PAL task). On the other hand, in a much larger sample of over 50 individuals, we observed a significant relationship between depression scores and pattern separation, but no relationship between depression scores and any of our control tasks. Another caveat is that we included in our non-responder group two participants who did not complete the exercise training. Nonetheless, just looking at the responder group alone, we observed a statistically significant, selective improvement in pattern separation pre- vs. post-exercise with no effect on PAL. We plan to expand on the results of our exercise study in future work by including a non-exercising control group that can be compared to exercisers (regardless of whether they are responders or non-responders), as opposed to performing a median split-based analysis. Though, it should be noted that our median split-based analyses and correlational analyses yielded consistent results.

Another variable that warrants further investigation is the timescale over which exercise or stress may enhance or inhibit neurogenesis and affect memory in humans. The timeline for newly generated granule cells to mature and functionally integrate into the DG-CA3 network in humans is unknown, although estimates from rodents suggest that the process may take 2–4 weeks (Snyder et al., 2009). Evidence from rhesus monkeys suggests that the process may take even longer in primates (Ngwenya et al., 2006). If our exercise program had lasted 12 weeks instead of 6, we may have observed larger or more consistent increases in VO_{2peak} and neurogenesis-dependent memory functions. It has been posited that sustained exercise maximizes the proliferation potential for activity-induced DG neurogenesis, while acute bouts of exercise may only partially restore the neurogenic potential of the subgranular zone (Kempermann, 2010). An important issue is whether a relatively short-term exercise intervention such as the one used here could have comparable effects on neurogenesis and cognition in populations at known risk for depression, such as those with mild depressive symptoms who do not yet meet criteria for a full threshold episode of depression, or even those at high genetic liability. Whether exercise could act via a restoration of neurogenesis to prevent a depressive episode remains to be confirmed, but such an approach has appeal as a health-related disease prevention strategy.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Craig E. L. Stark for the use of his stimuli on the pattern separation task. This research was funded by grants from NSERC to Suzanna Becker and Martin Gibala and CIHR to J. Martin Wojtowicz and Astra Zeneca to Glenda MacQueen.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 26 January 2013; paper pending published: 21 February 2013; accepted: 14 April 2013; published online: 30 April 2013.

Citation: Déry N, Pilgrim M, Gibala M, Gillen J, Wojtowicz JM, MacQueen G and Becker S (2013) Adult hippocampal neurogenesis reduces memory interference in humans: opposing effects of aerobic exercise and depression. Front. Neurosci. 7:66. doi: 10.3389/fnins.2013.00066

This article was submitted to Frontiers in Neurogenesis, a specialty of Frontiers in Neuroscience.

Postscript

Taken together, the studies outlined in Chapter 2 suggest that lifestyle factors associated with increased or decreased neurogenesis may be used to study putative neurogenesis-dependent cognition in healthy young adults (Déry et al., 2013). Specifically, we found a significant positive correlation between change in fitness (assessed by VO_2peak) following a highly controlled six week-long aerobic exercise program and change in BPS-O performance, specifically on lure trials (Déry et al., 2013). On the other hand, BDI scores demonstrated the inverse relationship with BPS-O performance – there was a significant negative correlation between BDI scores and accuracy on lure trials (Déry et al., 2013).

Unpublished data from the second study described in Chapter 2 reveal that accuracy on similar lure trials occurring in a different block from the target presentation (in a different temporal and visual context) significantly correlates with performance on the hippocampal-dependent CANTAB® PAL task, whereas there is no correlation between accuracy on lures appearing in the same block as the target image and performance on PAL (Figure 2.6). Taken together, these data suggest that distinct processing is required for identifying lures in the two different conditions (i.e. within block performance versus across block performance). Whereas lure trials occurring in a different block from the original target image requires global hippocampal processing, it seems lure trials occurring within the same block as the target image, which possess inherently higher levels of interference, require some sort of additional processing (such as pattern separation in the DG/CA3). The added benefit of contextual change afforded

by the change in visual background and time, which itself may be considered an aspect of context, might make it easier for participants to identify the lures as being "similar" as opposed to "old". Further, this relative lack of interference between the target image and its lure might render AHN unnecessary to separate the two images in memory.

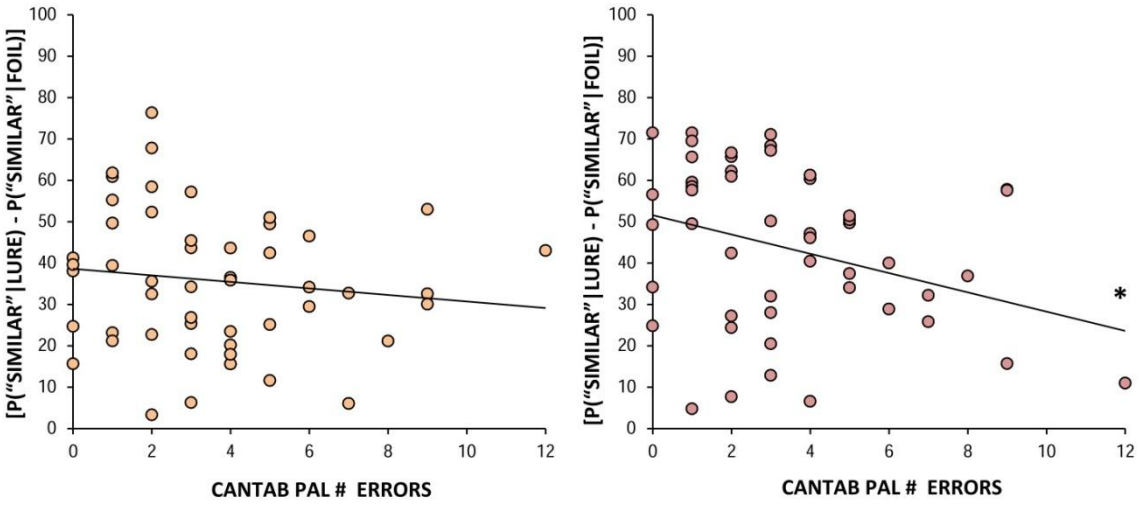

*Figure 2.6. Within- and across-block performance on lures vs. paired associates learning. Left: Accuracy on lures within blocks vs. performance on PAL; Right: Accuracy on lures across blocks vs. performance on PAL. * = $p \leq .05$*

A recent report by Shelton and Kirwan (2013) largely support our findings. They used a different, but nonetheless comparable, version of the BPS-O and assessed subjective levels of depression via the Depression Anxiety Stress Scales (DASS) as opposed to the BDI. Despite these differences Shelton and Kirwan (2013) also found a significant negative correlation between perceived level of depression and performance at identifying similar lures. They controlled for other factors known to affect neurogenesis, such as the participants' recent exercise and sleep habits. Prior exercise level, anxiety scores, and sleep quality all failed to influence behavioural pattern separation

performance, indicating that the observed deficits were primarily associated with self-reported levels of depression (Shelton and Kirwan, 2013). Further, Fujii and colleagues (2014) recently found that higher activity in the DG/CA3, assessed by high resolution fMRI, was associated with correct responses in a delayed match to sample pattern separation task. Moreover, BDI scores negatively correlated with activity in the DG/CA3 region (Fujii et al., 2014). These data suggest that subclinical depression scores, assessed by BDI, are able to predict impaired activity in the DG/CA3 and behavioural pattern separation deficits, lending support to our claim that high BDI scores may be a non-invasive, indirect measure of impaired or downregulated neurogenesis. Thus, adding to the already well-established data demonstrating a decline in behavioural pattern separation performance as people age (Toner et al., 2009; Yassa et al., 2010a, 2010b, 2011; Stark et al., 2010, 2013; Yassa and Stark, 2011; Holden et al., 2012), our study implicates two more factors associated with AHN (exercise and stress) in overcoming interference between highly similar stimuli in humans. Although the evidence we provide is correlational, it is nonetheless encouraging that our results support what has been observed in pre-clinical animal studies and are consistent with previous findings in humans. Thus, behavioural pattern separation performance may be a valid indicator of pattern separation function and, therefore, may hold some predictive value in assessing the level of AHN humans.

Other factors that have been linked to regulating neurogenesis in animals, such as antidepressant pharmaceuticals or alcohol should also be investigated for their ability to ameliorate or worsen BPS-O performance, respectively. Besides its role in pattern

separation, there is also recent evidence to suggest that a secondary, perhaps non-mutually exclusive, role for AHN exists. That role is in memory persistence: the process of preserving or clearing memories.

Chapter Three

Preamble

Following the studies described in Chapter 2, we decided to test another proposed function of adult neurogenesis in humans, namely, remote memory. We used the same approach as before, having participants fill out self-report questionnaires assessing perceived levels of depression (BDI score) as well as a battery of cognitive tests. In this study, we analyzed performance on the BPS-O as a function of PSS scores in addition to the BDI scores. PSS scores are considered to be a measure of stress experienced more recently, in contrast to the BDI, which is considered to be a measure of stress experienced over the more distant past.

In the study outlined in Chapter 3, we also incorporated a two week-long delay between the learning phase of certain repetitions and lures and the subsequent recognition phase. In this way, we would be able to 1) replicate our previous finding that BDI scores negatively correlate with BPS-O performance, specifically on lure items; 2) test if PSS scores also share a negative relationship with lure trial performance; and 3) elucidate the relationship between BDI or PSS scores and accuracy at identifying repetitions or lures tested two weeks after presentation of the original target images. Replicating our previous findings would provide additional support to the hypothesis that BDI scores represent a lifestyle-based correlate of AHN. In turn, it would help justify our using the same measure (i.e. BDI scores) to assess the role of AHN in remote memory. Testing the two proposed functions of AHN, pattern separation and memory persistence, within the same

population holds many advantages. For instance, it would allow us to test whether or not there is a relationship between accuracy on highly similar lures versus remembering repetitions across two weeks. Importantly, since neither BDI nor PSS scores were normally distributed, we opted to use Spearman's rho (r_s) for all correlative analyses as opposed to Pearson's *r*.

Abstract

Adult hippocampal neurogenesis (AHN) is perhaps the most striking example of ongoing plasticity in the human brain. AHN is modulated by a number of lifestyle factors including chronic stress, which acts as downregulator (Warner-Schmidt and Duman, 2006). Although the process of AHN has been the subject of intensive investigation over the last decade or so, its functional significance remains the basis of speculation. Computational models and mounting empirical evidence point to a role for immature neurons in minimizing interference between similar memories (pattern separation; Becker, 2005; Aimone, Wiles and Gage, 2006, 2009). Interestingly, opposing views have been put forward regarding the role of neurogenesis in remote memory. Some have proposed that neurogenesis promotes the clearance of old memories within the hippocampus and their consolidation elsewhere, while others have proposed that neurogenesis promotes long-term retention of memories within the hippocampus. Here, we used a modified version of Kirwan and Stark's (2007) behavioural pattern separation task, which tests recognition of images of objects, some of which are repeated across trials and some of which are highly similar lures. The correct classification of lures may tax pattern separation processes in the hippocampus and thus AHN. To investigate the

potential role of AHN in remote memory, we also introduced a two week-long delay between the presentation and recognition trials of certain stimuli. Across short time delays, as found in previous studies (Déry et al., 2013; Shelton and Kirwan, 2013), those with higher depression and stress scores made significantly more errors at identifying lure stimuli as "similar" relative to those with lower levels. Importantly, following a two-week delay, performance at classifying lures dropped to chance levels for all groups, whereas those with lower stress and depression scores were significantly more accurate at classifying exact repetitions as "old", suggesting a role for AHN in the stabilization of remote memories.

Introduction

Adult hippocampal neurogenesis (AHN) refers to the post-natal production of new neurons arising from stem-like cells in the subgranular zone. AHN has been found to occur in the dentate gyrus (DG) subregion in many different mammalian species (reviewed in Barker et al., 2011) including humans (Eriksson et al., 1998; Knoth et al. 2013; Spalding et al., 2013), and persists throughout life. These new cells have been implicated in some forms of learning and memory (Feng et al., 2001; Shors et al., 2001; Kempermann, 2002; Becker, 2005; Snyder et al., 2005; Aimone et al., 2006, 2009; Wiskott et al., 2006; Becker and Wojtowicz, 2007; Appleby and Wiskott, 2009; Becker et al., 2009; Weisz and Argibay 2009, 2012; Appleby et al., 2011) as well as in the regulation of emotional and stress responses (Abrous et al., 2005; Becker and Wojtowicz, 2007; Sahay and Hen, 2007; Becker et al., 2009; Snyder et al., 2011).

Newborn neurons in the DG are critical for performance on a variety of different memory tasks in rodents. For example, many groups have described the importance of AHN in distinguishing between similar contexts (Saxe et al., 2006; Winocur et al., 2006; Warner-Schmidt, Madsen and Duman, 2008; Wojtowicz et al., 2008; Hernandez-Rabaza et al., 2009; Kitamura et al., 2009; Ko et al., 2009; Guo et al., 2011; Kohman et al., 2012; Nakashiba et al., 2012). Further, a large number of tasks specifically designed to test the pattern separation function of newborn neurons implicate AHN in pattern separation performance, regardless of whether the stimuli were similar odours, visual objects, spatial locations or environments (McHugh et al., 2007; Creer et al., 2010; Guo et al., 2011; Sahay et al., 2011; Luu et al., 2012; Pan et al., 2012a; Tronel et al., 2012; Winocur et al., 2012). AHN is also required for the long-term retention of platform location in the Morris Water Maze (MWM; Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009) and the long-term persistence of memories for where an aversive shock was received (Pan et al., 2012a, 2012b, 2013). Moreover, pattern separation deficits observed in rodents lacking AHN occur across both short and long timescales. Rodents with ablated neurogenesis also display deficiencies in tasks that require cognitive flexibility (Saxe et al., 2007; Burghardt et al., 2012, Pan et al., 2012a). Undoubtedly, there is a wide range of tasks that seem to require AHN. However, each of these tasks could be categorized in two ways: overcoming interference or memory persistence. Learning a new platform location in the MWM, learning a context-shock association in the contextual fear conditioning apparatus, and distinguishing between highly similar odour pairs, neighbouring spatial locations or highly similar visual objects all require overcoming interference. Indeed,

even cognitive flexibility, or forgetting previously learned information in order to respond appropriately to new task demands requires overcoming proactive interference from previously learned task demands. On other tasks that do not explicitly have a high proactive interference component, but are still shown to be deficient in those animals with ablated neurogenesis, there is generally a long time interval (>24–48h) between study and test (e.g. Snyder et al., 2005; Jessberger et al., 2009; Kitamura et al., 2009). The mere passage of time between study and test could itself be considered a source of retroactive interference. The more time that passes, the more the opportunity there is for learning material that could interfere with previously stored memories. On the other hand, AHN could be required for memory persistence, dictating which memories are reinforced and which memories are cleared by redistributing or adjusting the strength of connections in CA3.

Adult-born granule cells are thought to be important for pattern separation, the process of decorrelating similar inputs. Without pattern separation, less distinctive memory traces will be formed that fail to preserve the unique or novel details of an event, increasing the chance of memory retrieval errors. Cortical inputs project to the hippocampus primarily via the entorhinal cortex (EC). Within the hippocampus proper, EC reaches the CA3 and CA1 subregions both via direct perforant path projections as well as indirectly via the mossy fibre projections of the DG. The latter projections are postulated to be the predominant route for which novel incoming information is transmitted through the circuit, having a strong influence over what is encoded in CA3 (Marr, 1971; McNaughton and Morris, 1987; Treves and Rolls, 1992). In this way,

information can be orthogonalized, in part because of newborn granule cells in the DG, before reaching the CA3. The associative pathways within the hippocampus, including the CA3 recurrent collaterals and the Schaffer collaterals projecting from CA3 to CA1, are postulated to perform associative retrieval, allowing the current stimulus to cue the retrieval of stored memories (Rolls, 2007). The process of eliciting a full memory trace from partial retrieval cues is termed pattern completion. The retrieval of similar stored memories could either lead to memory enhancement or interference. For example, the retrieval of a similar memory, when recognized as being distinct from the current input, may facilitate the separate encoding of new material (Hardt, Nader and Nadel, 2013). Conversely, if the retrieved trace is not recognized as being distinct from the current input, the old trace may be modified or corrupted by details of the current stimulus via reconsolidation (Hupbach et al., 2007; Hardt et al., 2013). While this type of interference may be viewed as undesirable, pattern completion may also serve to maintain efficient retrieval, by reducing pathological focusing on irrelevant details (Sahay et al., 2011; Kheirbek et al., 2012). AHN is postulated to be an important mechanism that balances the two mnemonic processes, pattern separation and pattern completion (O'Reilly and McClelland, 1994).

Neurogenesis has been implicated in memory consolidation, the process by which a memory transitions from a labile to a relatively permanent state, in two distinctly different ways. First, some evidence supports a role for AHN in the long-term retention of some kinds of memory, on tasks such as the MWM that are permanently hippocampal-dependent (Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009). This suggests a

role for adult-generated neurons in cellular consolidation within the hippocampus, which we will refer to as the "memory retention hypothesis". Second, a role for adult-born granule cells has been proposed in "systems consolidation", a process by which memories become independent of the hippocampus, as their traces are repeatedly strengthened in the cortex (McClelland et al., 1995; Squire and Alvarez, 1995; Maviel et al., 2004; Squire and Bayley, 2007). More specifically, it has been suggested that AHN accelerates one component of systems consolidation: the process of clearing older memories from the hippocampus (Deisseroth et al., 2004; Josselyn and Frankland, 2012; Frankland et al., 2013). This could facilitate the encoding of new incoming information by freeing up neural circuitry for subsequent memory formation. In support of this "memory clearance hypothesis", it has been reported that elevated neurogenesis levels accelerate the process of systems consolidation of contextual fear conditioning (Kitamura et al., 2009; Akers et al, 2014), a type of memory that is initially hippocampal-dependent, but can also be supported by extra-hippocampal structures. Similarly, neurogenesis is a proposed mechanism underlying infantile amnesia, the loss of memories acquired early in life, in non-precocious neonatal species including humans and rats; the high levels of neonatal neurogenesis in these species may make the continuously rewiring infantile brain more vulnerable to interference (Josselyn and Frankland, 2012; Frankland et al., 2013). Does this mean that adults with upregulated or intact neurogenesis would perform worse on tests of remote memory as compared to those with downregulated or ablated neurogenesis? The answer to this question may depend on the type of memory being tested. Some forms of memory, including allocentric spatial memory (O'Keefe and

Nadel, 1978) and episodic memory (Tulving, 1983), seem to be permanently hippocampal-dependent (Vargha-Khadem et al., 1997; Mayes et al., 2001; Fortin et al., 2002; King et al., 2002). Several labs have demonstrated in rodents that AHN is required for long-term retention of spatial memories in the MWM (Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009). In contrast, as noted above in the case of contextual fear memory, neurogenesis could facilitate systems consolidation by helping to clear memories from the hippocampus as they are consolidated in extra-hippocampal structures.

In contrast to rodent studies of neurogenesis and memory that are typically administered over a period of days to weeks, most studies that have examined the potential contribution of neurogenesis to human memory have employed tasks administered within a single experimental session. Several studies have found that lifestyle factors associated with increased or decreased AHN in rodents correlate with performance on analogous, putative neurogenesis-dependent, behavioural tasks in humans. One potentially neurogenesis-dependent task that has gained attention in recent years is the behavioural pattern separation task – object version (BPS-O), which was originally designed by Kirwan and Stark (2007) and has since evolved into numerous other versions (reviewed in Yassa and Stark, 2011). The BPS-O task includes several blocks of study and test phases. In each study phase, a set of images of everyday objects is presented one at a time. In the test phase the participant views another series of images of objects, which are either 1) identical copies (repetitions), 2) highly similar, but non-identical stimuli (lures), or 3) completely novel objects (foils). For each image, the

participant is asked to judge whether the object is old, similar or new compared to one previously observed. The similar items, or lures, introduce a high interference component to the task and, hence, accuracy on lure trials (identifying them as "similar" as opposed to "old") should benefit from the pattern separation function attributed to the DG (Marr, 1971; McClelland et al., 1995; Kesner, 2007) and to adult-born granule cells in particular (Becker, 2005; Aimone et al., 2006, 2009; Wiskott et al., 2006; Becker and Wojtowicz, 2007; Appleby and Wiskott, 2009; Becker et al., 2009; Appleby et al., 2011). An indirect line of evidence that the BPS-O may be neurogenesis-dependent is that performance on this task is affected by several established up-regulators and down-regulators of neurogenesis. For example, following a six week-long exercise program, those exhibiting a greater change in fitness (assessed by VO_2*peak*) also showed greater increases in pattern separation performance (Déry et al., 2013). On the other hand, those who scored high on the Beck Depression Inventory-II (BDI) demonstrated a marked deficit in correctly identifying lures as "similar" as compared to their less-depressed counterparts, whereas there were no differences between groups in their ability to correctly identify repetitions or novel objects (Déry et al., 2013). Similarly, higher depression inventory scores as assessed by the Depression Anxiety Stress Scale were negatively correlated with behavioural pattern separation performance (Shelton and Kirwan, 2013). Another lifestyle factor associated with downregulated neurogenesis in humans is aging (Imayoshi et al., 2009; Knoth et al., 2010; Spalding et al., 2013). High-resolution fMRI and diffusion tensor imaging (DTI) studies have demonstrated that hyperactivity in the DG/CA3 region and perforant path degradation as well as mild cognitive impairment

(MCI) manifest with old age, both of which negatively correlate with pattern separation performance (Yassa et al., 2010b, 2010c, 2011; Yassa and Stark, 2011). Thus, three lifestyle factors that have been shown consistently to influence neurogenesis in rodents, exercise, stress and aging, have also been shown to correlate with putative neurogenesis-dependent cognition in humans in the same manner.

To further elucidate the potential role of AHN in reducing memory interference at short versus long time scales, we decided to extend our previous findings by incorporating a long-term, remote memory aspect to the BPS-O. Thus, in addition to the traditional within-day testing that we and others have investigated, we had participants complete a version of the BPS-O that incorporated a two week-long delay between the study of some targets and the subsequent testing of some repetitions and lures. Accordingly, while some participants were only presented with images of old, similar and new objects during same-day testing, others were asked to come back two weeks later for additional testing. During the two-week follow-up session, participants were presented with another set of images of everyday objects in a series of study–test phases. However, this time, some targets were originally presented on the same day, whereas other targets were from a study phase occurring two weeks earlier. We hypothesized that participants who scored high on depression and stress inventories would be impaired at identifying lures as "similar" on within-day testing, as in our previous study. At the longer time scale, the alternative theories described above make different predictions. According to the memory retention hypothesis, neurogenesis promotes long-term memory retention within the hippocampus, and thus, predictors of neurogenesis such as stress and

depression scores should also correlate negatively with performance across a two week delay. Those with high neurogenesis levels, on the other hand, should maintain high fidelity memory representations of the studied images across the two-week delay. Alternatively, the memory clearance hypothesis predicts that those with higher neurogenesis levels should exhibit a more efficient clearance of hippocampal-dependent memories. This might be coupled with faster systems consolidation of those memories, assuming that memories of the BPS-O images can be supported by extra-hippocampal structures. Under this assumption, the consolidated memories of the BPS-O images should be less detailed and more "schematized" (Frankland et al., 2013). Thus one might expect reasonably good recognition of the original targets, but increased confusion between similar lures and targets studied two weeks ago relative to lures tested within the same day as target presentation.

In order to control for differences in pattern separation performance in the BPS-O that may have been due to factors unrelated to neurogenesis, such as deficits in global hippocampal processing (in addition to the repetition trials within the BPS-O itself, which act as an internal control for traditional recognition performance) or more global cognitive deficits, we administered two very different control tasks: paired associates learning (PAL) from the Cambridge Neuropsychological Test Automated Battery (CANTAB®; Cambridge Cognition, Cambridge, United Kingdom) and reverse digit span. PAL is a visuo-spatial associative learning task that is sensitive to hippocampal pathology, but was hypothesized not to be neurogenesis-dependent. The patterns presented in PAL lack a high interference component, as they have very little visual

similarity and are presented at a small number of well separated spatial locations. Furthermore, in our previous studies, neither BDI scores nor post-exercise improvements in fitness predicted performance on the PAL task (Becker et al., 2009; Déry et al., 2013). The second control task, reverse digit span, is a test of working memory and was also hypothesized to be non-neurogenesis-dependent. Verbal working memory span is unimpaired in patients with medial temporal lobe lesions (Drachman and Arbit, 1966; Jeneson and Squire, 2012), but instead relies upon regions of the auditory phonological loop including the superior temporal gyrus, anterior cingulate and fronto-insular cortex (Li et al, 2012). Moreover, Saxe et al (2007) found a paradoxical improvement in working memory after ablation of neurogenesis, which could mean that a dysfunctional hippocampus lacking neurogenesis permits other structures more relevant to working memory to dominate in these tasks.

Methods

All aspects of our protocol were approved by the Hamilton Integrated Research Ethics Board. Our study population consisted of healthy young adults from the McMaster University undergraduate student pool. All participants gave informed consent after being screened for the following inclusion criteria: normal or corrected to normal vision and no history or previous diagnosis of any psychiatric disorder. Although we measured subjective levels of depression and stress in this study, we wanted to exclude anyone with a long-term history of major depression, as severe or multiple episodes of depression can lead to gross hippocampal pathology, including hippocampal volume loss (Campbell et al., 2004), therefore overshadowing any cognitive deficits that may be caused by down-

regulated AHN. In addition, chronic reductions in AHN have been shown to compromise the cell morphology and function of other hippocampal subregions, including the CA3 (Schloesser et al., 2014). Thus, we only wanted to recruit participants ranging from those who did not have any signs of depression to those who were at risk for developing their first depressive episode. We recruited 109 participants in total (age 17-27, 21 male, 88 female) and 44 returned for the second session (age 17-26, 7 male, 37 female). The data obtained from 57 of the participants have been published elsewhere (Déry et al., 2013) and re-used here in combination with an additional set of participants that were recruited subsequently. Each participant's data was linked to a unique code and their identifying information was kept anonymous to the experimenters. However, each participant's identifying information and BDI score were forwarded to a third party for assessment. If any student was flagged as being at risk for major depression or suicide then their contact information was sent to a psychological counselor at the McMaster University Student Wellness Centre who would then contact the participant to discuss possible treatment options.

In each visit, participants completed the Beck Depression Inventory-II (BDI; Beck et al., 1996) and Cohen's Perceived Stress Scale (PSS; Cohen et al., 1983) in a private testing room. Based on the well-established association between chronic stress and the onset of depression, we considered the BDI to be an indicator of enduring stress as it is not overly sensitive to daily fluctuations in mood. However, a BDI score is susceptible to inflation based on a participant's current physical condition (e.g. due to illness) as it is heavily reliant on questions relating to physical symptoms such as fatigue (Moore et al.,

1998). Accordingly, we also administered the PSS as a predictor of more transient stress, experienced over the past month, since its predictive value drops off after two to four weeks. Furthermore, it has been previously demonstrated that stress can, under some circumstances, improve hippocampal excitability, long-term potentiation and hippocampal-dependent memory (Kim and Diamond, 2002; Joëls et al., 2007; Kirby et al., 2013) as well as transiently upregulate AHN (e.g. Kirby et al., 2013). Thus, the BDI and PSS could provide not only complimentary, but also differing predictive value in terms of AHN and associated cognition. Nonetheless, since chronic stress is a major predisposing factor for unipolar depression, we might predict that those who score high on both the BDI and PSS have either experienced high levels of stress for longer or have a predisposition to developing depression (perhaps resulting in lower AHN) than those who have, for instance, a low BDI, but high PSS score. Following the emotional questionnaires each participant was asked to complete our version of the BPS-O including the two-week delayed recognition test, as well as two control tasks, the CANTAB® paired associates learning (PAL) which is thought to be hippocampal-dependent but not neurogenesis-dependent, and a reverse digit span test as an indicator of working memory and executive functions.

The BPS-O used here was adapted from the task as first described by Kirwan and Stark (2007). In this study, we used the same version of the BPS-O as in our previous study (Déry et al, 2013), with eight blocks in total, but with one important difference. In our former study, eight blocks were presented over one day. However, in the current study, we present four blocks on the first day of testing and the remaining four blocks on

the second day of testing two weeks later. Each block includes a study phase and a test phase, which we also refer to as a presentation phase and a recognition phase, respectively. Another way in which we modified Kirwan and Stark's task, in our previous study (Déry et al., 2013), as well as in the present experiments, was to introduce visual context as an additional variable. Each presentation phase includes 16 images of every objects displayed for 2,500 ms each, with a 500 ms inter-trial interval, and all of the images are seen for the first time (that is, there are no repetitions or lures). Each recognition phase includes a different number of images, depending on the block number. For instance, the recognition phase within the first block contains 38 images in total: 8 repetitions, 8 lures and 22 unrelated foils. Each subsequent block contains the same number of images; however, one repetition and one lure are added from each proceeding block, as well as two additional foils. Thus, in the fourth recognition phase, there are 8 repetitions and 8 lures from the fourth presentation phase, 1 repetition and 1 lure from each of the 3 proceeding blocks and 28 unrelated foils for a total of 50 images. On the second day blocks five through eight contain the exact same proportion of trial types as blocks one through four, respectively. However, blocks five, six, seven and eight also contain one additional repetition and one additional lure from each block that was completed on day one. During each test phase the participant is explicitly asked to judge whether the image being displayed is "old", "similar" or "new" compared to the images previously viewed. If the image is exactly the same as one previously seen, they are instructed to select "old." If the participant recognizes the object as being similar to, but not the exact same as, one previously viewed then they are instructed to select "similar".

Finally, if the participant does not remember seeing the image before, they are instructed to select "new". A correct response is said to occur when the participant identifies a repetition as "old", a lure stimulus as "similar" or an unrelated foil as "new". When a participant correctly identifies a lure as "similar", we can assume that pattern separation has occurred, whereby the participant has overcome the interference generated between stimuli (i.e. the object originally viewed during the presentation phase and the similar item being viewed during recognition) in order to appropriately discern between what is similar and what is old (or new). As previously stated, we are not saying that correctly recognizing a lure item as "similar" is the behavioural analogue or a definition of pattern separation. Rather, we are saying that correctly identifying lures as "similar" requires discriminating between two highly confusable items and thus *likely* requires pattern separation in the DG. On the other hand, when a participant misclassifies a lure as "old" then pattern completion has *likely* occurred, whereby the observation of a lure stimulus has generated sufficient overlap with the original target for the two objects to be considered one and the same (leading to an error in recognition). Accordingly, the correct identification of a lure stimulus constitutes a correct rejection (i.e. correctly rejecting the lure stimulus as being similar, but not identical, to the target image) whereas incorrectly classifying a lure as "old" constitutes a false positive (i.e. falsely identifying a lure as being identical to the target). The more similar an object is to one previously viewed, the harder it becomes to identify it as "similar" as opposed to "old". Accordingly, one could see how performance varies as a function of similarity between targets and lures (see

Yassa et al., 2010a; Lacy et al., 2011; Motley and Kirwan, 2012; Déry et al., 2013; or Stark et al., 2013 for examples).

Constructing the task in such a way allowed us to vary the amount of interference between target stimuli and lures. Each target object was seen only once during a given study phase, but a lure could appear in the same block and the same context, a varying number of blocks later in a unique visual context or two weeks later in a different visual context. This afforded us the opportunity to analyze differences in traditional recognition (performance on repetition trials) or putative neurogenesis-dependent recognition (performance on lure trials) as a function of the degree of visual and/or temporal context change between the presentation of a target and a repetition or lure, and how performance on each of these conditions correlates with BDI and PSS scores. Importantly, the long temporal context change across the two-week delay also allowed us to measure the effect of stress and depression, presumably reflecting the contribution of adult-born granule cells, to remote memory formation and retrieval. We considered the identification of repetitions tested two weeks after their first presentation as a measure of remote memory. Similarly, correct identification of lures when the original similar items were presented two weeks earlier would be an even more stringent measure of remote memory. We expected that participants who had putatively higher rates of AHN, those with lower BDI and PSS scores, would better overcome the interference generated by lure stimuli and, thus, outperform those with putatively lower rates of AHN, those with high BDI and PSS scores, at correctly identifying lure objects as "similar". In contrast, we did not expect that there would be any difference between groups in their ability to identify repetitions

as "old" or foils as "new", since these are relatively low interference items. As mentioned, in the current version of the BPS-O we asked some participants to return precisely two weeks following their first visit so they could complete another four blocks of the task. Importantly, each repetition was presented three times in total (once during study, once during test and once again during a subsequent block either on the same day or two weeks later), while each lure was only presented once (albeit as three highly similar images over three different trials). Thus, each presentation of a repetition item could have been introducing non-overlapping aspects to the memory (e.g. visual context) in those with reduced neurogenesis, while these aspects may have been cleared in those with higher neurogenesis. At the same time, overlapping features of the memory (e.g. the repetition itself) would be the only aspect strengthened in those with higher levels of AHN. Therefore, we posit that participants who score relatively high on the BDI or PSS will recognize fewer repetitions as "old" when viewed two weeks after learning by incorrectly mistaking them as similar or novel i.e. due to memory intrusions or inaccessibility of the original memory trace.

Since the length of each recognition trial is determined by the participant, we were able to eliminate those who were clearly not putting forth the effort required to perform the task. Specifically, we eliminated participants who scored more than two standard deviations below the mean on low-interference foil trials on either day 1 or day 2. Nine participants were eliminated based on this criterion. Consequently, there were 100 participants left in day 1 analyses (17 male, 83 female, average age 19.5, *SD* = 2.1) and 41 in day 2 (7 male, 34 female, average age 19.3, *SD* = 1.9).

In order to control for differences in pattern separation performance in the BPS-O that may have been due to factors unrelated to neurogenesis, such as deficits in global hippocampal processing, executive function or working memory, we implemented two control tasks: PAL and reverse digit span. PAL assesses the cued recall of pattern-location pairs. White squares are displayed on the computer screen and, one at a time, they momentarily disappear. One or more of them will contain an abstract pattern. The same patterns are then displayed in the center of the computer screen, one at a time, and the participant is asked to select the square where the pattern was originally located. If an error is made, then the pattern-location pairs are repeated. The task becomes increasingly difficult as pattern-location pairs grow from one in the first iteration to eight in the last. In reverse digit span participants are shown a series of numbers on the computer screen, and are subsequently they are required to type those numbers in reverse order. There are two numbers to remember at the beginning, with one more added every time that a criterion of correct responses is reached. The task ends following five trials, with eight numbers in each trial.

Since neither BDI nor PSS scores were normally distributed, we used Spearman's rho (r_s) for all correlative analyses. For all comparisons between groups scoring in the lower and upper ranges on the BDI and PSS questionnaires we used the student's *t*-test with equal variance assumed. For repeated measures comparisons within groups we used the paired version of Student's *t*-test. Since the data were re-analyzed in two different ways (BDI versus PSS) that highly correlate with one another, we opted not to correct for

multiple comparisons. For each statistical test a p value (two-tailed) ≤ 0.05 was considered significant.

Results

On the first day of testing, participants were highly accurate at correctly identifying foil images as "new" (92.3%) and repetitions as "old" (87.6%). In contrast, participants had greater difficulty identifying lure stimuli, correctly classifying only 45.5% of them as "similar", while incorrectly categorizing 45.5% of lures as "old". Interestingly, participants performed worse on repetition trials when they occurred in a separate, visually-distinct block from the target item, going from 87.6% (SD = 8.8%) correct within blocks to 78.5% (SD = 23.0%) across blocks ($t_{(99)} = 3.87$, $p < .001$, $d = 0.78$). On the other hand, consistent with our previous findings (Déry et al., 2013), participants were better able to identify lures as "similar" when they were shown in a different block and within a unique visual context compared to the original target (going from 45.5% [SD = 17.6%] within blocks to 53.8% [SD = 27.8%] correct across blocks; $t_{(99)} = 2.87$, $p = .005$, $d = 0.58$). At the same time, participants made fewer pattern completion errors on lure trials when they were tested across blocks (as opposed to within blocks), committing pattern completion errors on 45.5% (SD = 16.1%) of lure trials within blocks, to 32.5% (SD = 23.1%) across blocks ($t_{(99)} = 5.54$, $p < .001$, $d = 1.11$).

When lures were displayed against a visually unique background and two weeks had elapsed since the original target presentation (as opposed to being displayed on a different background, but on the same day) participants performed near chance levels (31.5% correct), misidentifying 24.1% of lures as "old". We also analyzed performance

on repetitions that were presented across a two week-long delay and found that, interestingly, participants performed about the same as they did on lure trials separated by two weeks – correctly identifying 35.8% of repetitions as "old".

Prior to analyzing pattern separation performance as a function of self-reported depression (BDI) or stress (PSS) scores we first corrected for any response bias among participants. Specifically, if a participant was biased towards selecting the "old" response option (regardless of trial type), then their proportion of correct responses to repetition trials would be inflated. Similarly, if a participant was biased towards selecting the "similar" response option (regardless of trial type), then their proportion of correct responses to lure trials would be exaggerated. Accordingly, we applied the following correction formulae to repetition and lure trials, respectively:

Formula 1. [*p*("Old"|Target) – *p*("Old"|Foil)]

Formula 2. [*p*("Similar"|Lure) – *p*("Similar"|Foil)]

where *p* = the proportion of responses. Next, we divided participants into two groups based on the median BDI score and, separately. On the basis of this median split, we created a "low" depression group (*n* = 50, mean BDI = 3.6, 0–7) and a "high" depression group (*n* = 50, mean BDI = 15.8, range 8–43) and compared them with an independent samples *t*-test. Similarly, we performed a median split on stress scores to create a "low" stress group (*n* = 52, mean PSS = 10.4, range 1–16) and a "high" stress group (*n* = 48, mean PSS = 22.9, range 17–35). "Low" and "high" BDI groups had significantly different depression scores ($t_{(98)}$ = 10.86, $p < .001$, d = 2.19), but did not differ in age or gender. Likewise, "low" and "high" PSS groups were significantly different with respect

to self-reported levels of stress ($t_{(98)}$ = 14.95, p < .001, d = 3.02), but not for age or gender.

We used independent samples *t*-tests to compare pattern separation performance between groups. Consistent with findings in previous studies (Déry et al., 2013; Shelton and Kirwan, 2013), there was a significant difference between "low" and "high" BDI groups in their ability to correctly identify lures as "similar" when presented in the same block as the first presentation ($t_{(98)}$ = 2.06, p = .04, d = 0.42), with the "low" BDI group outperforming the "high" BDI group (42.8% correct vs. 35.2% correct, respectively). Similarly, those with lower stress scores outperformed those with higher stress scores at correctly recognizing lures as "similar" when they appeared in the same block as the original target ($t_{(98)}$ = 2.17, p = .03, d = 0.44). Importantly, there were no differences between "low" and "high" BDI groups or between "low" and "high" PSS groups in their ability to detect foils or repetitions occurring within the same block as their first presentation, suggesting that subjective levels of depression and stress exclusively influence performance on lure trials.

In agreement with our previous findings (Déry et al., 2013), the extended delay (on the order of minutes) and the change in visual background improved pattern separation performance in both "low" and "high" BDI groups by 9% ($t_{(49)}$ = 2.10, p = .04, d = 0.60) and 13% ($t_{(49)}$ = 2.76, p < .01, d = 0.79), respectively. In contrast to our previous findings, there was no significant difference in pattern separation performance between "low" and "high" BDI groups when lure trials were presented in a different block (in the same day) and in a different visual context from the original targets ($t_{(98)}$ =

0.89, $p = .38$, $d = 0.18$). However, the slight difference in performance between groups was in the hypothesized direction, with the "low" BDI group performing marginally better than the "high" BDI group. There was also no difference in pattern separation performance across blocks between "low" and "high" PSS groups ($t_{(98)} = 1.34$, $p = .18$, $d = 0.27$). Thus, we were unable to replicate our previous findings, whereby the "low" BDI group outperformed the "high" BDI group at identifying lures displayed across on the same day (Déry et al., 2013). In fact, we found that performance on lure trials across blocks actually correlated negatively with the number of errors in CANTAB® PAL, whereby the more errors participants made in PAL, the worse they performed at appropriately classifying lures as "similar" when the target appeared in a different block as "similar" ($r_{(98)} = -.20$, $p = .04$, 95% CI [.02, −.39]). In contrast, there was no significant relationship between performance on CANTAB® PAL and behavioural pattern separation performance on lure trials presented in the same block as the original target or presented following a two week-long delay (both $p > .50$). These results suggest that identifying lures that follow the original target by a longer period of time, but on the same day, and in a unique visual context does not require AHN (at least to the same degree as lures appearing in the same block as the original target) but, rather, relies on the same sort of global hippocampal processing that paired associates learning does.

We also examined how a two week-long delay between study and test phases affected performance at detecting lures and repetitions. There was no difference between "low" and "high" BDI groups, or between "low" and "high" PSS groups at identifying lures across two weeks. Interestingly, the "low" BDI group outperformed the "high" BDI

group at identifying repetitions across a two week delay (Figure 3.1; $t_{(39)} = 2.25$, $p = .03$, $d = 0.72$). The same difference was found between "low" and "high" PSS groups ($t_{(39)} = 2.37$, $p = .02$, $d = 0.76$). In both cases it seems that those with higher depression and stress scores more often classified repetitions as "new" as opposed to "similar" (high BDI group: $t_{(19)} = 2.46$, $p = .02$, $d = 1.13$; high PSS group: $t_{(19)} = 2.63$, $p = .02$, $d = 1.21$). Moreover, the "high" BDI group incorrectly identified repetitions as "new" 47% of the time versus 34% of the time in the "low" BDI group (Figure 3.2; $t_{(39)} = 1.57$, $p = .13$, $d = 0.50$). Similarly, the "high" PSS group misclassified repetitions as "new" 49% of the time versus 32% in the "low" PSS group ($t_{(39)} = 2.16$, $p = .04$, $d = 0.69$). These data suggest that low BDI and PSS scores are associated with enhanced memory for repeated images of objects across longer delays, in addition to better behavioural pattern separation across shorter delays (Déry et al., 2013; Shelton and Kirwan, 2013).

Next, we analyzed the level of pattern separation (orthogonalization) versus pattern completion (overgeneralization) between groups. To do so, we subtracted the proportion of pattern completion errors from the proportion of correct responses to lure trials according to the following formula:

Formula 3. [(p("Similar"|Lure) − p("Similar"|Foil)) − (p("Old"|Lure) − p("Old"|Foil))]

Consistent with previous reports (Déry et al., 2013; Shelton and Kirwan, 2013), an independent-samples *t*-test comparing "low" and "high" BDI groups on this measure of separation versus completion, we found a trend towards those with lower BDI scores

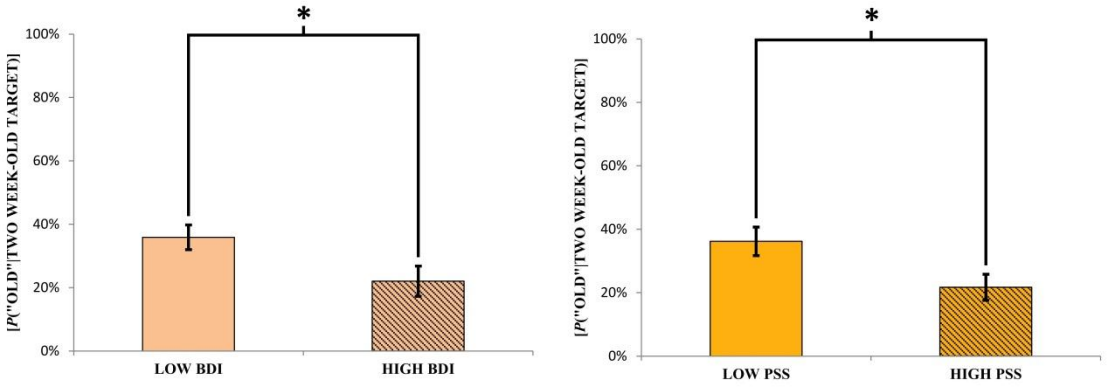

*Figure 3.1. Proportion correct responses to repetitions tested two weeks after presentation. (A) Participants were split into groups based on median Beck Depression Inventory-II score. (B) Participants were split into groups based on median Cohen's Perceived Stress Scale score. * $p \leq .05$.*

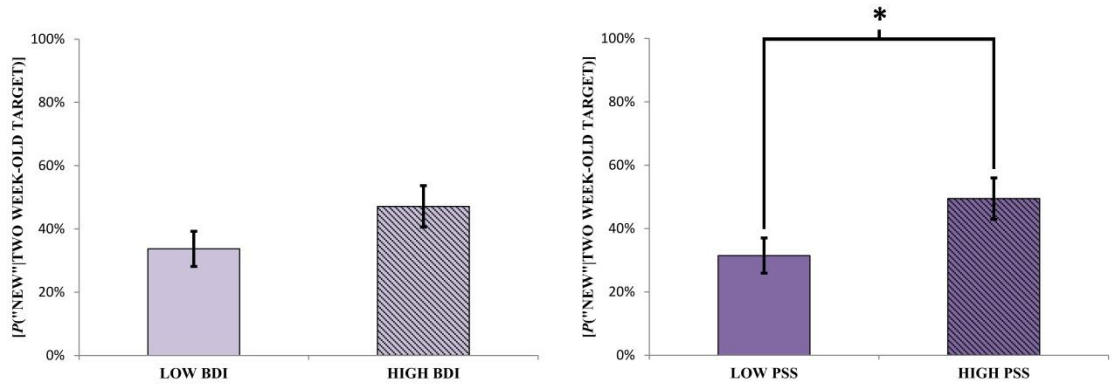

Figure 3.2. Proportion incorrect "new" responses to repetitions tested two weeks after presentation. (A) Participants were split into groups based on median Beck Depression Inventory-II score. (B) Participants were split into groups based on median Cohen's Perceived Stress Scale score.

being more biased towards pattern separation than those with higher depression scores ($t_{(98)}$ = 1.78, p = .08, d = 0.36). We found a similar result when comparing groups that were split via PSS score, with those experiencing lower levels of stress more biased towards pattern separation than those who self-reported having relatively higher levels of stress ($t_{(98)}$ = 1.61, p = .11, d = 0.33). When lures were presented across blocks, there was

no longer a significant difference in this measure of pattern separation versus pattern completion between "low" and "high" depression groups or between "low" and "high" stress groups. There were also no differences between BDI or PSS groups in bias towards orthogonalization versus overgeneralization on lures that were tested two weeks after the relevant study phase.

In addition to the median split analyses described above, we also performed correlational analyses using raw BDI and PSS scores as continuous measures of depression and stress, versus our measures of interest, pattern separation and remote memory. Since both BDI and PSS scores violated Shapiro–Wilks test for normality, we performed the non-parametric Spearman's rho (r_s) for all correlational analyses. We found a significant negative correlation between BDI scores and pattern separation performance within blocks ($r_{s(98)} = -.21$, $p = .04$, 95% CI [.00, −.39]). There was also a significant negative correlation between PSS scores and percent correct identifications of lures within blocks ($r_{s(98)} = -.26$, $p < .01$, 95% CI [−.07, −.43]). These data suggest that both subjective levels of depression and perceived stress affect pattern separation performance. Namely, the more depression-like symptoms or stress that individuals have recently experienced, the worse they are at orthogonalizing highly similar information.

In contrast to our previous findings (Déry et al, 2013), we did not find a significant correlation between depression scores and the proportion of correct responses to lure trials when they appeared in a distinct block but on the same day as first presentations ($r_{s(98)} = -.12$, $p = .28$, 95% CI [0.09, −.31]). Likewise, there was no correlation between PSS scores and behavioural pattern separation performance on the

lures that appeared in a different block from the original target ($r_{s(98)} = -.15$, $p = .15$, 95% CI [.05, −.33]).

We did not find any correlation between depression scores and percent correct identifications on lure trials tested two weeks following the appropriate study trials. Likewise, there was no relationship between stress scores and behavioural pattern separation performance when there was a two week gap between study and test trials.

We were also interested in how remote memory performance, the ability to identify a repetition as "old" following a two week-long delay, would correlate with depression and stress scores. We found a significant negative correlation between BDI scores and percent correct classifications of repetitions across two weeks ($r_{s(39)} = -.35$, $p = .03$, 95% CI [−.05, −.60]). We found a similar relationship between PSS scores and remote memory, whereby there was a significant negative correlation between stress and the ability to identify repetitions across a two week-long delay ($r_{s(39)} = -.41$, $p = .008$, 95% CI [−.13, −.63]). Therefore, it appears that the more depressed or stressed a participant is, the worse they are at recognizing repetitions when those objects are tested two weeks post-learning.

When repetitions and lures were presented within the same block, there was a marginal negative correlation between BDI scores and orthogonalization versus generalization scores ($r_{s(98)} = -.18$, $p = .07$, 95% CI [.01, −.36]). Moreover, there was a significant negative correlation between stress and pattern separation versus completion scores ($r_{s(98)} = -.22$, $p = .03$, 95% CI [−.02, −.40]). These data suggest that the more depressed or more stressed that someone is (i.e. those who presumably have lower levels

of AHN) the more biased they are towards overgeneralization, as opposed to pattern separation. On the other hand, when lures were presented in a different block that was visually distinct from the original target, there was no relationship between BDI or PSS and the pattern separation versus pattern completion score (both $p > .11$). We also investigated how the tendency to perform pattern separation versus pattern completion was affected by a two week-long delay between presentation and recognition. There was no correlation between BDI or PSS scores and the separation versus completion scores (both $p > .32$). Interestingly, when investigating the relationship between BDI scores and remote memory versus forgetting (i.e. p("Old"|Target) – p("New"|Target)) we found a marginal negative correlation ($r_{s(39)} = -.35$, $p = .07$, 95% CI [.03, −.55]), suggesting that the higher the depression score, the more likely the participant will be biased towards forgetting the original target. Whether or not the object is actually forgotten or merely not accessible remains to be determined and is certainly outside the scope of the present study. A significant negative relationship was found between PSS scores and this 'forgetting' index ($r_{s(39)} = -.42$, $p = .007$, 95% CI [−.14, −.62]).

Notably, there was no difference in performance between low and high BDI groups on CANTAB® PAL or in reverse digit span, suggesting that differences in performance on the BPS-O were not produced by global deficits in hippocampal processing or more general cognitive impairments. The same was found between low and high PSS groups, whereby there were no significant differences between groups in PAL or reverse digit span. There were also no significant correlations between BDI scores and

PAL or reverse digit span. Likewise, there were no significant correlations between PSS scores and performance on the CANTAB® PAL or reverse digit span tasks.

Discussion

In the present study, we found that lower stress and depression scores predicted improved visual object recognition memory, with less false recognition of lures as repetitions on same-day tests, and greater accuracy at recognizing old items as repetitions on two-week delayed retention tests. Interestingly, on two-week delayed retention tests, regardless of stress and depression levels, all participants were at near chance levels at correctly classifying lures, and were more likely to mistake them as being "new" rather than as "old". Thus, our results provide indirect evidence from human participants that AHN is important for pattern separation across shorter delays, while strengthening memory for individual items across longer delays.

According to the memory clearance hypothesis (Deisseroth et al., 2004; Frankland et al, 2013), in those with higher neurogenesis levels (here, lower stress and depression scores) memories should be cleared more rapidly from the hippocampus. At the same time, those memories might be consolidated in extra-hippocampal structures in a more schematized form. If successfully consolidated, the memories should be less detailed, and therefore more easily confused with similar incoming information, leading to inflated false memory rates for lures on the long-delay retention test relative to same-day tests. If on the other hand the memories were not consolidated, then they should simply be forgotten, resulting in poorer recognition of both old repetitions and similar lures on the delayed retention test. The findings of the present study were not consistent with either of

these predictions. We found that those with presumed higher neurogenesis levels (lower stress and depression scores) outperformed those with lower neurogenesis at long-term retention of repeated items, but this was not coupled with inflated rates of false memory for lures. Instead, regardless of stress and depression levels, participants tended to misclassify similar lures as new items. In fact, it was the groups who had higher stress and depression scores who more often misclassified repetitions across a two week gap as being "new" as opposed to misclassifying them as "similar", suggesting that their memories for the original targets were no longer accessible. Thus, in contrast to the memory clearance hypothesis, at least on the current task, it would seem that lower neurogenesis might actually lead to enhanced forgetting.

On the other hand, according to the memory retention hypothesis, higher neurogenesis favors long-term retention, leading to high-fidelity, long-lasting memories for items within the hippocampus. If this is the case, on long-term retention tests, repeated items should be more accurately recognized as "old", while lures should be more accurately recognized as "similar", in those with higher neurogenesis levels. These predictions were only partially confirmed by the findings of the present study. Those with lower stress and depression scores, hypothesized as having higher neurogenesis, recognized more old items across a two week gap. However, this same group was very poor at classifying lures as "similar" following a two-week delay. One reason for this could be that over time, the younger adult-generated neurons become increasingly dominated by inhibition, as they develop over a period of weeks from their young highly plastic stage to their final, less plastic form (Wang et al., 2000; Li et al., 2012). Thus, the

young neurons recruited at the time of encoding, in the course of a two-week delay, may have become less responsive, more sharply tuned, and less likely to be activated in response to similar items. Additionally, the change in internal and external context across the two-week delay may have made the lures seem much less similar to the original targets, explaining the increased likelihood of their being classified as "new". By the same token, the change in context could result in repetitions seeming less similar to the identical study items. This is consistent with the finding that, over time, patterns of activity elicited by even the same stimulus are never exactly the same (Freeman and Skarda, 1985). Thus, changing visual context and time across two weeks might be acting to further separate highly similar items (hence the high proportion of misidentifications of lures as "new"), while at the same time making repetitions (exact same items) less similar and requiring pattern separation for their correct identification as "old".

Pattern separation and memory persistence may not be unrelated processes. According to Competitive Trace Theory (CTT), higher pattern separation in the DG would lead to reduced interference in the hippocampus, but more interference for older memories that have become dependent on extra-hippocampal structures (Yassa and Reagh, 2013). Enhanced orthogonalization of events, resulting from a bias towards pattern separation as opposed to pattern completion, would result in a larger proportion of non-overlapping information introduced to the hippocampal network (Yassa and Reagh, 2013). In turn, the non-overlapping aspects of a previously stored memory would be subjected to enhanced interference in response to a similar event (Yassa and Reagh, 2013). Greater levels of interference being introduced to existing memories could be one

mechanism whereby older memories are altered or even cleared (Yassa and Reagh, 2013). At the same time, overlapping features between a new event (during encoding) and an existing memory would become strengthened (Yassa and Reagh, 2013). Such an account would explain why those with putatively higher rates of neurogenesis outperformed those with relatively lower rates of neurogenesis at identifying repeated items, but not lures, across two weeks. While those with lower stress and depression scores, hypothesized as having higher neurogenesis, would display superior behavioural pattern separation in the short term, there would be a higher proportion of non-overlapping information between the long-term memory for the target and the lure. On the other hand, those with higher neurogenesis would be able to weed out the interference generated by change in context between repeated items (e.g. background, time) and better consolidate the repeated image itself.

As overlapping aspects of a memory are reconsolidated, it is has been proposed that they become more schematized in nature. Therefore, it would follow that as memories for repeated items become less precise over time, you might expect there would be more "old"-"similar" confusions rather than a transition to confusing old items as "new". Future studies with various delays between study and test would be needed to see how incorrect selections of "similar" versus "new" on repetition trials changes over time.

AHN may enhance remote memory for repetitions in at least two other ways. First, it has been proposed that newborn neurons in the DG may represent the storage site of memories, thus the enhanced survival of adult-born neurons would result in the

enhanced preservation of memories. Indeed, while many adult-generated granule cells die off, many others persist for several months and might actually endure into old age (Eriksson et al., 1998; Dayer et al., 2003; Kempermann et al., 2003). Second, the exercise-induced upregulation of AHN has been shown to accelerate the consolidation process (Kitamura et al., 2009), reducing the vulnerability of memories to intrusions by shifting their dependence into more stable hippocampal-neocortical networks rather than remaining in less stable associative networks within the hippocampus. Although the underlying mechanisms remain unknown, our results are broadly consistent with the hypothesis that stress and depression are associated with a reduction in AHN, which in turn can lead to impaired pattern separation performance as well as remote memory.

The question of whether remote memory deficits in those with high stress and depression scores represent an inability to form new memories, an inability to retrieve older memories or a deterioration of previously learned memories remains open to investigation. Importantly, stress and depression scores showed no relationship with performance at identifying novel objects or repetitions within a given test day (within or across blocks), nor did they correlate with the number of errors committed in CANTAB® PAL or performance on reverse digit span. Thus, differences in performance between the low and high BDI or PSS groups on the BPS-O cannot be explained by generalized deficits in global hippocampal processing or by differences between groups in their working memory capacity.

In contrast to our previous work (Déry et al., 2013), we did not find a significant difference between low and high BDI groups with respect to their ability at identifying

lure items that were tested on the same day, but in a temporally- and visually-unique block from the original target. There are several reasons for why this might be the case. It would seem that the "high" BDI group in this study more strongly benefited from the change in context between target and lure than did the "high" BDI group in our earlier investigation (13% increase in performance versus 7%, respectively). Perhaps the change in temporal and visual context between targets and lures reduced interference to the point that AHN was no longer necessary for pattern separation processes to take place (or perhaps pattern separation was not required at all). Indeed, pattern separation has been reported in other regions besides the DG. Interestingly, in this study the ability to identify similar lures across blocks, though not correlating with depression or stress scores, did show a significant negative correlation with the number of errors committed in a known hippocampal-dependent task, CANTAB® PAL. Specifically, the more errors participants made on PAL, the worse they performed at identifying lures tested across blocks, suggesting that cross-block identification of lures could be based on long-term memory processes within the hippocampus that are not dependent upon the dentate gyrus, perhaps relying upon the associative pathways within the CA3 and CA1 regions. On the other hand, we replicated our previous finding that performance at identifying lures across blocks is superior to within-block performance, likely owing to the enhanced difference between target and lure afforded by the change in temporal and visual context (Déry et al., 2013). Together, our findings suggest that increasing the degree of change in temporal and/or visual context between the relevant study phase and lure trials allows more global (or neurogenesis-independent) hippocampal processing to assist in 'delayed' behavioural

pattern separation performance. In contrast, when study and test phases are presented closer together in time and within the same visual context, the greater interference makes performance more dependent upon pattern separation in the DG. Instead, pattern separation processes in extra-hippocampal regions or within the hippocampal network itself may be sufficient to orthogonalize similar images. Indeed, there is evidence to suggest that pattern separation is not unique to the DG. For instance, the direct pathway between EC and CA3 might be capable of contributing to pattern separation. It has been shown that CA3 cells, in the absence of input from the mossy fibre projection from dentate granule cells, are capable of differentially encoding unique spatial locations (McNaughton et al., 1989). When neurogenesis levels are low, the sparse firing of the DG, CA3 and CA1 regions may be able to compensate by performing some degree of pattern separation, but there will be an increased susceptibility to interference due to a lack of neuronal turnover. Lastly, it could also be the case that reducing the number of blocks tested in one day from eight to four reduced memory load and thus participants were better able to remember the targets. Fewer images presented between study and test phases would reduce the total amount of aberrant information experienced during the task and, hence, total interference.

Acute stress has an inverse U-shaped effect on performance on some tasks, with moderate stress levels providing a benefit, and high stress causing impairments. Although stress has, under some circumstances, been shown to enhance hippocampus-dependent cognition and related processes such as long-term potentiation and AHN (Kim and Diamond, 2002; Joëls et al., 2007; Kirby et al., 2013), we found that subjective levels of

stress at the time of testing were associated with impaired performance on putative neurogenesis-dependent recognition, with an increased false recognition of lures, and no benefit to traditional recognition of repeated items. Perhaps PSS scores represent more chronic, rather than transient, levels of stress occurring over the past few days to weeks, the cumulative effects of which impair performance. More physiologically-based, non-subjective measures of stress, such as salivary cortisol and alpha amylase, could more accurately reflect stress levels at the time of testing and thus might reflect a different relationship with hippocampus-dependent recognition than do PSS scores. Such a relationship remains to be investigated. However, we posit that this more biologically-based measure of stress would demonstrate a significant negative correlation with behavioural pattern separation performance.

Our results are largely consistent with a long line of previous research in both human participants and rodents. Older humans, who have reduced AHN compared to younger populations (Imayoshi et al., 2009; Knoth et al., 2010; Spalding et al., 2013) display deficits on a wide variety of tasks that likely require pattern separation (Toner et al., 2009; Stark et al., 2010; Yassa et al., 2011; Holden et al., 2012; Stark et al., 2013). As mentioned, our results also parallel those described by Déry et al (2013) and Shelton and Kirwan (2013), whereby higher scores on depression and stress inventories (the BDI and DASS) are associated with worse performance on behavioural pattern separation tasks. Much like the current study, the results reported in these studies are also correlational in nature since only lifestyle-based correlates of AHN were used, as opposed to any direct measurement. However, computational models have also

demonstrated that when CA3 neurons receive weak input from the DG (e.g. under circumstances of reduced AHN), they will respond more preferably and rapidly to direct input from the EC (Nolan et al., 2011). Direct input from the EC would bias the hippocampal network towards pattern completion by treating stimuli as familiar, rather than novel, and engaging in associate retrieval mechanisms via recurrent collaterals (Nolan et al., 2011). In addition, evidence from studies using rodents demonstrate that ablated AHN, either by genetic manipulation or by irradiation, results in severe spatial pattern separation deficits, particularly for highly similar spatial locations (Clelland et al., 2009, Guo et al., 2011; Pan et al., 2012a). On the other hand, aerobic exercise has been shown to increase AHN (Creer et al., 2010; Kohman et al., 2012) and leads to enhanced behavioural pattern separation performance. Likewise, genetic upregulation of neurogenesis also improves behavioural pattern separation (Sahay et al., 2011), suggesting that the exercise-induced enhancement of behavioural pattern separation is likely due to the positive effects of exercise on AHN, rather than the more pleiotropic benefits of physical activity. Further, behavioural testing in animals has demonstrated that AHN is associated with the persistence of remote memories for both place and context (Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009; Kitamura et al., 2009; Inokuchi, 2011; Pan et al., 2012a, 2012b, 2013). For example, rats that underwent irradiation prior to learning demonstrated normal acquisition on the Morris Water Maze, intact retention on one-week probe trials, a marked deficit at remembering the hidden platform location when tested two or four weeks post-training (Snyder et al., 2005). Despite these consistencies, our findings should nonetheless be considered preliminary

because depression and stress scores cannot be considered an accurate predictor of AHN. Further, our results are inherently noisy. However, it should be noted that remote memory performance in rats varied widely depending on the level of neurogenesis. For instance, Jessberger et al (2009) found that a slight (15%) depletion of hippocampal neurogenesis had no effect on recognition memory when tested three hours and one month post-training, whereas a higher degree of neurogenesis knockdown (85%) impaired memory retrieval on delayed retention tests. Thus, it would be expected that neurogenesis-dependent cognition in humans would demonstrate marked variability depending on the always fluctuating levels of AHN (due to influence from any combination of neurogenesis regulators). In addition, a method for measuring AHN in humans, at least non-invasively, remains to be discovered. At present, lifestyle-based correlates of AHN that have been reliably shown to regulate neurogenesis in immunohistochemical studies in rodents may nonetheless offer the best opportunity to indirectly measure rates of granule cell production and/or survival in humans. Our lab is currently exploring various physiological correlates that may provide improved predictive value compared to the subjective self-report questionnaires used here.

Future studies should be aimed at further investigating the hypothesis that AHN controls the level of pattern separation versus pattern completion in the DG, which may in turn contribute to the amount of overlapping versus non-overlapping information being coded or cleared in the hippocampus, respectively. One way to test this hypothesis in humans would be to develop a protocol similar to the one used here, except with many more trials occurring over a period of weeks to months. If those with putatively higher

rates of AHN (based on a variety of neurogenesis regulating lifestyle factors) are better at recognizing repetitions that were presented multiple times in multiple different contexts, then it would lend further support for the idea that AHN is important for maintaining remote memories that are subject to interference. Increasing the frequency of learning trials over successive days or weeks might also rescue performance in those with downregulated neurogenesis by increasing the opportunity for repetition items to be strengthened in memory and maintaining appropriate object-cue associations, thereby reducing the impact of a changing external environment on object recognition. Accordingly, the number of trials that participants are asked to complete may also affect the contrast in performance between those with putatively "low" versus those with putatively "high" levels of AHN. Such a study might elucidate a set of specific strategies that could be used to overcome deficits in long-term memory formation or retention in those with reduced neurogenesis and thus pattern separation. Interventions targeted at upregulating neurogenesis could also be used to improve behavioural pattern separation and remote memory performance. Indeed, we have previously demonstrated in sedentary but otherwise healthy young adults that taking part in a long-term aerobic exercise regime is sufficient to reverse the behavioural pattern separation deficits in those who had marginally lower fitness (assessed by VO_2*peak*) and marginally worse performance on the BPS-O at the onset of exercise (Déry et al., 2013). Thus, exercise may be one intervention that can be used to prevent the decline in behavioural pattern separation performance as humans age, as well as in younger adults who may also be at risk for downregulated AHN, such as those with stress-related psychiatric disorders.

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Postscript

The study described in Chapter 3 is the first to provide indirect evidence in humans that not only does neurogenesis contribute to pattern separation in the short-term, but also to recognition of repeated items in the long-term (over a period of two weeks). Specifically, both BDI and PSS score were negatively correlated with performance at identifying highly similar lures as well as with performance at identifying repetitions tested two weeks after the original targets were presented. More work is required before we can answer the questions that follow, such as: 1) how long does neurogenesis help you to remember old items before they are cleared; 2) how does varying the visual or environmental context change performance in those with relatively high or low rates of neurogenesis; or conversely 3) would remote memory for repetition items improve in those with relatively lower rates of neurogenesis if the visual context remained the same between the study and test phase?

Another outstanding question that follows from the study in Chapter 3 is: how come those with putatively higher rates of neurogenesis do not outperform those with lower rates when it comes to recognizing lure items tested two weeks post-learning? Perhaps the internal context provides a "time stamp" that helps to reduce interference among lures presented farther apart in time (Becker, 2005; Becker et al., 2009; Luu et al., 2012). If true, then those with lower neurogenesis could better discriminate between similar objects that are presented far apart in time versus discriminating between similar objects presented close together in time. This account is in line with previous theories stating that ongoing neural processes create an ever-changing internal (temporal) context

that becomes part of the memory (Manns et al., 2007; Polyn and Kahana, 2008), such that time itself could help separate events. The fact we changed the visual background between the study and test phase could have also helped those with lower rates of neurogenesis overcome interference between objects. Similarly, when rats with hippocampal lesions were tested in a different context, they were able to identifying similar odor pairs just as well as rats that were not lesioned, but were tested in the same context (Butterly et al., 2012). If the context becomes associated with the stimulus, then changing the context would provide a mechanism for separating events (Butterly et al., 2012).

Using a biologically plausible model of the whole hippocampus, Becker (2005) demonstrated that neuronal turnover benefits spaced learning trials when items are very similar and there is a high potential for interference. So, perhaps if we continued to show highly similar objects to participants over a series of learning trails, then we would see a divergence in performance among those with high neurogenesis versus those with low neurogenesis.

A final question that warrants further investigation is: what happens when neurogenesis is upregulated after learning has already taken place, but before testing has occurred? Behavioural work in rodents suggests that this would impair recognition (reviewed in Akers et al., 2014). This could be tested in humans by presenting them with a series of images to remember prior to starting an exercise intervention and then seeing how recognition of those items compares between those who underwent exercise versus those in a sedentary control group.

Despite all of the unanswered questions that follow, the study described in Chapter 3 provides an important first step in discerning the role for AHN in long-term memory. According to our results, it would seem that AHN is required for the persistence of memories. Future studies will be needed to confirm under what other circumstances neurogenesis aids long-term memory and under what circumstances neurogenesis may impair long-term memory.

Chapter Four

Preamble

Following up on the first study described in Chapter 2, we decided to run a larger cohort of healthy young adults through a long-term aerobic exercise training program. Again, we measured each participant's aerobic capacity and putative neurogenesis-dependent and -independent memory prior to and following the exercise program. In addition, we sampled peripheral blood at resting state from each participant pre- and post-exercise to see how the exercise regime changed the basal expression of various growth factors associated with AHN.

There is a tight coupling between neurogenesis and several growth factors including BDNF, IGF-1 and VEGF (reviewed in Cotman et al., 2007). Importantly, BDNF in serum may reflect levels of BDNF in the brain (Karege et al., 2002). It was estimated that 70-80% of neurotrophins found in peripheral blood is synthesized in the brain and transported into the periphery via the BBB (Rasmussen et al., 2009). It was found in a variety of different species that serum BDNF correlated with BDNF in the hippocampus (Klein et al., 2011). Further, there was a significant positive correlation between hippocampal IGF-1 and serum IGF-1 in both growth hormone-deficient mice and controls (Yan et al., 2011). The relationship between VEGF in blood and brain is less clear. Nonetheless, growth factors measured from peripheral blood may shed light on the level of growth factors in the brain and, thus, their influence on neurogenesis. Accordingly, with the intention of identifying several growth factors as potential

biomarkers for AHN in humans, we measured BDNF, IGF-1 and VEGF in blood and putative neurogenesis-dependent memory pre- and post-exercise.

Abstract

Our awareness of the cognitive benefits imparted by physical activity has been growing in recent decades. However, the physiological basis for such benefits remains poorly understood. Hippocampal neurogenesis, perhaps the most striking example of ongoing plasticity in the adult mammalian brain, is upregulated by aerobic exercise. Converging evidence from theoretical, electrophysiological, behavioural and imaging studies in both human and non-human animals suggest that adult-born neurons in the hippocampus have a functional role in pattern separation, the process of making similar information more distinct. As such, AHN is an ideal candidate mechanism for the study of cognitive changes following physical activity. We have previously found that six weeks of high intensity aerobic exercise leads to improved performance on a putatively neurogenesis-dependent visual pattern separation task. In this study, we investigated whether such an exercise training program would similarly affect established biomarkers of neurogenesis in humans and neuropsychological assays of mood in concert with improvements in cognition. Growth factors like brain-derived neurotrophic factor (BDNF), insulin-like growth factor type-1 (IGF-1) and vascular endothelial growth factor (VEGF) are, at least in part, responsible for the exercise-induced increase in AHN. When circulating IGF-1 or VEGF is blocked from entering the brain during bouts of physical activity, so too is the exercise-induced increase in AHN. A large body of evidence suggests that peripheral levels of these neurotrophins reflect levels measured in the

hippocampus. Here, we measured aerobic capacity, depression scores and peripheral BDNF, IGF-1 and VEGF in healthy young adults prior to and following six weeks of high-intensity interval training. We also assayed performance on several cognitive tasks, including a modified version of Kirwan and Stark's (2007) behavioural pattern separation task, pre- and post-exercise. The task involves the presentation of a series of images of objects, some of which are repetitions, and some of which are lures that are highly similar to images previously viewed. Lures are hypothesized to require pattern separation for their appropriate classification as "similar", as opposed to "old". Accordingly, we hypothesized that young adults who underwent chronic exercise training would demonstrate improvedVO_2*peak*, the gold-standard measurement of aerobic capacity, and that changes in VO_2*peak* and BDNF, IGF-1 and VEGF would predict lower depression scores and improved performance at correctly identifying lures as "similar". We found that fitness percentile was a significant positive predictor of lure trial accuracy, both at baseline and following six weeks of interval training. Change in depression scores pre- versus post-exercise also strongly predicted improvements on classification of lures. Finally, serum BDNF and change in serum IGF-1 post- minus pre-exercise were significant positive predictors of lure trial performance at baseline and following six weeks of physical activity, respectively. These data are consistent with the hypothesis that exercise-associated improvements in pattern separation are mediated, at least in part, by neurogenesis.

Introduction

The physical benefits of aerobic exercise are widely recognized. In contrast, the benefits of aerobic activity on the brain are relatively unknown. Although we know that exercise is generally good for brain health, the underlying mechanisms for such benefits remain elusive. One candidate mechanism underlying the cognitive benefits of exercise is the up-regulation of neurogenesis. Contrary to popular belief, we are not born with a finite number of neurons, which then die off as we age. Rather, the post-natal production of new neurons, adult neurogenesis, occurs in at least two regions of the healthy brain: the sub-ventricular zone (from where they migrate to the olfactory bulb) and the subgranular zone of the dentate gyrus (DG) in the hippocampus; the current paper focuses on the latter. Since their 'rediscovery' nearly 15 years ago (Eriksson et al., 1998), scientists have been attempting to answer the question: what are these new neurons good for? Given the well-accepted critical role of the hippocampus in episodic and spatial memory, it is natural to ask what if any contribution the new neurons make to these types of memory. Several different theories have been put forward as to the functional significance of neurogenesis. For example, it has been suggested that the addition of new neurons may serve to clear older memories (Feng et al., 2001; Deisseroth et al., 2004; Josselyn and Frankland, 2012; Frankland et al., 2013). On the other hand, it has been proposed that the integration of newborn neurons is critical for the formation of new memories, allowing the hippocampus to deal with the continuous input of novel information (Kempermann, 2002). More specifically, Becker (2005; Becker et al., 2009) showed through a computational model of the whole hippocampus that adult-generated

granule cells may facilitate the distinct representation of highly similar events, thereby protecting older memories from both proactive and retroactive interference. Adult-born neurons in the DG might help reduce interference between memories in a number of ways. For instance, newborn granule cells in the DG are transiently hyperexcitable (Schmidt-Hieber et al., 2004; Marin-Burgin et al., 2012) and are preferentially activated during excitatory stimulation. Immature neurons also more readily undergo long-term potentiation (LTP), and it is longer-lasting, compared to that of more mature neurons (Snyder et al., 2001; Ge et al., 2007). These properties suggest that the new neurons may be important for laying down new long-term memory traces. Indeed, Clelland and colleagues (2009) employed two different non-match-to-place tasks that had unique behavioural requirements, but tested the same psychological construct: spatial pattern separation. AHN was ablated by either focal irradiation (IRR) or inhibition of *Wnt* signalling specifically in the DG. IRR kills proliferating cells (e.g. Snyder et al., 2005), while *Wnt* signaling is critically involved in the generation of newborn neurons in the DG (Lie et al., 2005; Clelland et al., 2009; Jessberger et al., 2009). Accordingly, both IRR and inhibition of *Wnt* signalling significantly reduced AHN while inducing spatial discrimination deficits in both tasks (Clelland et al., 2009). Importantly, these cognitive deficits were only manifest on trials where stimuli were presented in close spatial proximity, consistent with a deficit in spatial pattern separation.

There are several lifestyle factors that can affect the proliferation and/or survival of newborn neurons in the hippocampus (reviewed in Kempermann, 2011). For instance, aerobic exercise has been shown to promote adult hippocampal neurogenesis (AHN) in a

variety of species (van Praag et al., 1999a, 1999b, 2005; Trejo et al., 2001; Cotman and Berchtold, 2002; Fabel et al., 2003, 2009; Eadie et al., 2005; Ernst et al., 2006; Kronenberg et al., 2006; Olson et al., 2006; Wojtowicz et al., 2008; Snyder et al., 2009; Creer et al., 2010; Josselyn and Frankland, 2012; Kohman et al., 2012; Winocur et al., 2012; Voss et al., 2013). In turn, upregulated neurogenesis may underlie some of the benefits of physical activity on mood and cognition. For example, aerobic exercise via wheel running improves spatial discrimination in mice, such as in the delayed non-match to place task using a touch screen apparatus (Creer et al., 2010). Behavioural pattern separation in rodents also improved when AHN was selectively increased by targeted genetic manipulation, suggesting that the exercise-induced improvement in pattern separation was likely due to changes in AHN, rather than the more pleiotropic benefits of physical activity (Sahay et al., 2011). On the other hand, chronic stress (e.g. social defeat, social isolation, restraint, electric shock, or a combination) has been shown to downregulate AHN in rodents (Malberg and Duman, 2003; Pham et al., 2003; Alonso et al., 2004; Heine et al., 2004; Joëls et al., 2004; Lee et al., 2006; Stranahan et al., 2006; Yap et al., 2006; Czéh et al., 2007; Oomen et al., 2007; Xu et al., 2007; Silva et al., 2008; Dagyte et al., 2009; Ferragud et al., 2010). In turn, downregulated neurogenesis via chronically elevated stress hormones may underlie some of the cognitive deficits observed in stress-related disorders such as major depression.

While neurogenesis cannot be measured directly and non-invasively in the human brain, non-human animal studies have provided great insight into the functional significance of AHN in memory and have narrowed the search for lifestyle-based

correlates of neurogenesis. We have previously used some of these lifestyle-based correlates of AHN as an indirect means of quantifying neurogenic activity in humans. Consistent with the findings in rodents, we found that sedentary but otherwise healthy young adults who took part in a controlled six week-long aerobic exercise training program exhibited fitness-related improvements in cognition. Specifically, those participants who exhibited the greatest post-exercise change in fitness (assessed by VO_2*peak*) exhibited significant improvement on a putative neurogenesis-dependent behavioural pattern separation task (Déry et al., 2013). In contrast, participants with higher depression scores, drawn from the same healthy, non-clinical population (i.e. those who scored above the group median on the Beck Depression Inventory-II), some of whom may be in a pre-clinical first episode of depression, performed significantly worse than those with relatively low BDI scores on the same task (Déry et al., 2013). Similarly, others have reported that depression scores as measured by the Depression Anxiety Stress Scale negatively correlated with behavioural pattern separation performance (Shelton and Kirwan, 2013). Further, young adults with moderate to severe depression scores (on the BDI-II) had selective deficits on the Cambridge Neuropsychological Test Automated Battery (CANTAB®) delayed match-to-sample task (DMS) at the longest delay, a test that requires rapid encoding and subsequent recognition of complex and unfamiliar visual patterns amongst highly similar lures (Becker et al., 2009). Importantly, these 'pre-clinically depressed' participants performed within the normal range on a battery of other memory and attention tasks that do not have a high interference component (Becker et al., 2009; Déry et al., 2013). While the evidence from these human studies of self-reported

stress and depression levels is merely correlational, it is consistent with the findings from the controlled exercise study mentioned above, and with evidence from non-human animal studies that implicate neurogenesis in high interference memory tasks. That being said, more physiologically-based markers of neurogenesis in combination with these lifestyle-based assessments could provide researchers with even greater confidence in making predictions about the level of AHN and associated cognition in humans.

Measuring AHN directly in the human brain has only been possible in post-mortem tissue assays (e.g. Eriksson et al., 1998; Boldrini et al., 2009, 2012; Knoth et al., 2010). However, several indirect or correlative methods for quantifying rates of AHN using magnetic resonance imaging (MRI) have recently been described (Manganas et al., 2007; Pereira et al., 2007). Proton magnetic resonance spectroscopy (MRS), a method frequently used to evaluate the concentration of various metabolites, has been suggested as one way to indirectly quantify neural progenitor cells in the living human (Manganas et al., 2007). Using MRS, Manganas and colleagues (2007) identified a peak at 1.28 ppm that was postulated to represent neural progenitor cells. Another way to indirectly assess changes in neurogenesis using MRI is by measuring blood volume, based on the well-established link between increased neurogenesis and angiogenesis, the growth of new vasculature (Palmer et al., 2000; Louissant and Sudha, 2002). Endothelial cells and neural progenitors divide together in a vascular niche, a microenvironment of capillaries that allows for the passage of nutrients and trophic support, essential for the proliferation, maintenance and survival of neural progenitor cells (Palmer et al., 2000; Yang et al., 2011). Aerobic exercise can lead to angiogenesis in the hippocampus (van Praag et al.,

2005), which can be accurately quantified by certain MRI perfusion techniques (Dennie et al., 1998; Sugahara et al., 1998; Aronen et al., 2000). Pereira and colleagues (2007) took advantage of this connection and were the first to use a gadolinium enhanced MRI perfusion technique to measure blood volume changes in the hippocampus of both mice and older humans following exercise. Increased blood volume was observed selectively in the DG/CA3 region (and not the CA1 or subiculum) in older adults who completed 12 weeks of aerobic exercise (Pereira et al., 2007). These participants also exhibited an exercise-related improvement on the Rey Auditory Verbal Learning Test. Importantly, in mice, exercise-induced increases in DG/CA3 blood volume correlated with increased hippocampal neurogenesis as confirmed in post-mortem assays (Pereira et al, 2007), validating the MRI measure as a potential non-invasive correlate of neurogenesis in humans (Pereira et al, 2007). We used a similar MRI perfusion method in a small pilot study of healthy young adults and found that change in VO_2*peak* following chronic exercise significantly correlated with change in blood volume in the right DG/CA3 (Déry et al., 2010). Moreover, these blood volume changes in the right DG/CA3 marginally correlated with post-exercise improvements on a behavioural pattern separation task (Déry et al., 2010). Unfortunately, we were unable to follow-up on this pilot study because the research-dedicated MRI scanner at McMaster University was shut down for an extended period of time. To extend the findings of Pereira et al and our pilot study, we therefore conducted the current study in which we assayed putative cognitive and peripheral biomarkers of AHN in circulating blood pre- and post-exercise.

Several growth factors including brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) are recognized as being primary mediators of AHN (Zigova et al., 1998; Aberg et al., 2000; Jin et al., 2002; Lee et al., 2002). BDNF and VEGF synthesis are fairly evenly distributed throughout central and peripheral locations, while IGF-1 is principally produced in the liver and released into the periphery (Balu and Lucki, 2009). Importantly, peripherally generated BDNF and IGF-1 can cross the blood-brain barrier (BBB) and impact plasticity in the brain (Podulso and Curran, 1996; Pan et al., 1998; Armstrong et al., 2000; Carro et al., 2000; Pan and Kastin, 2000; Yan et al., 2011). While evidence is mixed as to whether VEGF crosses the BBB (Fabel et al., 2003; Lopez-Lopez et al., 2004), it can nonetheless impact BBB permeability (Mayhan, 1999; Zhang et al., 2000) and promote the uptake of other neurotrophic factors. BDNF, IGF-1 and VEGF appear to be members of a common activity-dependent signalling cascade that work synergistically to regulate brain plasticity including AHN (Cotman et al., 2007; Llorens-Martin et al., 2008). All three neurotrophic factors have major high-affinity receptors in the hippocampus (Cotman et al., 2007). Genetically altered mice that display roughly half the normal levels of BDNF in the brain have reduced levels of proliferation and survival of adult-generated neurons at baseline (Lee et al., 2002). On the other hand, BDNF infusion into the hippocampus increases the number of newborn granule cells (Scharfman et al., 2005). Klein and colleagues (2011) demonstrated that there is a positive correlation between BDNF in blood and hippocampal BDNF in a variety of species. Thus, a wide range of evidence supports the hypothesis that BDNF sampled from peripheral blood reflects BNDF levels in the brain.

The close linkage between serum and brain BDNF levels and neurogenesis may be especially relevant in human depression. Serum BDNF is reduced in patients suffering from major depression (Sen et al., 2008), a disorder typically characterized by downregulated neurogenesis in humans with depression (Boldrini et al., 2009, 2012) and in animal models (Malberg and Duman, 2003; Pham et al., 2003; Heine et al., 2004; Joëls et al., 2004; Lee et al., 2006; Yap et al., 2006; Czéh et al., 2007; Oomen et al., 2007; Xu et al., 2007; Dagyte et al., 2009). In contrast, BDNF levels in peripheral blood (Sen et al., 2008; Brunoni et al., 2008; Cattaneo et al., 2010) and neurogenesis in the hippocampus (Malberg and Duman, 2003; Czéh et al., 2007; Oomen et al., 2007; Li et al., 2008; Santarelli et al., 2008) are normalized following antidepressant treatment. Similarly, post-mortem assays of humans with major depressive disorder reveal elevated neurogenesis levels in those who were treated with SSRIs relative to non-medicated individuals (Boldrini et al., 2009, 2012). BDNF mRNA synthesis from leukocytes is also lower in drug-free depressed patients and these levels, along with behaviour, are normalized following treatment with antidepressant pharmaceuticals (Karege et al., 2005; Lee and Kim, 2009). Importantly, in animal models, the behavioural effects of antidepressant treatment require BDNF binding on the TrkB receptors located on adult-born cells in the DG (Li et al., 2008) as well as intact neurogenesis (Santarelli et al., 2008). Thus, it is clear that peripheral BDNF may regulate, at least in part, the effects of antidepressant treatment (including aerobic exercise) on BDNF activity and synaptic plasticity in the brain as well as the behavioural recovery from depression (Cotman and Berchtold, 2002; Berchtold et al., 2005; Cotman et al., 2007; Christie et al., 2008). Alterations in BDNF

levels may also underlie changes in hippocampal volume observed in depression and ageing. The duration and severity of major depressive episodes is correlated with reduced hippocampal volumes (e.g. MacQueen et al., 2003; Campbell et al., 2004). Conversely, chronic exercise results in increased levels of BDNF in serum as well as larger hippocampal volumes in older adults (Eriksson et al., 2011).

Serum BDNF may also be a good predictor of exercise-induced cognitive enhancements. The interaction between baseline aerobic fitness and serum BDNF measured in healthy young adults was found to predict their performance on a recognition memory task that included a high interference component (Whiteman et al., 2014). Thus, BDNF seems to be an essential regulator of hippocampal function and plasticity, including neurogenesis, both at baseline and following physical activity. Since BDNF in the blood reflects levels of BDNF in the brain (Klein et al., 2007), peripherally measured BDNF may be a good predictor of AHN levels and associated memory function.

It is also important to consider the interactive effects of different neurotrophins acting in concert. Interestingly, the exercise-induced enhancement of BDNF expression in the hippocampus may be dependent on peripheral IGF-1 (Iwamoto et al., 1996; Carro et al., 2000; Trejo et al., 2001; Ding et al., 2006; Koopmans et al., 2006; Chen and Russo-Neustadt, 2007; Cotman et al., 2007). Basal levels of BDNF mRNA in the hippocampus are reduced (Koopmans et al., 2006) and the exercise-induced increase in BDNF protein in the hippocampus is abolished (Ding et al., 2006) upon infusion of an anti-IGF-1 antibody. In contrast, intracarotid infusion of IGF-1 enhances BDNF expression in the brain, similar to the way that exercise does (Iwamoto et al., 1996). Thus, IGF-1 acting on

BDNF may be one mechanism underlying plasticity following exercise (Cotman et al., 2007), as BDNF-TrkB signalling has been shown in numerous studies to play a direct role in long-term potentiation (Soulé et al., 2006). Just as is the case for BDNF, levels of IGF-1 in the brain may also depend on levels of IGF-1 in the periphery, at least during adolescence (Yan et al., 2011). 30 days of peripheral growth hormone (GH) replacement, which mediates the release of IGF-1 from the liver, increased hippocampal BDNF in GH-deficient mice (Yan et al., 2011). Importantly, Yan and colleagues (2011) found a significant correlation between serum IGF-1 and hippocampal IGF-1 in both GH-deficient and control mice. There is also a strong association between IGF-1 and neurogenesis. Serum IGF-1-deficient mice have downregulated AHN and display cognitive impairment, deficits which cannot be reversed by physical activity (Trejo et al., 2007, 2008). However, when serum IGF-1-deficient mice were administered IGF-1 subcutaneously, neurogenesis was restored to control levels and cognitive performance improved (Trejo et al., 2007, 2008). Peripheral infusion of antibodies for IGF-1 also reduces AHN and blocks the exercise-induced upregulation of adult-born granule cells in both mice and rats (Trejo et al., 2001; Llorens-Martin et al., 2010). On the other hand, peripheral or intracerebroventricular injection of IGF-1 increases both the proliferation and survival of adult-born granule cells in rats (Aberg et al., 2000, 2003; Lichtenwalner et al., 2001). Thus, it is reasonable to assume that IGF-1 released in the periphery following exercise may cross the BBB and, either directly or indirectly (by interfacing with BDNF), regulate levels of brain plasticity and AHN. Finally, converging evidence also implicates VEGF in regulating AHN. Injecting VEGF into the ventricles is sufficient to increase

AHN (Jin et al., 2002; Schanzer et al., 2004), while blocking peripheral VEGF entry into the brain prevents the exercise-induced proliferation of new neurons in the hippocampus (Fabel et al., 2003). However, blockade of peripheral VEGF had no effect on basal AHN (Fabel et al., 2003), suggesting that VEGF likely mediates the exercise-induced upregulation of AHN but does not regulate neurogenesis at baseline. Indeed, whereas IGF-1 and BDNF seem to mediate the effects of exercise on learning and recovery from depression, exercise-dependent enhancement of neurogenesis seems to be regulated by IGF-1 and VEGF (Cotman et al., 2007). Taken together, the above-mentioned studies suggest that assessing BDNF, IGF-1 and VEGF in the periphery following long-term aerobic exercise might provide an indirect measure of neurogenic activity in the hippocampus.

Although extensive work in non-human animals has implicated the exercise-induced enhancement of growth factors in hippocampal neurogenesis and improved learning and memory performance, few such studies have been conducted in humans (but see Déry et al., 2013; Whiteman et al., 2014). The rodent and human hippocampus are homologous in terms of anatomical sub-regions (Seress, 2007), and are essential for spatial and episodic memory in both humans and rodents (Winocur et al., 2010). Therefore, investigating growth factors and other putative biomarkers of neurogenesis in humans might be useful for gaining information about changes in AHN and associated cognition. In this study, we utilize a known lifestyle correlate of AHN from the animal literature, long-term aerobic exercise, hypothesized to stimulate AHN in the DG of healthy young adults. We measure aerobic fitness and peripheral biomarkers as well as

emotional questionnaires and a large battery of putative neurogenesis-dependent and -independent cognitive tasks pre- and post-exercise. We hypothesize that changes in fitness and putative biomarkers of neurogenesis (i.e. BDNF, IGF-1 and VEGF) found in peripheral blood will selectively predict improved performance on neurogenesis-dependent cognitive tasks that have a high interference component.

Methods

Participants

All aspects of our aerobic exercise training program were approved by the Hamilton Integrated Research Ethics Board (HIREB). A total of 37 participants were recruited (26 female and 11 male, mean age = 21.8, *SD* = 2.3; see table 1) from the McMaster University student population using ethics board approved advertisements that were posted across campus. The data obtained from 13 of the participants have been published elsewhere (Déry et al., 2013) and re-used here in combination with 24 additional participants that were recruited subsequently. Each participant underwent fitness testing, which consisted of treadmill running until peak oxygen uptake (VO_2peak) was obtained, blood draws and a battery of cognitive and mood tests prior to and following completion of our six week-long study. However, only 24 participants consented to providing blood samples and completing mood questionnaires. Fourteen of the 37 participants acted as non-exercising controls (10 female and 4 male, mean age = 21.2, *SD* = 2.6), while the remaining participants underwent 6 weeks of high intensity interval training (HIT; 16 female, *M* age = 21.9, *SD* = 2.1). All participants provided written informed consent and met the inclusion criteria for our study: a healthy body

mass index (≤ 25), a sedentary lifestyle (no more than one hour of physical activity per week), no known predispositions for cardiovascular, pulmonary, or metabolic disease, and no prior history of psychiatric illness.

Fitness testing

VO_2*peak* is the gold-standard measure of aerobic fitness and is obtained by continuously measuring oxygen uptake during an incremental ramp test to exhaustion. A baseline VO_2*peak* score was obtained from each participant during the week prior to study commencement. A second VO_2*peak* score was obtained from each participant in the week following study completion, six weeks later. In order to control for gender bias in aerobic capacity, we then transformed VO_2*peak* scores into fitness percentile scores as described by Whiteman et al (2014).

Exercise intervention

The high intensity interval training (HIT) program used in the present study was the same as that used in our previously published study (Déry et al, 2013). Briefly, exercisers ran on an indoor track three times per week for six weeks. Participants' heart rates were displayed on Timex Ironman® Road Trainer™ watches and monitored periodically throughout each training session by one of the experimenters to ensure that target heart rates were being achieved. Prior to each session, participants completed a 5 minute warm-up by running at a rate that allowed them to maintain 50% of their heart rate reserve (HRR), followed by sport-specific stretching in order to prevent injury. During a cool-down period following each HIT session, participants also completed a 5 minute jog at 50% of HRR and sport-specific stretching. Each running session was

separated from the last by at least 24 hours. In weeks 1 and 2, sessions lasted for 15 and 20 minutes, respectively, and the intensity remained at 65% of HRR for the entire duration. In weeks 3 to 6 we had runners start at 75% of HRR for 5 minutes, followed by alternating sprint and rest phases of 95% HRR and 50% HRR respectively. The number of sprints in each session started at three on the third week and increased by one each subsequent week.

Emotional questionnaires

We had a subset of participants complete the Beck Depression Inventory-II (BDI) because of the established negative relationship between depression scores and pattern separation performance (Déry et al., 2013, Shelton and Kirwan, 2013). The BDI is a self-report questionnaire that assesses each individual's subjective level of depression. Due to the tight association between stress and neurogenesis in animals (e.g. Malberg and Duman, 2003) we hypothesized that depression scores would provide additional predictive value in terms of pattern separation performance, reflecting levels of neurogenesis.

Cognitive testing

Putative neurogenesis-dependent cognitive task

The neurogenesis-dependent test used here was the same as that described by Déry et al (2013) and is a modified version of the behavioural pattern separation – object task (BPS-O) originally described by Kirwan and Stark (2007). In each session, participants completed four blocks (four in pre-testing and four in post-testing for a total of eight blocks). Each block included one presentation/study and one recognition/test

phase. Within the study phase, participants were shown images of everyday objects, one at a time for 2,500 ms with a 500 ms inter-trial interval. Immediately following each study phase participants completed a test phase, in which they were shown another series of images of everyday objects, including some repetitions, some similar lures, and some completely new foils, and they were asked to identify whether each image was "new", "old" (repetition of a previously viewed object) or "similar" (highly similar, but not identical, to an object previously seen). We considered pattern separation to occur when a participant correctly identified a lure as "similar" (i.e. "similar"|lure). On the other hand, a pattern completion error was said to have occurred when a lure was miscategorised as "old" (i.e. "old"|lure). Each block had a unique visual background. BPS-O results for both the "old" and "similar" trial types were first corrected for selection bias prior to analysis according to the following formulae: % correct on "old" trials = [*p*("Old"|Target) – *p*("Old"|Foil)], whereas; % correct on "similar" trials = [*p*("Similar"|Lure) – *p*("Similar"|Foil)].

Neurogenesis-independent cognitive tasks

As control tests, we selected three subtasks from the Cambridge Neuropsychological Test Automated Battery (CANTAB®): pattern recognition memory (PRM), spatial recognition memory (SRM), and paired associates learning (PAL). These controls tasks were selected because they are considered to be hippocampal-dependent, however, we hypothesized that they would be neurogenesis-independent because they lack a high-interference component. Moreover, we have previously shown that young adults with elevated stress and depression scores who showed a deficit on the CANTAB®

delayed match to sample (DMS) task performed normally on the same three CANTAB® control tasks used here, suggesting that DMS may be neurogenesis-dependent, but that PRM, SRM and PAL are not (Becker et al., 2009). In CANTAB® PRM task, in each study phase the participant is presented with a series of patterns, one at a time. Following each study phase, the participant is shown a series of image pairs, and asked which of the two patterns was previously seen. In SRM, in each study phase the participant is shown a white square that successively appears in different locations on the computer screen. Following each study phase they are shown a series of pairs of white squares in different spatial locations, and for each pair, they are asked to select the one that appears in a previously viewed location. Finally, in PAL, in each study phase participants are shown six white squares on the computer screen, and one at a time they view an object hidden under one or more of the squares. Then in each following test phase participants are presented with those same abstract images, one at a time in the center of the screen, and are instructed to select the white square which covered that particular image.

Blood and serum collection and processing

Blood samples were collected between 10:00 and 12:00 in order to control for diurnal fluctuations in BDNF and VEGF levels (e.g. Berchtold et al., 1999; Choi, Bhang and Ahn, 2011; Jensen and Cao, 2013). Peripheral whole blood was collected into two PAXgene™ Blood RNA tubes per participant and stored at room temperature for one day prior to further processing. Following the incubation period, RNA was purified according to the manufacturer's directions included with the PAXgene™ Blood RNA Kit (Qiagen, Mississauga, ON, Canada). RNA was eluted with 80 µl of elution buffer. In an effort to

digest any remaining genomic DNA, the RNA was further purified and DNase treated using an RNeasy MiniElute Cleanup Kit (Qiagen). The concentration and purity of each RNA sample was determined by absorbance at 260 and 280 nm using a Thermo-Fisher Scientific NanoDrop 2000c (Thermo-Fisher Scientific, Toronto, ON, Canada) or Multiskan GO UV/Vis microplate spectrophotometer and SKANIT: 3.2 software (Thermo-Fisher Scientific). Purified RNA samples had an A260/A280 that fell between 1.98 and 2.1. Purified samples were frozen at -80°C until required for reverse transcription.

To obtain serum samples, peripheral whole blood was collected in BD Vacutainer SST tubes (BD, Franklin Lakes, NJ, USA), immediately chilled on ice for one hour and then centrifuged at 3000 rpm (1500 g) for 15 minutes at 4°C. The supernatant was extracted immediately after centrifugation and aliquoted into cryovials before being placed in a freezer at -80°C, where they were stored until needed for further analysis.

Reverse Transcription

Two micrograms of each RNA sample was reverse transcribed in a 10 µl reaction using Superscript III (Life Technologies, Burlington, ON, Canada) following the manufacturer's protocol. Reverse transcription was conducted in the GeneAmp PCR system 2400 thermal cycler (Applied Biosystems, Streetsville, ON, Canada). Negative controls lacking reverse transcriptase were included to confirm lack of genomic DNA contamination.

RT-qPCR

PCR primers for BDNF were designed with PRIMER3 software (freeware program online: http://www.frodo.wi.mit.edu/; Rozen and Skaletsky, 2000; see table 1) and were synthesized in the MOBIX facility at McMaster University. The primer sequence for VEGF was obtained from Helena S. Isaksson and corresponds to those used in (Isaksson and Nilsson, 2006). For each PCR reaction, 300 nM of the forward and reverse primers were used. Each 20 µl real-time PCR reaction contained 10 µl of SYBR Green qPCR SuperMix UDG (Life Technologies), 30 nM of ROX reference dye (Life technologies) and either cDNA derived from 200 ng of RNA per sample (10 ng for β-actin) or standard dilutions. Standards for BDNF and VEGF were PCR products generated using target-specific primers. PCR products were gel purified using a Qiagen kit and quantified by spectrophotometric reading. Standards for β-actin were generated from a commercially-available plasmid (Life technologies). Real-time amplifications were carried out in duplicate using the MX3000P PCR system (Stratagene, La Jolla, CA, USA) using the following thermal profile: 2 minutes at 50°C, 2 minutes at 95°C, 40 cycles of 95°C for 30 seconds, 58°C for 30 seconds and 72°C for 45 seconds for both BDNF and VEGF. The thermal profile for β-actin was: 2 minutes at 50°C, 2 minutes at 95°C, 40 cycles at 95°C for 15 seconds, 58°C for 30 seconds, and 72°C for 30 seconds. Standard curve R values were above .995 and efficiencies were above 90%. Following the amplification cycles, a dissociation curve was added to check for unwanted secondary products.

Table 4.1. PCR primer sequences. A list of the PCR primer sequences used in this study.

Target and accession number	Forward primer	Reverse primer	PCR product bp
BDNF NM_001709	5'-AAA CAT CCG AGG ACA AGG TG-3'	5'-AGA AGA GGA GGC TCC AAA GG-3'	250
VEGF	5'-GCA CCC ATG GCA GAA GG-3'	5'-CTC GAT TGG ATG GCA GTA GCT-3'	90
B-actin NM_001101	5'-CTC TTC CAG CCT TCC TTC-3'	5'-TGT TGG CGT ACA GGT CTT-3	109

ELISA

Serum BDNF and IGF-1 protein were measured according to kit specifications. All samples and standards for each ELISA were run in duplicate. Absorbency of samples was 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). BDNF was measured using the human BDNF DuoSet ELISA kit (R&D Systems; Minneapolis, MN, USA; cat #DY248), while IGF-1 protein levels in serum were quantified using the human IGF-1 Quantikine® ELISA kit (R&D Systems, cat #DG100). Samples run through the BDNF ELISA were diluted 75x using Reagent Diluent (R&D Systems). The dilution factor for samples run through IGF-1 ELISAs was 100x, due to the necessary acid pre-treatment recommended in the manufacturer's protocol.

Statistical procedures

All statistical analyses were performed using IBM SPSS statistics version 21. For comparisons between exercisers and controls we used independent samples *t*-tests assuming equal variance, whereas for comparisons between pre- and post-exercise scores within groups we employed paired samples *t*-tests. Fitness scores were not normally

distributed. Therefore, comparisons between low and high fit groups (determined by median change in VO2peak) were carried out using the Mann-Whitney *U* test assuming equal variances. The Wilcoxon signed rank test was used to compare exercise and control groups to themselves post- minus pre-exercise. For each Mann-Whitney *U* test and Wilcoxon signed rank test, *p*-values were determined using Monte-Carlo simulations with 10,000 samples. We considered a two-tailed *p*-value $\leq .05$ to be significant.

For all regression analyses, hierarchical multiple regression was used. We used partial regression plots to check for non-linearity prior to proceeding with OLS regression to ensure a linear model would be the best fit. In all cases the relationships looked like they would be reasonably well fit using linear regression models. All continuous input variables were first centered to zero, except for fitness percentile measured at baseline, which was centered to the 50^{th} percentile for easier interpretability. All continuous variables were then standardized by dividing by two times their standard deviation so as to reduce multicollinearity between variables. We also checked our data for multicollinearity following each regression analysis by ensuring the variance inflation factor fell below a value of 3. Prior to regression analyses, data were tested for normality and log-transformed wherever normality was violated. Each regression model that included fitness percentile or hormones as one of the independent variables was corrected for the possible influence of age, gender and body mass index (BMI) by including them as covariates. Interactions between continuous variables were calculated by taking the product of two centered variables in order to reduce the potentially high correlations between them. Interaction effects were tested by including each independent variable and

their product in the regression model, such that the dependent variable was modeled by the formula:

$$D = b_1 X_1 + b_2 X_2 + b_3 X_1 X_2 + \varepsilon$$

where D is the dependent variable, X_1 is the first variable, X_2 is the second, X_1X_2 is their product, b_1, b_2, and b_3 are the beta coefficients for the first, second and interaction variables, respectively and ε is the error term.

Due to the smaller number of participants that provided blood samples and because there were random occurrences that affected collection or processing, such as phlebotomist or experimenter error, we imputed those values where they were considered missing completely at random. In all, 3 of 24 values were generated for serum protein measures (2 from the exercise group) and 4 of 24 values were generated for whole-blood mRNA measures (none from the exercise group). We included age, gender, BMI, fitness percentile and the measure to be imputed (BDNF, BDNF mRNA, IGF-1 or VEGF mRNA) into the imputation model. We decided to use these values because of their assumed connectedness and because Collins and colleagues (2001) suggest that when missing values represent a small proportion of the total sample size (i.e. less than 25%), variables correlated with the outcome of interest, rather than their missingness, are more impactful. The multiple imputation function in SPSS was used to automatically generate the missing data based on the constructed model. Only five iterations were performed because previous studies have demonstrated that anything greater than three to ten iterations are considered redundant (Rubin, 1987; Carpenter and Kenward, 2008). Once all imputed datasets were obtained, we combined them to produce one overall set of

estimates. Finally, we considered values above Cook's d using the conservative 4 / (n-k-1) (where n = observations, k = independent variables) cut-off to be highly influential cases. Accordingly, those values were eliminated from the analysis.

Results

Analyses of cognitive and bioindicators at baseline

At baseline, there was no difference in fitness percentile scores between the control and exercising groups [$t_{(35)} = 0.48$, $p = .64$, $d = 0.16$]. There was also no difference in baseline fitness between experimental females and control females [$t_{(24)} = 0.45$, $p = .66$, $d = 0.18$], nor was there a significant difference in fitness between experimental males and control males [$t_{(9)} = 0.24$, $p = .81$, $d = 0.16$] (see table 2). Given the lack of baseline gender differences, gender was not included as a factor in any subsequent analyses reported here.

Table 4.2. Participant fitness levels at baseline (Total N = 37). There were no significant differences in aerobic capacity between the experimental and control groups at baseline.

		N	Group range[a]	
			Female	Male
VO_2*peak*	Experimental	23	23.7–42.6	34.4–58.3
	Control	14	24.5–40.6	36.0–48.7
		N	Group range	
			Female	Male
Fitness percentile	Experimental	23	0.9–74.6	8.2–95.9
	Control	14	1.2–65.7	12.7–72.6

[a] VO_2*peak* summarized as range in mL/kg^{-1} min^{-1}

There were four participants who performed greater than two standard deviations below the mean on the easiest, low interference baseline condition of the BPS-O (i.e.

correctly identifying novel objects as "new") either at baseline or following the six week-long gap. Thus, their BPS-O data were excluded from subsequent analyses. Results from cognitive testing are summarized as mean percent correct ± standard deviation. As expected, most participants had near perfect accuracy when identifying novel images (91.8 ± 5.6) (see figure 1), and were also highly accurate at identifying repetitions (83.4 ± 11.2). On the other hand, lures were quite difficult to identify correctly as "similar", with participants making just as many correct selections as they did incorrect (49.9 ± 18.3). Importantly, there were no significant differences on the BPS-O between control participants and those destined for our long-term exercise intervention at baseline.

Accuracy was quite high on all three control tasks: CANTAB® PRM, SRM and PAL (see table 3). Performance did not differ significantly between control and experimental groups at baseline on PAL. However, the experimental group outperformed controls on PRM ($t_{(35)} = 2.07$, $p = .05$, $d = .70$) and SRM ($t_{(35)} = 2.97$, $p = .005$, $d = 1.00$) at baseline.

Table 4.3. Performance on neurogenesis-independent cognitive tasks at baseline (Total N = 37). Unexpectedly, there were significant differences in pattern recognition memory and spatial recognition memory between the experimental and control groups at baseline.

	Percent correct[a]		*p*-value
	Experimental	Control	
PRM	92.0 ± 8.8	85.8 ± 8.9	.05
SRM	83.9 ± 11.1	72.3 ± 10.9	.01
PAL	91.8 ± 6.6	90.4 ± 5.4	.50

[a] Summarized as mean ± SD

There was no difference between low fit (fitness percentile < 50th) and high fit (fitness percentile > 50th) individuals at baseline at identifying foils and repetitions, although the higher fit group marginally outperformed the less fit group at identifying highly similar lures [Mann-Whitney U-test: $U_{(23)} = 81$, $Z = 1.68$, $p = .10$]. Results from OLS regression adjusted for age, gender and BMI, with fitness as the predictor variable and performance on one of the BPS-O trial-types as the dependent variable demonstrated that fitness percentile was not a significant predictor of accuracy on novel items [$R^2 = .01$, $F_{(1,29)} = 0.51$, $p = .48$], a marginal negative predictor on repetitions [$R^2 = .11$, $F_{(1,29)} = 4.10$, $p = .06$], and a significant positive predictor on highly similar lures [$R^2 = .26$, $F_{(1,29)} = 10.81$, $p = 0.003$]. Thus fitness level as assessed by VO_2*peak* is a strong positive predictor of recognition that requires overcoming high levels of interference, and may reflect individual differences in neurogenesis levels at baseline. In contrast, there were no differences between low fit and high fit individuals in performance on CANTAB® PRM, SRM or PAL at baseline (all $p > .23$). Moreover, age-, gender- and BMI-corrected hierarchical multiple regression analyses indicated that fitness was not a significant predictor of performance on PRM, SRM or PAL (all $p > .20$). Thus, the baseline difference in performance between controls and exercisers on one of the control tasks, SRM, was not likely caused by a difference fitness level.

Serum BDNF protein was not a significant predictor of accuracy on novel image or repetition trials (both $p < .12$), however, it significantly predicted accuracy on the high interference lure trials [$R^2 = .30$, $F_{(1,15)} = 7.71$, $p = .01$]. BDNF mRNA was not a significant predictor of performance on foils, repetitions or lures (all $p > .39$). Thus,

although related, BDNF protein and BDNF mRNA might differ in their predictive power. Neither serum IGF-1 protein nor VEGF mRNA were significantly related to performance on the BPS-O, regardless of trial type (all $p > 0.08$). Taken together, these data indicate that serum BDNF levels are significantly related to baseline performance on BPS-O lure trials, but none of the other neurotrophin measures were significant predictors on any trial type in the BPS-O task at baseline. Serum BDNF protein was not a significant predictor of baseline accuracy on PRM or PAL (both $p = .12$), however, it significantly predicted performance on SRM [$R^2 = .12$, $F_{(1,19)} = 5.47$, $p = .03$]. BDNF mRNA was not a significant predictor of performance on PRM or SRM (both $p > .58$), but significantly predicted baseline performance on PAL [$R^2 = .32$, $F_{(1,16)} = 15.96$, $p = .001$]. Serum IGF-1 and VEGF mRNA were not significant predictors of performance on CANTAB® PRM or SRM (all $p > .18$), but both were marginal predictors of performance on PAL (both $p = .07$). Thus, BDNF protein was a significant positive predictor of baseline performance on SRM, while BDNF mRNA was a significant predictor (and serum IGF1 and VEGF-mRNA were marginal predictors) of baseline performance on PAL.

Given the significant relationship between baseline BDNF levels and classification of lures, we examined the interactive effect of fitness percentile and BDNF on BPS-O performance. Age-, gender- and BMI-corrected regression analyses with each of the BPS-O trial types (new, old and similar) as the dependent variable, with fitness percentile, serum BDNF and the interaction between the two as the independent variables, indicated no main effects of fitness or BDNF on any aspects of BPS-O performance, and no interactions. Overall, while baseline BDNF levels selectively predict

performance on the high interference trials in the BPS-O task, there is no evidence that this relationship is moderated by fitness levels. Regression analyses were also performed with fitness percentile, serum BDNF (or BDNF mRNA) and the interaction between fitness and serum BDNF (or BDNF mRNA) as independent variables and the CANTAB® control tasks as dependent variables. The only significant effect in these analyses was a positive relationship between BDNF mRNA and PAL accuracy [β = 1.22, t = 2.30, p = .04, 95% CI = .01, .27]. Thus, our data indicate that BDNF is a positive predictor of baseline cognitive performance on SRM and BDNF mRNA is a strong predictor of performance on PAL, but neither effect is mediated by baseline fitness.

Analyses of cognitive and bioindicators post-exercise

For the non-exercising control group, following six weeks of continuing their regular lifestyle habits, $V0_2peak$ scores remained fairly stable (see table 4), whereas for exercisers, after six weeks of intense physical activity there was a marked change in $V0_2peak$ scores. An independent samples *t*-test comparing the pre- minus post-study difference scores of exercisers to controls revealed that exercisers experienced a significantly greater change in percentile fitness than controls [$t_{(35)}$ = 2.96, p = .005, d = 1.00]. Similarly, paired samples *t*-tests within each group, comparing pre-study to post-study fitness, revealed that the fitness of the exercising group improved significantly during that span [$t_{(22)}$ = 4.89, p < .001, d = 2.09], whereas for controls, fitness levels at study completion were not significantly different from those at baseline [$t_{(13)}$ = 1.67, p = .12, d = 0.93].

Table 4.4. Change in participant fitness levels (Total N = 37). There was a marked change in aerobic capacity within the experimental group pre- versus post-exercise. On the other hand, the non-exercising control group did not undergo a marked change in aerobic capacity following six weeks of continuing their regular lifestyle habits.

		N	Mean change[a]	
			Female	Male
VO_2*peak*	Experimental	23	+17.0	+13.5
	Control	14	+4.2	+5.6
		N	Mean change	
			Female	Male
Fitness percentile	Experimental	23	+21.3	+23.3
	Control	14	+5.4	+8.4

[a]Mean change in VO_2*peak* summarized as mL/kg^{-1} min^{-1}

The control group's ability to identify novel images or lures did not significantly change over the course of our study (both $p > .14$). In contrast, exercisers' accuracy at identifying both novel foils [$t_{(21)} = 2.23$, $p = .04$, $d = 0.98$; see figure 1] and highly similar lures [$t_{(21)} = 2.56$, $p = .02$, $d = 1.12$] improved significantly pre- to post-exercise. Unexpectedly, both the controls [$t_{(10)} = 3.48$, $p = .01$, $d = 2.20$] and exercisers [$t_{(21)} = 2.53$, $p = .02$, $d = 1.10$] performed significantly worse on repetitions following the six week-long delay. There was no significant difference between groups in the extent to which their performance on repetitions worsened [$t_{(31)} = 1.69$, $p = .10$, $d = 0.61$], thus, both groups experienced a comparable pre- versus post-test decline in performance on repetitions. In contrast, it was only the exercisers whose accuracy on lure trials improved significantly from their pre-exercise scores (see figure 2). Therefore, our data suggest that physical activity specifically improves accuracy on highly similar lure items. Following six weeks, neither the controls nor the exercisers were more accurate on PRM, SRM or

PAL than they were at baseline (all $p > .11$). Thus, accuracy does not significantly improve on any of our hippocampal-dependent control tasks lacking a high level of interference.

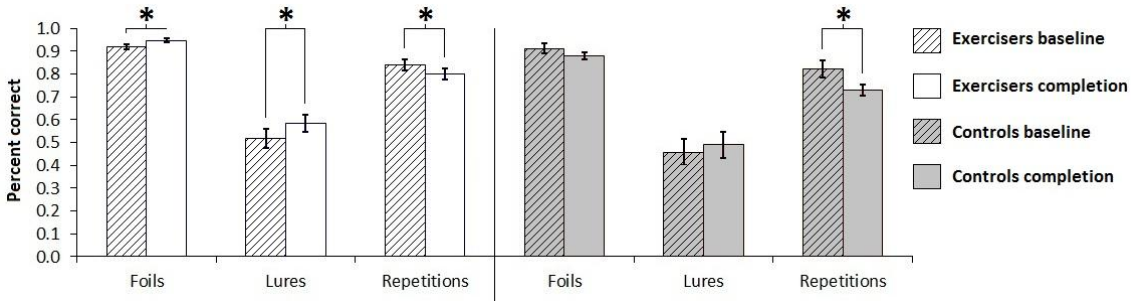

Figure 4.1. BPS-O performance pre- versus post-exercise. Comparing performance on the BPS-O at baseline versus study completion, and according to trial type (foil, lure, repetition), in both (A) exercisers and (B) non-exercising controls.

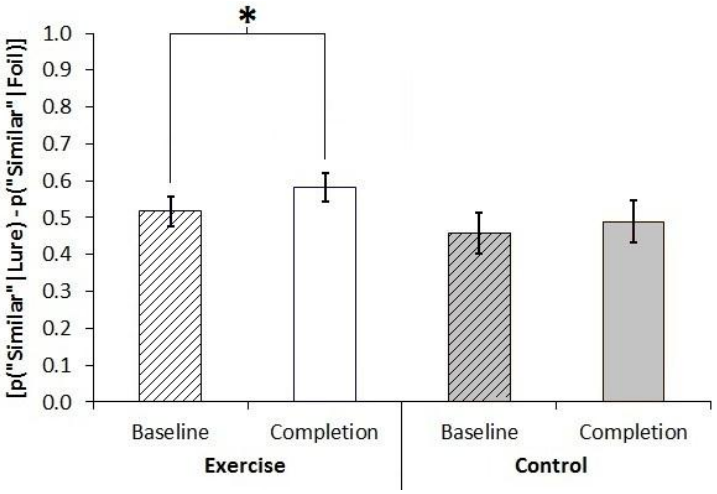

Figure 4.2. Accuracy on lures pre- versus post-exercise. Comparing performance on lure trials at baseline versus study completion in both (A) exercisers and (B) non-exercising controls.

Change in fitness percentile over the course of the six weeks was a significant predictor of change in performance on novel items [$R^2 = .21$, $F_{(1, 28)} = 9.72$, $p = .004$] and a marginal predictor of performance on lures [$R^2 = .11$, $F_{(1, 28)} = 3.56$, $p = .07$], but did not predict performance on repetitions [$R^2 = .001$, $F_{(1, 28)} = 0.02$, $p = .88$]. When looking at

exercisers only, their change in fitness post- minus pre-exercise was a significant positive predictor of change in performance on novel items [R^2 = .27, $F_{(1, 17)}$ = 7.18, p = .02]. Importantly, change in fitness percentile in exercisers was also a significant positive predictor of change in accuracy on lure trials [R^2 = .21, $F_{(1, 17)}$ = 4.72, p = .04], but did not predict performance on repetitions [R^2 = .001, $F_{(1, 17)}$ = 0.02, p = .88]. Thus change in aerobic fitness, assessed by VO_2*peak* and converted to fitness percentile, is not a significant predictor of change in traditional recognition (identifying old items), but it is a significant predictor of change in recognition of high interference items, indicating that it may reflect increased neurogenesis. However, it is unclear why the change in fitness would also predict change in performance on novel items. After six weeks between pre- and post-testing, participants' changes in accuracy on our three control tasks, PRM, SRM and PAL, were not significantly related to their change in fitness percentile, whether including both exercisers and controls, although the relationship between change in fitness percentile and change in SRM did approach significance [R^2 = .12, $F_{(1, 28)}$ = 3.84, p = .06]. However, there were no moderate or significant relationships between change in fitness percentile and change in performance on any of the CANTAB® tasks when looking only at exercisers (all p > .43). Thus, it would seem that change in aerobic fitness does not significantly predict change in cognitive performance on hippocampal-dependent tasks that lack a high interference component (although see discussion section).

In contrast to BDNF levels at baseline, the change in serum BDNF protein following six weeks was not a significant predictor of performance on lure items, novel

items or repetitions, whether combining exercisers and controls (all $p > .30$) or looking at the exercising group alone (all $p > .14$). The same was true of the changes in BDNF mRNA and VEGF mRNA (all $p > .12$). The change in serum IGF-1 protein following our six-week long study was not a significant predictor on novel or old items, either when combining exercisers and controls or when analyzing only the exercisers (all $p > .31$), but it significantly predicted the change in performance on highly similar lures, both when combining exercisers and controls [$R^2 = .34$, $F_{(1, 16)} = 8.67$, $p = .01$] and when looking at exercisers only [$R^2 = .62$, $F_{(1, 6)} = 14.71$, $p = .009$; see figure 3]. Thus, changes in serum IGF-1 following chronic exercise (or even sedentary behaviour) are associated with improved performance on the BPS-O, specifically at correctly identifying highly similar lures. There were no significant relationships between change in peripheral BDNF and change in performance on any of the control tasks, either in the combined groups or when excluding the controls (all $p > .09$). Similarly, the change in BDNF mRNA did not predict a change in accuracy on PRM, SRM or PAL (all $p > .31$), and when controls were excluded, it did not predict a change in PRM or SRM (both $p > .19$). However, when looking at exercisers only, BDNF mRNA was a significant positive predictor of performance on CANTAB® PAL [$R^2 = .37$, $F_{(1, 7)} = 9.37$, $p = .02$]. This was unexpected, but is consistent with the significant relationship we found between BDNF mRNA and PAL at baseline (see above). In contrast, there were no significant relationships between change in VEGF mRNA and change in accuracy on PRM, SRM or PAL, either when groups were combined (all $p > .27$) or when controls were excluded (all $p > .11$). There was also no relationship between the change in serum IGF-1 and change in accuracy on

PRM, SRM or PAL (all $p > .10$). The same was true for PRM and PAL when looking at exercisers only (both $p > .21$). However, in the exercising group, change in serum IGF-1 was a significant positive predictor of change in performance on SRM [$R^2 = .39$, $F_{(1, 6)} = 18.40$, $p = .01$]. In sum, BDNF mRNA positively predicted performance on the hippocampal-dependent PAL task both at baseline and following exercise, while a change serum IGF-1 positively predicted post-exercise improvements in performance on SRM.

Given the significant relationship between change in IGF-1 and change in BPS-O performance on lure trials, we were interested in whether this interaction was mediated by a change in fitness. Therefore, we modeled accuracy on the BPS-O separately for each trial type: new, old and similar, using changes in fitness percentile, serum IGF-1 and the interaction between the two as independent variables, while controlling for age, gender, and BMI. Results of the adjusted model indicated a main effect of change in IGF-1 [$\beta = .62$, $t = 3.41$, $p = .004$, 95% CI = .06, .28] and a strong positive interaction between the effects of change in fitness percentile and serum IGF-1 on the change in performance on lure items only [$\beta = .60$, $t = 3.55$, $p = .003$, 95% CI = .09, .36; overall model: $R^2 = .63$]. However, when we included only exercisers in the model, the change in IGF-1 only marginally predicted change in performance on lures [$\beta = .69$, $t = 2.34$, $p = .07$, 95% CI = -.02, .46], while the interaction between change in fitness and IGF-1 was not significant [$\beta = .54$, $t = 1.09$, $p = .33$, 95% CI = -.30, .74; overall model: $R^2 = .73$]. Nonetheless, these data suggest that change in the serum concentration of IGF-1 protein is a strong positive predictor of change in accuracy on the BPS-O, specifically on lure trials, and that this relationship is moderated by the effect of a change in fitness percentile. Follow-up

studies with additional participants would be required to further tease apart the separate effects and relationships amongst these variables. Given the significant relationships we observed between changes in BDNF mRNA and PAL as well as between IGF-1 and SRM, we assessed change in fitness as a possible modulator of these relationships. There were no significant main effects or interactions. The same was true when each model only included data obtained from exercisers. These results indicate that the observed relationships between changes in BDNF mRNA and PAL, as well as between changes in IGF-1 and SRM, are not modulated by exercise-induced changes in fitness.

When we included both exercisers and controls in regression analyses of mood scores versus cognitive performance, we did not find any significant relationships between change in BDI scores and change in performance on the BPS-O ($p > .12$ for all trial types). However, when we only included exercisers in the regression analyses, we found that change in BDI scores was a significant negative predictor of change in the BPS-O, specifically on lure trials [$R^2 = .63$, $F_{(1, 6)} = 15.25$, $p = .008$], but not on novel items or repetitions (both $p > .84$). Thus, the larger the drop in BDI score following exercise, indicative of lower depression levels, the greater the increase in performance on lure trials. In contrast, the change in BDI from study onset to study completion was not a significant predictor of change in performance on any of the three control tasks, CANTAB® PRM, SRM or PAL, either when exercisers and controls were combined (all $p > .55$) or when controls were excluded from the analysis (all $p > .11$).

The change in serum IGF-1 was a significant negative predictor of change in BDI score post- minus pre-six weeks, but only in the exercising group [$R^2 = .55$, $F_{(1,7)} = 9.99$,

p = .02], and not for the entire study sample [R^2 = .001, $F_{(1, 16)}$ = 0.03, p = .88]. Changes to the other neurotrophin measures were not significant predictors of change in mood scores. Thus, an increase in serum IGF-1 following long-term physical activity is associated with a reduction in subjective depression ratings. We then investigated whether the interaction between change in BDI score and IGF-1 could predict change in accuracy on lure trials. Results of the adjusted model indicated a main effect of change in IGF-1 [β = .83, t = 2.92, p = .03, 95% CI = .03, .50] on lure trial accuracy, but no significant interaction between change in BDI and IGF-1 (p > .87). To assess whether IGF-1 was a mediating factor in the influence of BDI on pattern separation performance, we analyzed a model that included the product of the betas for BDI predicting IGF-1 (-.40) and IGF-1 predicting lure accuracy (1.29) divided by the combined standard error (.23). This model was significant ($t_{(9)}$ = 2.24, p = .05), suggesting that the influence of changes in depression levels on changes in pattern separation performance following exercise is modulated by IGF-1.

Discussion

The current study was undertaken to elucidate relationships between aerobic capacity, mood, putative peripheral biomarkers (BDNF, IGF-1 and VEGF) and cognitive markers (BPS-O performance on lure trials) of AHN in healthy young adults both at baseline and following six weeks of high intensity aerobic exercise. We found that at baseline, both fitness levels and serum BDNF levels predicted behavioural pattern separation performance (lure trial accuracy in the BPS-O). Further, consistent with our previous findings (Déry et al., 2013), in those who underwent six weeks of physical

activity, change in aerobic capacity was significantly related to pattern separation performance. Moreover, in the exercisers, change in serum IGF-1 predicted post-exercise change in pattern separation performance. On the other hand, exercisers exhibited a negative relationship between change in depression scores and pattern separation performance, an effect that may be mediated by a change in IGF-1.

The improved accuracy on lure items post-exercise was expected and consistent with the findings of our previous study in a smaller sample of exercising participants (Déry et al., 2013). However, we did not expect that both exercisers' and controls' accuracy on repetitions would worsen following the six week-long delay. Although there was no overlap or obvious similarity between any of the images used at baseline compared to those used in the post-test, there may nonetheless have been some proactive interference from the pre-test at baseline. On the other hand, it could be that the older images were simply harder to remember in the post-exercise version of the task. Counterbalancing what version of the task is presented first might avoid this issue in the future. Nonetheless, since both groups experienced a significant decline in performance on repetitions, it seems unlikely that exercise was the cause. Alternatively, given the increased experience that participants would have with the task at post-test, they may have shifted their strategy or bias toward classifying uncertain items as "similar" rather than as "old". This would increase the correct classification rate of similar items while inflating the error rate for classification of repetitions. However, the data do not support this alternative interpretation. Neither group showed elevated error rates in calling old items "similar". In fact both groups had slight post-test increases in the probability of

incorrectly calling both old and similar items "new"; in the exercisers, this was coupled with a small but significant improvement in correct classification of novel foils as "new". This slight shift in bias after more experience with the BPS-O task makes sense, given that there was an equal proportion of new items to the combined number of old and similar items. Importantly, however, only the exercisers' accuracy of classifying lures as "similar" improved post-test.

Our data are consistent with other studies demonstrating a tight relationship between baseline fitness and cognition (Whiteman et al., 2014) and improved cognitive performance following physical activity in humans (Hillman et al., 2008; Klusmann et al., 2010; Erickson et al., 2011; Ruscheweyh et al., 2011; Déry et al., 2013). The reasons BDNF was a stronger predictor of performance at baseline, while IGF-1 was a stronger predictor of performance following long-term aerobic exercise remain to be determined. Whiteman and colleagues (2014) recently reported a strong interaction between fitness and BDNF predicting performance on a recognition memory test at baseline. Although we did not find the same fitness by BDNF interaction to be a significant predictor of cognitive performance, we did nonetheless find that both fitness and serum BDNF are strong individual predictors of recognition memory at baseline, specifically for memory tests that may require pattern separation. Since most of our participants were closer to the bottom of the fitness percentile range, it makes sense that we would not find the same non-linear interaction that Whiteman et al (2014) report, with an apparent inflection point at the 75^{th} fitness percentile. Another peculiarity is that at low fitness levels, Whiteman et al (2014) reported a negative relationship between resting BDNF and recognition

memory performance. In contrast, we found a significant positive relationship between resting BDNF and performance on the BPS-O, specifically on lure trials. Given that peripheral BDNF fluctuates with circadian rhythm (Berchtold et al., 1999; Choi, Bhang and Ahn, 2011), one possible reason for the discrepancies between our study and Whiteman's is the time at which blood was sampled (8:00 to 9:45 in Whiteman's study and 10:00 to 12:00 in ours). Another obvious methodological difference is that we used entirely different cognitive tasks. Although the BPS-O and the recognition memory test used in Whiteman et al (2014) share a high interference component, the tasks vary in multiple ways that may contribute to our discrepant findings. The version of the BPS-O we used has a passive study phase whereas Whiteman's had an active study phase – participants were required to select if each scene was new or old. Further, results from animal studies have demonstrated that varying the level of interference or memory load using even the same task can change its level of dependence on AHN. Ablation of neurogenesis has often resulted in the inability of rodents to form shock-context associations (Hernandez-Rabaza et al., 2009; Ko et al., 2009; Saxe et al., 2007; Warner-Schmidt et al., 2008; Winocur et al., 2006; Winocur et al., 2008). However, many others failed to find the same effect (Shors et al., 2002; Clark et al., 2008; Dupret et al., 2008; Pollak et al., 2008; Zhang et al., 2008). Shorter training intervals or lower shock intensities may make it harder for animals to separate study and test contexts, making it more likely that adult-born neurons are engaged in memory formation (Drew et al., 2010; Pan et al., 2013). On the other hand, when the study phase is prolonged or higher shock intensity administered, neurogenesis no longer seems to be required for the animal to

differentiate study-shock associations (Drew et al., 2010; Pan et al., 2013). Thus, standardization of cognitive tasks used to test the hypothetical role of adult neurogenesis in human memory should lead to more consistent results.

The strong positive relationship between change in IGF-1 and change in putative neurogenesis-dependent memory was expected given the established relationship between circulating IGF-1 and AHN (Aberg et al., 2006). For instance, peripheral administration of IGF-1 crosses the BBB and increases the number of neurons born in the adult DG (Reinhardt and Bondy, 1994; Aberg et al., 2000; Trejo et al., 2001). The release of liver-derived IGF-1 following exercise is required for the exercise-induced upregulation of neurogenesis (Carro et al., 2000; Trejo et al., 2001, 2008). Given this apparently tight relationship between circulating IGF-1 and neurogenesis, it is surprising that we did not find a relationship between serum IGF-1 and pattern separation performance at baseline. However, while IGF-1 can stimulate neurogenesis following peripheral administration of exogenous IGF-1 or following exercise, the basal rate of neurogenesis does not appear to depend on IGF-1. For instance, the injection of IGF-1 antibodies that specifically prevent IGF-1 from entering the brain abolishes the exercise-induced upregulation of AHN, but has no effect on the basal rate of neurogenesis (Trejo et al., 2001).

We found that the change in fitness by IGF-1 interaction was a significant positive predictor of performance on the BPS-O. Thus, it is likely that the relationship between IGF-1 and lure trial performance is dependent on change in fitness percentile. In contrast to IGF-1 and behavioural pattern separation, there was no interaction effect between

fitness and BDNF mRNA on PAL at baseline and following exercise. Thus, it would seem that the relationship between BDNF mRNA and PAL does not depend on physical activity and thus the change in AHN in response to long-term exercise. Our data lend support to the proposal by Cotman et al (2007) that the activity-induced expression of peripheral IGF-1 and VEGF cross or alter the BBB before affecting central levels of IGF-1, BDNF and VEGF. IGF-1, BDNF and VEGF in the brain could then enhance hippocampal plasticity and AHN (Cotman et al., 2007). Thus, according to Cotman et al (2007), while the expression of IGF-1, BDNF and VEGF would all be enhanced in the brain following exercise, it is principally IGF-1 and VEGF that are enhanced in the periphery. Further, it has been suggested that IGF-1 may represent the best candidate for an activity-sensing gene (reviewed in Trejo et al., 2002) and is perhaps the most important factor in stimulating central BDNF signalling and AHN in response to exercise (reviewed in Cotman et al., 2007). However, it should also be noted that the changes to IGF-1 in serum or plasma following exercise reported in the literature have been inconsistent. Some have shown an increase in peripheral IGF-1 following exercise (Schwarz et al., 1996; Alemany et al., 2008; De Palo et al., 2008), while others report no change (Stokes et al., 2005; Wahl et al., 2010, 2013) or even a decline (Koistinen et al., 1996). Discrepancies between studies may be attributable to a variety of factors including duration and intensity of exercise, delay between the last bout of exercise and blood sampling and differences between the demographics or fitness level of participants. Standardizing population characteristics and collection criteria to avoid these confounding factors in future studies will be critical for ensuring consistency between

studies and is ultimately necessary in order to verify IGF-1 (and other neurotrophins) as a peripheral biomarker of neurogenesis.

A potential limitation of the present study is that we are measuring basal levels of the neurotrophins, taken several days after the last exercising session. Although basal levels of serum BDNF protein, for instance, showed no significant change with the exercise intervention, this does not necessarily mean that BNDF was not released during each bout of physical activity before return to baseline values. Indeed, it has been shown that BDNF expression in serum is transiently increased following exercise (Tang et al., 2008). Thus, it is possible that peripheral BDNF and VEGF still play important roles in activity-induced brain plasticity and subsequent memory improvement, even though change in peripheral levels of BDNF and VEGF were not predictive of changes in putative neurogenesis-dependent cognition at the time scales measured in the current study.

An alternative interpretation of our findings is that the observed exercise-induced enhancement in cognition was due to factors other than or in addition to neurogenesis. Physical activity has been shown to enhance other forms of hippocampal plasticity, such as synaptic density and dendritic arborization (Dietrich et al., 2008; Lin et al., 2012; Stranahan et al., 2009). Prolonged exercise also increases capillary density in the hippocampus (Murugesan et al., 2011). Therefore, we cannot rule out the possibility that the relationship between change in IGF-1 and change in behavioural pattern separation performance observed here is at least partly attributable to these other forms of plasticity. However, given the theoretical role of neurogenesis in pattern separation and the

abundance of both animal and human data implicating the DG and neurogenesis in pattern separation processing, the preponderance of evidence implicates DG neurogenesis in the behavioural pattern separation improvements experienced in the exercising group. Besides memory enhancement, running has also been shown to reduce depressive-like symptoms in rodents (Duman et al., 2008) as well as having well-established anti-depressant effects in humans (e.g. Lawlor and Hopker, 2001). The antidepressant effects of aerobic exercise in rodents have been shown to rely on increased expression of neurotrophins (Li et al., 2008) and hippocampal neurogenesis (Bjornebekk et al., 2005; Olson et al., 2006; Yi et al., 2009; Brandt et al., 2010). These data suggest that prolonged physical activity, perhaps working through the activity-dependent regulation of neurotrophins, promotes AHN and behavioural recovery from depression. Similarly, we have shown that change in peripheral neurotrophins, notably IGF-1, following chronic exercise in healthy young adults positively predicts accuracy on a memory task that putatively requires AHN. Moreover, we found a negative relationship between subjective measures of depression and accuracy on the same task – a relationship seemingly mediated by the change in IGF-1 following physical activity. We have previously found in a larger group of young adults that BDI scores are negatively correlated with pattern separation performance at baseline, while change in aerobic capacity is positively correlated with pattern separation performance (Déry et al., 2013). Thus, the relationship between BDI scores and accuracy on lure trials in response to long-term physical activity is not surprising and is consistent with the hypothesis that accuracy on lure trials may

require pattern separation in the DG. Additional studies are required to determine if the effects of IGF-1 on mood and cognition are direct or indirect.

Unexpectedly, we also observed significant relationships between serum neurotrophin levels and performance on some of our control tasks. Serum BDNF protein was a significant positive predictor of performance on SRM at baseline. Moreover, change in serum IGF-1 protein positively predicted post-exercise change in SRM. Finally, BDNF mRNA predicted performance on PAL both at baseline and following long-term aerobic exercise. Given the widespread effects of BDNF on the brain and cognition, including dendritic spine density in CA1 (Tyler and Pozzo-Miller, 2001; Alonso et al., 2004), it was not entirely surprising that we found that BDNF mRNA predicted performance on one of our control tasks, PAL, both at baseline and following chronic exercise in sedentary, but otherwise healthy young adults. Interestingly, performance on CANTAB® PAL may be an early indicator of dementia, including Alzheimer's Disease (O'Connell et al., 2004). Moreover, Alzheimer's patients show post-mortem reductions in hippocampal BDNF mRNA (Phillips et al, 1991). Thus, BDNF mRNA may reflect more global hippocampal processing, rather than selectively reflecting AHN levels. Unexpectedly, we found that serum BDNF at baseline and the change in serum IGF-1 following chronic exercise were significant positive predictors of SRM at baseline and change in SRM performance following physical activity, respectively. We initially branded SRM as "neurogenesis-independent" because it is a well-known hippocampal-dependent task that lacks a high interference component. However, different trials do have different degrees of interference, as the two squares

shown during a given test trial are presented at locations that are varying distances apart. The higher level of spatial interference present in some of the SRM trials could be one reason why accuracy on the task demonstrated a significant positive correlation with performance on lure trials in BPS-O at baseline [$r_{(32)} = .57$, $p < .001$]. Future studies employing both visual and spatial pattern separation tasks (two tasks with different behavioural requirements but testing the same psychological construct – pattern separation) would be important for supporting the proposed role of AHN in behavioural pattern separation in humans.

A correlation between BDNF mRNA in peripheral whole-blood and serum BDNF protein has been reported at baseline and following antidepressant treatment (Karege et al., 2005). However, we did not observe a significant correlation between the two. Even though BDNF mRNA and protein are fundamentally related, it does not necessarily mean their concentrations would be proportional. BDNF is synthesized as a longer protein called proBDNF before being cleaved into mature BDNF by a variety of cleavage enzymes such as plasmin (Pang et al., 2004; Mizoguchi et al., 2011). While all BDNF mRNAs are translated into proBDNF (the precursor form of mature BDNF; not measured here), not all proBDNF is cleaved into mature BDNF (Barker, 2009). As such, a different ratio of BDNF mRNA to protein could result. Moreover, it remains to be determined how exactly exercise regulates BDNF gene transcription. One study demonstrated that exercise may selectively target promoter region IV, but not all promoter regions were assayed in this study (Gomez-Pinilla et al., 2011). All BDNF mRNAs coded from the various BDNF transcripts are translated into identical proteins, but each of these proteins

has different targets and, therefore, can influence plasticity in a variety of regions and in a variety of ways (Pruunsild et al., 2007; reviewed in Tongiorgi, 2008). It is also feasible that BDNF mRNA and protein found in peripheral blood may have different targets in the brain and thus may exert different effects on plasticity. If this were true, it might in part explain our discordant findings, such that BDNF mRNA and protein predict different aspects of cognition.

Another way to investigate the potential pattern separation function of the DG in humans is via functional neuroimaging. An fMRI study found that activity in the hippocampus, specifically in the DG/CA3 region, changes according to whether participants performing a BPS-O task correctly identified an old item as "old", a similar item as "similar" or falsely identified a similar item as "old" (Kirwan and Stark, 2007), suggesting that the hippocampus plays a role in distinguishing between old and similar information. Moreover, the DG/CA3 subregion is equally strongly activated by both novel and highly similar items (Bakker et al., 2008; Lacy et al., 2011), suggesting that the DG subregion much more strongly treats similar items as novel relative to the CA1 or subiculum. Moreover, when images of objects were parametrically varied in similarity via rotation, while participants' behavioural pattern separation performance increased with the degree of rotation, their hippocampal response functions were similar regardless of rotation, suggesting that pattern separation is the default operation of the hippocampus (Motley and Kirwan, 2012). Although not specific enough to measure activity at the cellular level, the results from studies such as these provide converging evidence that the

human hippocampus, and specifically the DG/CA3 region, may play a critical role in distinguishing between old and similar information.

Given the results presented here, it is tempting to conclude that circulating growth factors mediate the effects of exercise on AHN and relevant cognition. However, there is still not enough evidence to make any definitive conclusions. For instance, there are mixed findings with regards to change in serum BDNF following exercise. While some have reported that the brain may be the principal source of the exercise-induced increase in peripheral BDNF, others have shown that chronic stress impaired spatial memory and reduced hippocampal BDNF as well as AHN, but did not affect circulating BDNF (Yau et al., 2014). Therefore, although inter-related, circulating growth factors do not always share a clear relationship with central growth factors and/or AHN. Future multimodal studies are needed, combining measures of putative peripheral biomarkers of neurogenesis (such as BDNF, IGF-1 and VEGF) with MRI-based measures of brain plasticity in order to better characterize the relationship between peripheral hormones, angiogenic changes in the neurogenic niche or perforant path integrity and putative neurogenesis-dependent memory performance. Although the specifics have yet to be determined, results from a number of human studies, including those reported here, implicate neurotrophins such as BDNF and IGF-1 as potential biomarkers for memory performance, even in those who are healthy (Whiteman et al., 2014).

Rene Hen described adult neurogenesis as the fulcrum on a teeter-totter, controlling the balance between pattern separation and pattern completion (Hen, 2013). According to this view, our results suggest that increasing neurogenesis via exercise can

adjust the fulcrum in such a way that pattern separation dominates processing. Further, we have shown in humans that one mechanism underlying the ability of exercise to improve behavioural pattern separation is the activity-dependent release of IGF-1 in the periphery. Animal studies have shown that IGF-1 generated in the periphery may cross the BBB and bind to its receptors in the hippocampus to initiate plasticity directly (Reinhardt and Bondy, 1994; Aberg et al., 2000; Carro et al., 2000; Trejo et al., 2001, 2008), and also indirectly by regulating additional neurotrophins such as BDNF and VEGF (reviewed in Cotman et al., 2007). Our study has major implications for stress-related psychiatric disorders where neurogenesis may be reduced, such as major depression. It has already been demonstrated that BDNF is reduced in serum during a depressive episode and rebounds following treatment with various types of antidepressants (Sen et al., 2008). Further, exercise may exert its antidepressant potential by regulating the release of IGF-1, which in turn may act on BDNF in the brain to enhance neurogenesis – switching off the negative processing bias and allowing depressed individuals to ascribe the appropriate context to various events, thereby promoting recovery (Becker and Wojtowicz, 2007). Thus, increasing neurogenesis via exercise holds tremendous promise as a relatively side effect-free way of ameliorating cognitive and mood deficits in healthy aging and in psychiatric illness. Such promise underscores the need to find neurobiological and cognitive markers of neurogenesis in humans, the primary goal of the research described here.

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Postscript

The study described in Chapter 4 is the first to measure both an array of putative biomarkers and a cognitive correlate of AHN, pattern separation, in humans. The data obtained in Chapter 4 lend indirect support to the idea that IGF-1 is the principle activity-dependent regulator of AHN, providing a body to brain signaling mechanism for future adaptation to the present physical and cognitive demand (Llorens-Martin et al., 2008). Specifically, we demonstrated that change in serum IGF-1 protein was a significant positive predictor of change in accuracy on a memory task that taxes pattern separation processing in the DG – one of the proposed functions of adult-born granule cells. These data also provide support for the idea that AHN is required for overcoming interference. Further, the data outlined in Chapter 4 provide evidence for one of the underlying mechanisms for the exercise-induced increase in cognition, an activity-dependent increase of AHN, and demonstrate that exercise can improve behavioural pattern separation performance in humans. However, peripheral IGF-1 influences brain plasticity in a number of ways beyond its actions on AHN. For instance, IGF-1 is responsible for the running-induced increase in spine density on CA1 pyramidal neurons in mice (Glasper et al., 2010). Therefore, we cannot rule out the possibility that changes in behavioural pattern separation performance are not exclusively due to enhanced neurogenesis. However, given the relative specificity of the cognitive enhancements observed across our battery of cognitive tasks (i.e. only the high interference tasks hypothesized to require neurogenesis were the ones that benefited from exercise and

specifically the running-induced increase in IGF-1), we can conclude, with some confidence, that AHN underlies these benefits.

It has been suggested that IGF-1 may be preferentially increased in response to resistance training, as opposed to endurance training (Vale et al., 2009). In the study outlined in Chapter 4, we use a high-intensity interval training program, which has been shown to induce skeletal muscle and cardiovascular adaptations at low volume (Gibala et al., 2012). Therefore, high-intensity training may provoke a greater activity-dependent release of growth factors in the periphery and, hence, neurogenesis centrally than would traditional endurance training. Further, physical activity and demand of information processing might improve neurogenesis to a greater extent than exercise alone. Follow-up studies would need to elucidate what types of exercise (e.g. frequency, duration, intensity) provide the best intervention for improving putative neurogenesis-dependent memory. Another interesting follow-up study could incorporate a pattern separation training program that is given concurrently with physical exercise, such that study groups include: an exercise group; a cognitive training group, an exercise combined with cognitive training group and a no exercise, no cognitive training control group. In this way, similar to rodent studies, we could tease apart the effect of exercise versus enrichment on neurogenesis-dependent cognition and see whether or not there is a synergistic relationship between the two.

Our results are consistent with a number of human studies showing a positive correlation between serum IGF-1 and mental ability (Rollero et al. 1998; Aleman et al. 1999; Kalmijn et al. 2000; Dik et al. 2003; Arwert et al. 2005; Okereke et al. 2007). Our

results are also consistent with others who show that there are no inter-individual changes in basal levels of BDNF, IGF-1 or VEGF following exercise, but who do show a correlation between change in these growth factors and change in hippocampal volume, cognitive performance or functional coupling between the bilateral temporal gyrus (Erickson et al., 2011; Voss et al., 2013). Cotman and colleagues (2007) suggest that while BDNF and IGF-1 seem to mediate the effects of physical activity on learning and memory, the exercise-induced upregulation of neurogenesis seems to rely more heavily on IGF-1 and VEGF. Indeed, we have shown that BDNF is a predictor of several cognitive domains at baseline, including behavioural pattern separation performance, while change in IGF-1 following exercise is a significant and specific predictor of change in behavioural pattern separation – a putative indicator of neurogenesis in the brain.

General Discussion and Closing Thoughts

Self-renewing stem-like cells in the adult hippocampus have captured the imagination of neuroscientists and clinicians for decades. Unfortunately, there have been relatively few studies investigating the functional role of AHN in humans. Throughout this thesis, I have described a number of studies in which we undertook the challenge of identifying indirect correlates of AHN in humans as well as elucidating the functional role of adult-born granule cells in everyday memory. We accomplished this by assessing various lifestyle- and blood-based factors known to influence neurogenesis from the animal literature and comparing these factors to behavioural performance on tasks which tested the proposed roles for AHN in learning and memory.

5.1 Functional role of neurogenesis in humans (Chapters 2, 3)

There has been much speculation regarding the functional role of neurogenesis in humans. Computational modellers and theorists have proposed several distinct roles for AHN in cognition based on what is known of their location in the brain, functional connectivity with surrounding regions and physiological properties. Some have proposed that the constant turnover of newborn cells in the hippocampus would allow memory storage for novel events, while avoiding interference with older memories, a computational process termed pattern separation (Becker et al., 2005; Chambers and Conroy, 2007; Appleby and Wiskott, 2009; Becker et al., 2009; Weisz and Argibay, 2009; Aimone and Gage, 2011). However, events occurring close together in time may be

subject to enhanced interference because the same population of cells would be firing in response to each event encountered. This process of increasing interference between events occurring close together in time is referred to as pattern integration (Aimone et al., 2006). Indeed, some studies have shown paradoxical improvements in working memory tasks following ablation of neurogenesis (Saxe et al., 2007). However, working memory circuits outside of the hippocampus may be responsible for such improvements. Across short timescales, the majority of behavioural evidence from rodents has actually demonstrated that the role for neurogenesis in cognition, although seemingly varied across several tasks, converges on one function in particular. The formation of context-shock associations is impaired in animals lacking neurogenesis (Saxe et al., 2006; Winocur et al., 2006; Imayoshi et al., 2008; Warner-Schmidt et al., 2008; Wojtowicz et al., 2008; Hernandez-Rabaza et al., 2009; Ko et al., 2009; Guo et al., 2011; Nakashiba et al., 2012; Pan et al., 2012b), especially when the shock is relatively weak or training paradigm relatively short (Drew et al., 2010; Pan et al., 2012a, 2013). Animals lacking neurogenesis are also impaired at discriminating between overlapping odor pairs (Luu et al., 2012) or between nearby, but not far apart spatial locations (Clelland et al., 2009). In contrast, upregulating neurogenesis via aerobic exercise or genetic manipulation has been shown to increase AHN and leads to enhanced behavioural pattern separation or CFC performance (Creer et al., 2010; Sahay et al., 2011; Kohman et al., 2012). While it seems like a wide variety of tasks require adult-born granule cells, many, if not all of these tasks require overcoming interference. All of these tasks require the animal to form separate representations of similar stimuli, regardless of whether the stimuli are different contexts,

objects, spatial locations or odours. This is why I say that AHN is required for a widely varied, yet specific set of memory tasks. The behavioural requirements of tasks shown to depend on neurogenesis have differed substantially, but the psychological construct shown to rely on AHN has been fairly consistent.

Neurogenesis may further help separate similar events occurring over longer time periods (Becker, 2005; Aimone et al., 2006; Becker and Wojtowicz, 2007). A distinct pool of newborn neurons would help to add a degree of contextual novelty to similar events that are separated by a sufficient amount of time. Without new cells being added to the hippocampal network, the same populations of cells would end up representing multiple different memories, leading to catastrophic interference (Wiskott et al., 2006). This account of the role for neurogenesis in learning and memory has generally been referred to as the memory retention hypothesis throughout this thesis. In contrast, others have proposed that the addition of newborn cells to the hippocampus would result in existing connections being altered in such a way that information is lost (Feng et al., 2001; Deisseroth et al., 2004; Frankland et al., 2013). This account of the role for neurogenesis in learning and memory has generally been referred to as the memory clearance hypothesis throughout this thesis. Behavioural evidence from non-human animal studies has supported the memory retention hypothesis, especially for spatial or context-rich memories. For instance, rodents with ablated neurogenesis display marked deficits in remembering the platform location following MWM training across long, but not short timescales (Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009; Kitamura et al., 2009; Inokuchi, 2011; Pan et al., 2012a, 2012b, 2013). In contrast, some

studies have shown impaired long-term retention of fear memories in younger mice with relatively high rates of neurogenesis compared to their older counterparts (Akers et al., 2012). When older mice had wheel-running- or antidepressant drug-induced enhancement of neurogenesis, they were impaired on tests of remote memory compared to control mice (Akers et al., 2014). Therefore, it would seem that behavioural evidence from rodents has also supported the memory clearance hypothesis. However, it is important to note that these manipulations were applied post-learning, thereby changing the balance of young versus old neurons after the learning took place – a rather artificial situation. It could be that a post-learning increase in AHN caused a change in overall activity levels, which masked the memory rather than causing the memory to be cleared. If instead one compares two groups of animals, one with high and one with lower levels of immature neurons (e.g. using ablation, inactivation etc.) one does not see support for the memory clearance hypothesis. Further, the persistence or clearance of memories as a result of ongoing neural turnover in the DG may depend on the type of memory. There is evidence to suggest that spatial memories are always dependent on the hippocampus (Snyder et al., 2005; Deng et al., 2009; Jessberger et al., 2009). For these memories that are permanently hippocampal-dependent, AHN may help keep overlapping events distinct from one another, thereby promoting long-term retention of the original memory. On the other hand, fear memories may be supported by regions outside of the hippocampus (Kitamura et al., 2009). Thus, for those memories that can be supported by extrahippocampal structures, AHN may accelerate the process of systems consolidation (Kitamura et al., 2009), shifting the dependence of the memory from the hippocampus to neocortical

regions (McClelland et al., 1995; Squire and Alvarez, 1995; Maviel et al., 2004; Squire and Bayley, 2007). While animal studies have provided valuable clues as to the importance of AHN in learning and memory, it has nonetheless become apparent that studying the functional role of neurogenesis directly in humans is the critical next step that must be taken in order to reconcile some of the discrepant findings from non-human animal studies.

In the set of studies that comprise this thesis, we have demonstrated that change in aerobic capacity following chronic physical activity correlates with change in performance on a putatively neurogenesis-dependent visual pattern separation task. On the other hand, stress and depression scores had opposing effects on behavioural pattern separation performance. Importantly, neither exercise response nor depression scores predicted performance on other trial types within the BPS-O, repeated or novel items, nor the visuo-spatial CANTAB® PAL task. We have also shown that lower stress and depression scores are associated with improved visual object recognition on repeated items following a two-week delay from the study phase. Further, on two-week delayed retention tests, participants scored near chance at identifying lures as "similar", regardless of stress and depression levels. Interestingly, they more often misclassified these items as "new", as opposed to "old". Our results provide indirect evidence from human participants that AHN is important for pattern separation across shorter delays, while contributing to the persistence of memories for repeated items across extended time intervals. Future studies could explicitly test the memory clearance hypothesis in humans

by measuring recognition memory across longer timescales with a pro-neurogenic intervention, such as long-term exercise, in between study and test.

Pattern separation and memory retention (or clearance) may be coexisting phenomena. The addition of immature neurons to the hippocampus may at first bias the network towards pattern separation, as opposed to pattern completion, thereby reducing interference between events (Yassa and Reagh, 2013). In turn, the amount of pattern separation may determine what information will be subject to reconsolidation and what information will be cleared. Reconsolidation is the process whereby an existing memory becomes re-activated and susceptible to change. If an event is considered similar, but not the same as, a previously stored event then the original memory may be modified to accommodate the discrepant information. Thus, the constant addition of adult-born neurons to the hippocampus may serve as a means of adding contextual information to existing memories. However, the original memory may be altered so drastically during reconsolidation that it is no longer accessible in its original form (essentially cleared), depending on the amount of interference between the original memory and the novel event. Indeed, a number of computational models predict that the addition of newborn neurons to an existing circuit would hinder retrieval of previously stored memories (Deisseroth et al., 2004; Weisz and Argibay, 2009, 2012). On the other hand, if two events are considered one and the same, then the memory trace may be strengthened, although more generalized in nature. As these adult-born neurons that once contributed to pattern integration or pattern separation continue to mature and establish new synaptic connections with the pre-existing circuitry, they may destabilize previously established

memories in the hippocampus, leading to the loss of previously stored information (Josselyn and Frankland, 2012; Frankland et al., 2013; Yassa and Reagh, 2013). In turn, the clearance of older memories would make room for new ones and the newborn neurons would become part of the physical storage site for new memories (Josselyn and Frankland, 2012). Thus, both processes may be beneficial in their own way. Whether or not memory clearance is a benefit or detriment to memory performance really depends on the relative importance of information that is being cleared.

5.2 Correlates of Neurogenesis in Humans and Animals (Chapter 4)

Neurogenesis is down-regulated in a variety of neuropsychiatric disorders, so being able to characterize AHN in vivo is critical for better disease prevention and/or treatment. Unfortunately, there is no way to non-invasively quantify newborn cells in the living human hippocampus. Therefore, it has been difficult to improve our understanding of how neurogenesis influences the onset or recovery from certain disorders that may be associated with downregulated neurogenesis, such as depression. In addition, we cannot assess the specific contribution of newborn neurons to learning and memory.

The studies outlined in this thesis suggest that lifestyle factors and growth factors associated with neurogenesis hold promise as indirect cognitive and blood-based biomarkers of neurogenic activity. We were the first to show that stress, associated with downregulated neurogenesis, negatively correlated with behavioural pattern separation performance in human participants (Déry et al., 2013). We were also the first to show in a group of healthy young adults that chronic, high-intensity aerobic exercise, a potent upregulator of neurogenesis, positively correlated with accuracy at identifying visual

images susceptible to high levels of interference (Déry et al., 2013). The results outlined in this thesis are consistent with a wide range of evidence implicating higher levels of neurogenesis with enhanced pattern separation performance in rodents (Creer et al., 2010; Sahay et al., 2011; Kohman et al., 2012). Moreover, the results outlined in Chapter 4 suggest that the physical activity-induced enhancement in pattern separation performance may be mediated, at least in part, by changes in serum IGF-1. At baseline, both aerobic fitness and serum BDNF predicted accuracy on lure trials in the BPS-O. However, BDNF mRNA also predicted performance on PAL at baseline and following six weeks of exercise. Therefore, the specificity of BDNF as a marker for neurogenesis-dependent cognition remains open to investigation. On the other hand, change in aerobic capacity was a significant predictor of change in pattern separation performance in those who underwent six weeks of physical activity. Moreover, change in serum IGF-1 was a significant positive predictor of change in performance at identifying highly similar lures post- minus pre-exercise, an effect that was moderated by change in fitness. Importantly, serum IGF-1 is required for the activity-dependent increase in hippocampal neurogenesis in mice (Trejo et al., 2001). Thus, Chapter 4 provides perhaps some of the strongest evidence to date from humans for the proposed role of neurogenesis in reducing interference between similar items. Thus, assessing serum IGF-1 expression in combination with accuracy on high interference cognitive tasks before and after pro- or anti-neurogenic interventions may help shed light on how that particular intervention is affecting AHN. Despite these advances, much more work remains to be done when it comes to identifying reliable biomarkers of neurogenesis in humans.

5.3 Limitations

The outlined work has focused on elucidating the functional role of AHN in human memory, through the use of putative lifestyle- and peripheral blood-based biomarkers of neurogenesis. Despite the knowledge gained from these studies, several limitations must be pointed out. First, both exercise and depression are known to act on multiple forms of brain plasticity besides neurogenesis. For instance, physical activity can enhance synaptic density, dendritic arborisation and capillary density (Dietrich et al., 2008; Stranahan et al., 2009; Murugesan et al., 2011; Lin et al., 2012). On the other hand, stress can cause dendritic atrophy in the CA3 (McEwen et al., 1995). Without any direct measure of adult-born neurons in our studies, there is no way to know with 100% certainty that the relationship between aerobic fitness and pattern separation performance as well as that between stress and depression scores and pattern separation performance are specific to AHN. However, we attempted to mitigate this issue by incorporating several control tasks within our studies. For instance, we used the hippocampal-dependent PAL task from the CANTAB® battery. If a change in hippocampal plasticity other than neurogenesis contributed to change in behavioural pattern separation performance, than we likely would have observed similar changes in accuracy on PAL – we did not. Further, the correlation between aerobic capacity and accuracy on the BPS-O task was specific to one trial type in particular, accuracy on lures. There was no relationship between aerobic fitness and traditional recognition, identifying repetitions as "old". The same pattern of results was observed when comparing stress and depression to BPS-O performance. Stress and depression scores negatively correlated with accuracy on

lures, but not with accuracy on novel items or repetitions. Further, since it is not possible to measure AHN directly in the human brain, we cannot rule out the possibility that change in accuracy on lure trials following exercise occurred in the absence of changes in neurogenesis.

A potential drawback of the studies outlined in this thesis is that the study populations were quite small and restricted to university-aged students, and thus did not represent the full spectrum of possible fitness or depression scores. Although we specifically recruited sedentary participants under the assumption that they would undergo the greatest improvement in fitness and concomitant enhancement of AHN, it may have been beneficial to recruit a larger population with a more varied level of baseline fitness. Such a recruitment strategy may have allowed us to determine the therapeutic potential of aerobic exercise on putative neurogenesis-dependent memory, which may in turn depend upon starting fitness. Specifically, we may have been able to identify a specific cut-off whereby any additional incremental gain in fitness would not have resulted in an increase in neurogenesis-dependent cognition. We also had good reason not to recruit clinically depressed participants in our studies. It has been shown that recurrent or multiple episodes of depression are associated with broader hippocampal pathology, such as grey matter volume loss (Campbell et al., 2004). Decreased grey matter volume could be attributable to a number of morphological changes besides loss of immature granule cells. Therefore, we recruited healthy young participants whose BDI scores typically ranged from mild to moderate, with very few scoring in the high range. If we would have been able to recruit more participants at risk for developing their first

episode of depression, scoring in the very high range on the BDI scale, then we may have been able to better ascertain how subjective depression scores affect neurogenesis-dependent cognition. While unlikely, we cannot rule out the possibility that there is a linear decrease in neurogenesis-dependent memory performance as BDI increases until a certain point at which very high depression scores are actually associated with an increase in behavioural pattern separation due to some sort of compensatory shift in brain regions recruited or strategies utilized to complete the task.

A limitation to the study outlined in Chapter 4 is that we did not control for certain factors known to influence BDNF levels due to a small sample size and lack of statistical power. For instance, plasma BDNF has been shown to fluctuate with menstrual cycle (Pluchino et al., 2009). Since most of our exercising population was female, but we did not control for cycle status when we sampled blood, it may have drastically affected our results. Future studies might want to consider using an exercise program that begins in a staggered fashion and runs for two months (as opposed to six weeks), so that all women recruited would begin and end the intervention at the same time in their menstrual cycle.

Given the results outlined in this thesis, it is tempting to conclude that circulating growth factors mediate the effects of exercise on AHN and relevant cognition. However, in light of related studies in the literature, there is still not enough evidence to make any definitive conclusions. For instance, there are mixed findings with regards to change in serum BDNF following exercise. Some have reported that the brain may be the principal source of the exercise-induced increase in peripheral BDNF (Rasmussen et al., 2009). On

the other hand, some have shown acute stress-induced improvements in spatial learning with a concomitant increase in hippocampal BDNF, but no change in AHN (reviewed in Yau et al., 2014). Further, chronic stress has been reported to impair spatial memory and reduce hippocampal BDNF and AHN, while not affecting peripheral BDNF (Yau et al., 2014). Indeed, many physiological and cognitive effects of stress show an inverted u-shaped curve with respect to amount of stress: mild amounts of stress may enhance whereas severe stress invariably impaires. Therefore, it seems that, although inter-related, circulating growth factors do not always share a linear relationship with central growth factors and/or AHN. More research investigating what other factors influence enhanced AHN following exercise besides circulating growth factors, and then controlling for those other variables, might help elucidate the complex relationship between the two. Perhaps, it would have also been beneficial to measure peripheral growth factors at regular intervals as opposed to just at the onset and completion of the exercise program. Chicharro and colleagues (2001) noted that serum IGF-1 increased following the first week of regular exercise, but then decreased back to normal levels following the third week. Therefore, it is possible that basal levels of certain growth factors increased at some point during our six week-long exercise program, but then tapered off by the sixth week due to homeostatic mechanisms.

Finally, much of what we know about the physiological properties of dentate granule cells comes from mice and rats. Therefore, when trying to design studies aimed at upregulating neurogenesis in humans through exercise, assumptions and generalizations must be made. The maturation process from stem-like cell to functional immature neuron

is about three to six weeks in rats (Piatti et al., 2011), but the duration of neuronal maturation in humans is unknown. Research has shown granule cells of a specific age make unique contributions to learning and memory. A probe trial following MWM training in mice preferentially activated those granule cells that were 4–6 weeks old at the time of MWM training and 10 weeks old at the time of testing (Kee et al., 2007), suggesting that 4-6 weeks may be the optimal age at which young neurons are recruited for novel encoding, while mature adult neurons are recruited during memory retrieval. In contrast we do not know the age of dentate granule cells that are preferentially recruited during neurogenesis-dependent learning in humans.

Relevant to our studies involving exercise, it is hard to equate running speed or distance in mice to that in humans. For example, if an animal study describes a two-fold increase in DG neurogenesis following two weeks of physical activity in mice, how long and for what duration would a human have to run in order to experience a comparable effect? Until a direct measure of neurogenesis is available in humans, this question shall remain unanswered. Therefore, it is very difficult to adapt the methods used in animal studies for use in humans. While changes to AHN measured in animals following pro- or anti-neurogenic interventions offer insight into what would happen to adult-born neurons in the human brain, we cannot: 1) design human studies to meet exactly the same specifications as those run in animals, and 2) know for sure that the effects of the intervention are comparable between rodents and humans. Indirect methods for measuring neurogenesis in humans offer a complimentary, macroscopic view of changes to the brain following stress, aging or exercise. More proof of concept studies comparing

indirect measures of AHN in humans, such as changes in peripheral BDNF, to immunohistochemistry cell counts in the rodent hippocampus would be needed to help justify the assumptions made when utilizing such methods in human populations.

5.4 Future Directions

There are several directions in which the research described in this thesis could be further explored. The pattern separation task that has been described throughout each chapter of this thesis, the BPS-O, uses visual objects. Extending the pattern separation task to other types of stimuli, such as spatial locations or word pairs would help elucidate the specific role of neurogenesis in cognition. Further, performing such tasks while in an fMRI scanner would uncover what brain regions are involved when spatial or verbal stimuli are the targets as opposed to visual objects. Moreover, in the particular version of the BPS-O we used, there was a contextual change between some study and test items presented in the same day. It was on these items that we found behavioural pattern separation performance to be spared in those with lower rates of neurogenesis (Chapter 3). One possible reason for this enhanced accuracy is that the contextual change between study and test helped those with low neurogenesis to make distinct representations of the study image and the highly similar lure in order to successfully discriminate between the two. Future studies should investigate the effects of visual context on pattern separation performance. It would be interesting to see if keeping the visual context exactly the same during study and test on items presented across long periods of time is enough to restore the stress-related difference in accuracy between those with putatively high versus low rates of neurogenesis. Perhaps the amount of time that passes between the study and test

phases of these particular items is enough to help keep them distinct in memory. This would be interesting considering that over one testing session a sufficient amount of time would not have passed in order for there to be a markedly new population of neurons available to represent the two memories. It could be the case that when there is enough separation between items, whether between the object features themselves, the visual background or time (as a feature of context), newborn cells are no longer required for pattern separation. Moreover, as memories are reconsolidated in response to repeated activation, it is thought they become more schematized. Accordingly, it is possible that repeated exposures to similar stimuli over a period of weeks might actually hinder recognition of the original item following each lure. Future studies utilizing behavioural pattern separation tasks across a variety of domains with varying contexts and timing between study and test would be required to tease apart the contribution of adult-born granule cells in reducing interference between similar stimuli at long time scales. Finally, the memory clearance hypothesis as proposed by Frankland et al (2013), suggests that increasing neurogenesis after learning would impede subsequent recognition. While we noted a significant positive correlation between change in aerobic fitness and change in pattern separation performance in exercisers, it would be interesting to see if long-term retention for those items learned at baseline would be impeded by the putative exercise-induced increase in AHN.

Since there is so little data concerning the effects of aerobic exercise on putative neurogenesis-dependent behaviour in humans, there is a need for more studies focusing on the effects of varying exercise program intensity, bout length, and program duration

on behavioural performance. Intermittently measuring physiological markers such as salivary cortisol and serum growth factors before, during and after the exercise program would help to provide valuable information concerning what might be happening to neurogenesis in the hippocampus. We cannot fully understand how activity-dependent changes in serum IGF-1 might reflect changes to AHN without assessing how exercise changes the expression of the various IGF-binding proteins, such as IGFBP-1 and IGFBP-3. Changes to circulating levels of IGF-binding proteins following exercise would modify the bioavailability of IGF -1. IGF-binding proteins change in response to physical activity (Koistinen et al., 1996; Chicharro et al., 2001). IGFBP-1 has been shown to inhibit the actions of IGF-1 (Cox et al., 1994). On the other hand, IGFBP-3 interacts with IGF-1 receptors to increase the biological effects of IGF-I (Cohich and Clemmons, 1993). Chicharro and colleagues (2001) have described an increase in total IGF-1 and IGFBP-1 following the first week of a chronic exercise training program. However, after three weeks there was no net increase in IGF-1 (Chicharro et al., 2001). Thus, it seems that IGFBP-1 and IGFBP-3 help regulate the actions of IGF-1 in order to maintain homeostasis. Increasing IGF-1 via exercise may only be one part of the puzzle. There would also need to be a concomitant decrease in IGFBP-1, for example, to ensure that upregulated serum IGF-1 remained available to bind to its receptors in the hippocampus and influence AHN. Serum IGF-1 diminishes as humans age. On the other hand, older adults that are more active have higher levels of IGF-1 (Onder et al., 2006; Cappola et al., 2009). Since animal studies have shown that exercise reverses the decline in neurogenesis even when started in middle or old age (Kuhn et al., 1996; van Praag et al., 2005;

Kronenberg et al., 2006; Kannangara et al., 2011) then perhaps running an exercise program in older adults would improve serum IGF-1 along with neurogenesis-dependent cognition.

There is a definitive need for large-scale, multi-centered and standardized studies with central oversight in order to achieve the statistical power required to control for factors known to affect neurogenesis, such as stress levels, cycle status, age, gender and BMI, while providing a pro-neurogenic intervention such as antidepressant pharmaceuticals (in a clinical population) or aerobic exercise and assaying for putative serum biomarkers. First and foremost, additional rodent and parallel rodent-human studies are needed to assess the relationship between various putative biomarkers of neurogenesis and actual immature granule cell counts. For example, Pereira et al (2007) demonstrated that exercise-induced changes in DG blood volume in rodents corresponded to immunohistochemical cell counts in post-mortem tissue. This type of proof of concept study is needed to both 1) provide supporting evidence for the assumptions made in human biomarker studies, and; 2) guide the search for other indirect correlates of neurogenesis in humans. MRI-based correlates of neurogenesis suffer from lack of specificity (e.g. there is no way of telling what structural changes actually underlie a change in hippocampal volume) and peripheral neurotrophins suffer from the same issue. Further, exercise, which has been shown to upregulate neurogenesis, influences plasticity in a number of ways besides the generation or survival of adult-born granule cells (Dietrich et al., 2008; Stranahan et al., 2009; Lin et al., 2012). Likewise growth factors are responsible for other morphological changes besides neurogenesis. IGF-1 expression

in response to exercise also stimulates the growth of dendritic spines on basal dendrites of CA1 pyramidal cells (Glasper et al., 2010). Combining a number of different biomarkers that converge on AHN may provide improved predictive value for estimating levels of neurogenesis in combination relative to any one of them alone. It has been shown that low serum IGF-1 is associated with abnormal brain vasculature in rats (Sonntag et al., 1997). Exercise-induced adaptations to brain vasculature are inhibited in mice lacking peripheral IGF-1 (Lopez-Lopez et al., 2004). Thus, perhaps a human study combining blood volume measurements in the DG with serum measures including IGF-1 would provide the greatest indirect assessment of neurogenesis in vivo. Interestingly, a relationship between serum BDNF and N-acetyl aspartate (NAA) as measured by MRS has been reported (Lang et al., 2007). NAA is a marker of neuronal integrity, reflecting neuronal loss (Lang et al., 2007). Further, Manganas and colleagues (2007) claim that a peak identified at 1.28 ppm represents neural precursor cells. Perhaps quantifying the NAA and 1.28 peaks in conjunction with serum BDNF may provide additional insight into levels of neurogenesis in the human brain over the course of depression treatment, for example. As mentioned earlier, first verifying the utility of these indirect correlates of AHN in animals before utilizing them in humans would be the best approach i.e. scanning rats in the MRI, taking serum BDNF measurements and performing post-mortem assays for neurogenesis. It would also be interesting to track how changes to neurogenesis relate to changes in hippocampal volume assessed by MRI. To my knowledge studies such as this have not yet been conducted. Scanning animals using high field strength scanners, such as 7 or 9.4 Tesla, would allow for subregion specificity so

that DG volume alone (rather than the whole hippocampus) could be compared to immunohistochemical cell counts of newborn neurons. Despite the inconsistencies in biomarker studies thus far, there remains a significant potential for using biomarkers such as growth factors from blood as indirect markers of neurogenesis. However, because putative biomarkers such as these are influenced by such a wide variety of factors, controlling for variables that may influence their expression is essential. Identifying and utilizing an array of biomarkers at the same time would likely provide the highest sensitivity when it comes to making estimates of AHN. The identification and standardization of biomarker assessment for neurogenesis hold tremendous value as a clinical tool for diagnosing various disorders whereby neurogenesis is downregulated as well as treatment response prior to any behavioural changes manifest. Computational models that incorporate more time-dependent roles for neurogenesis (recent vs. remote) on hippocampal processing are required to better elucidate the fundamental role of immature hippocampal neurons in recent and remote memory processes. Such models would help generate an avenue for future research in animals and humans by providing a new set of testable hypotheses. Lifestyle factors such as aging, stress and exercise have been shown to powerfully regulate AHN and putative neurogenesis-dependent cognition in humans. Until more direct and reliable methods for non-invasively quantifying AHN *in vivo* are developed, perhaps studies using such lifestyle-based correlates of neurogenic activity in humans can further our understanding of neurogenesis-dependent cognition in our own species. Indeed, our research has demonstrated that models of neurogenesis that

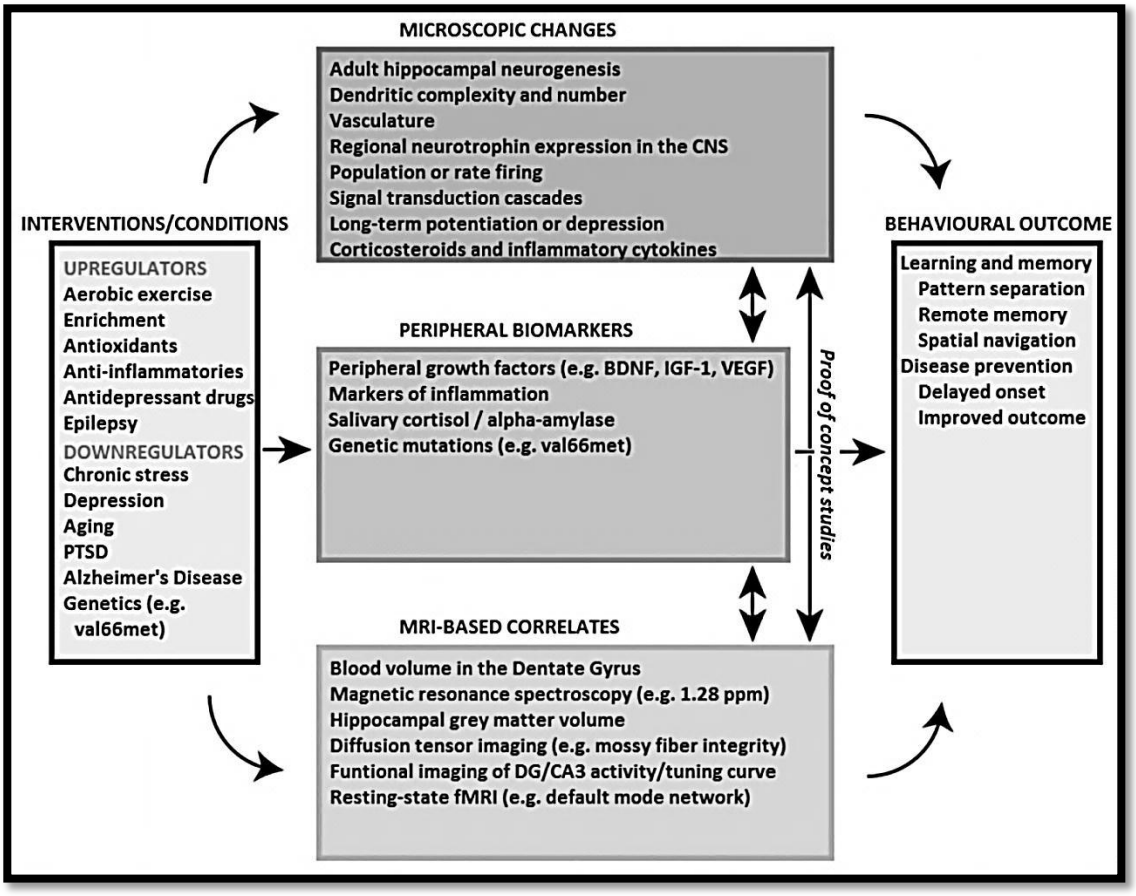

Figure 5.1. Searching for biomarkers of adult hippocampal neurogenesis in humans. Proof of concept studies in rodents are required to determine the validity and predictive value of putative peripheral biomarkers of neurogenesis in humans. Measuring an array of peripheral- or MRI-based correlates of neurogenesis in rodents and then using principal component analysis to determine which of those factors best predict immunohistochemical cell counts of immature neurons in the DG will be an important first-step in validating putative neurogenic biomarkers in humans. At the same time, measuring change in putative peripheral biomarkers of neurogenesis following large-scale interventional studies using upregulators of neurogenesis and comparing to changes in cognitive outcome may help elucidate the functional role of newborn neurons in the adult human hippocampus.

include, but are not limited to, neurogenesis effectors such as age, subjective measures of stress and depression as well as aerobic fitness may providean indirect means for estimating levels of AHN in humans. The primary goals of the research described here

were to elucidate the functional role of AHN in humans and test the utility of various theoretical biomarkers of AHN. Being able to indirectly track AHN in humans holds tremendous promise for preventing cognitive decline and improving mood in healthy aging and psychiatric illness. Such promise only underscores the need to find biomarkers of neurogenesis in humans. The studies outlined in this thesis provide indirect evidence that exercise may hold therapeutic potential for those disorders whereby neurogenesis in downregulated. With continued interdisciplinary and collaborative efforts, the development of increasingly effective biomarkers for AHN would help signal a new era in the field of behavioural neuroscience and it is my hope that the studies outlined in this thesis have helped bring us one step closer.

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