THE EFFECT OF LIFESTYLE FACTORS ON POTENTIAL MEASURES OF

NEUROGENESIS AND THE BEHAVIOURAL IMPLICATIONS

By MALCOLM PILGRIM, B.A.

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Abstract

Over long delays between events, evidence from computational models suggests that neurogenesis may be important for reducing the potential of interference between overlapping memories. These computational models also suggest that at shorter time scales, within a single memory episode, neurogenesis may play a role in binding together elements that share a common spatiotemporal context. Empirical evidence from animal research provides support for both of these hypothesized roles. Interestingly, results from recent research also suggest that depending on the task demands, neurogenesis may either aid or hinder performance.

In order to investigate this potential trade-off, we designed the Concentration Memory Task (CMT); a novel spatial memory task which subjected participants to trials where neurogenesis is hypothesized to aid performance and trials where neurogenesis is hypothesized to hinder performance. Furthermore, we tested undergraduates on this novel task and memory tests from the CANTAB battery, and administered neuropsychological mood inventories and a lifestyle questionnaire.

Our results suggest that measures on the CMT hypothesized to be dependent upon neurogenesis correlate with and predict performance on putatively neurogenesis-dependent tasks. Furthermore, individuals with potentially suppressed neurogenesis display selective deficits on these measures. However, our results failed to provide evidence for a working memory enhancement in these individuals.

The results from the present study provide strong encouragement for the continued development of this novel task. We provide evidence that as predicted, individuals with potentially suppressed neurogenesis display increased sensitivity to interference on the CMT. However, we failed to provide evidence that suppressed neurogenesis may enhance working memory performance. This null result may be due to shortcomings in the design of the CMT and a revised protocol that may resolve these shortcomings is discussed. With continued development, the CMT may serve as a tool for detecting early signs of cognitive impairment associated with suppressed neurogenesis.

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List of Abbreviations

Abbreviation

LTP	Long term potentiation
CMT	Concentration memory task
PSS	Perceived Stress Scale
BDI	Beck Depression Inventory
BAI	Beck Anxiety Scale
Old|Old	Correctly identified old item
Similar|Old	Old item misclassified as similar
New|Old	Old item misclassified as new
Similar|Similar	Correctly identified similar item
Old|Similar	Similar item misclassified as old
New|Similar	Similar item misclassified as new
New|New	Correctly identified new item
Old|New	New item misclassified as old
Similar|New	New item misclassified as similar
DMS	Delayed Match to Sample
PAL	Paired Associates Learning
RVP	Rapid Visual Learning
M	Mean
SD	Standard deviation

Introduction

1.1 Hippocampal Learning

It is well known that the hippocampus is an important structure involved in the processes of learning and memory. Most notably, the hippocampus has been cited as a crucial brain structure for the formation of episodic memories (Scoville & Milner, 1957). Episodic memories are memories of everyday events and involve the formation of complex associations between time and place to form single episodes. Evidence for the link between the hippocampus and episodic memory is demonstrated in cases of hippocampal damage, most famously in the case of patient H.M. (Scoville & Milner, 1957). H.M. had his hippocampus and some associated areas removed from both hemispheres as treatment for severe epilepsy. As a result, H.M. developed severe anterograde amnesia, an inability to store new episodic memories. Subsequent investigations of patients involving severe hippocampal atrophy further establish the role of the hippocampus in episodic memory as another patient, patient R.B., experienced localized hippocampal atrophy that resulted in marked anterograde amnesia (Zola-Morgan, Squire, & Amaral, 1986).

The hippocampal formation (Figure 1) consists of the dentate gyrus, entorhinal cortex, subiculum, and the cell fields (CA1/CA3), and functions closely with the adjacent perirhinal and parahippocampal cortices. The hippocampus lies at the top of the cortical processing hierarchy and receives the majority of its cortical projections from the entorhinal cortex. The entorhinal cortex in turn receives
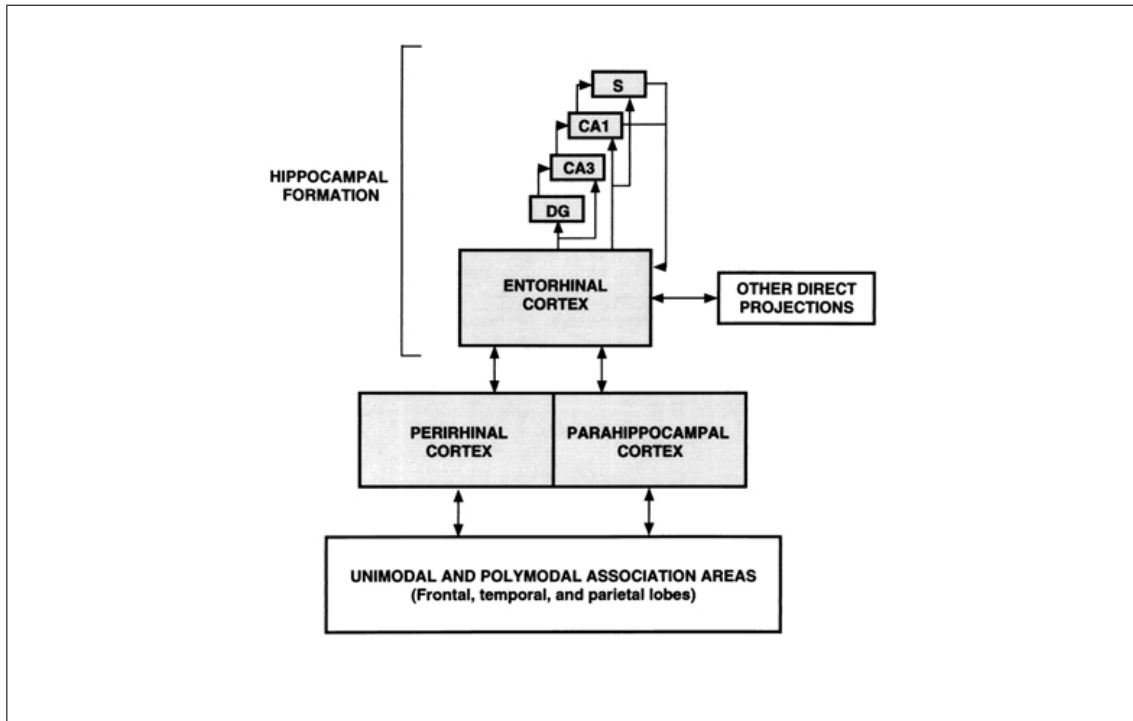
Figure 1: **Hippocampal system and projections**

projections from high level association areas in the frontal, temporal, and parietal lobes, and relays these projections to the hippocampus as well as back-projecting to all of these areas. It is these widespread projections that are believed to enable the hippocampus to perform tasks that depend on relating or combining information from multiple sources, such as tasks that require knowledge about specific events (episodic memory) or associative memory tasks that require different elements to be remembered as a pair (such as pairing a name with a face). More recently, strong evidence suggests that the dentate gyrus may play a crucial role in allowing the hippocampus to perform such tasks.

1.2 Dentate Gyrus and Pattern Separation

A large body of evidence suggests that the ability of the hippocampus to effectively form distinct memories relies on the ability to overcome high levels of interference between encoded events (e.g. Chadwick, Hassabis, & Maguire, 2011). This ability to overcome interference may be due to the orthogonalized manner in which the hippocampus encodes overlapping or similar information, which is often referred to as "pattern separation". Pattern separation refers to the differential representation of similar events within a memory store and it is believed that pattern separation is responsible for the avoidance of catastrophic interference between encoded information (Marr, 1971). It is believed to function by allowing new information to be encoded without interfering with or overwriting previously stored information (McClelland, McNaughton, & O'Reilly, 1995; Norman & O'Reilly, 2003).

An intuitive example of pattern separation is the ability to recall where one parked one's car today within a parking lot where one has parked numerous times before. Each individual episode is very similar and yet it is still possible, and relatively effortless, to differentiate where one's car is parked today from where it was parked on a previous day. Although all of the regions of the hippocampus have been suggested to play some role in the formation of memories, recent research and theory suggests that pattern separation is a key function specifically reserved for the dentate gyrus (Squire & Zola, 1996; Kirwan & Stark, 2007; Leutgeb, Leutgeb, Moser, & Moser, 2007).

A great deal of recent research focuses on the dentate gyrus because of its suggested role in pattern separation. This research presents compelling support for the role of the dentate gyrus in mediating pattern separation in the hippocampus. Here, I will present a brief summary of this research.

1.2.1 Location, anatomy, and connectivity

The dentate gyrus is located within the so-called "trisynaptic circuit" of the hippocampal formation in which information flows from the entorhinal cortex to the dentate gyrus, to the CA3 and CA1 subregions, and finally back to the entorhinal cortex. Information from the rest of the brain enters via the perforant path, which connects the entorhinal cortex and the dentate gyrus (Amaral, 2007). Each granule cell neuron within the dentate gyrus receives inputs from thousands of entorhinal cortex neurons (Coulter and Carlson, 2007). However, high inhibition levels within the dentate gyrus ensure that only those granule cells most activated by their inputs will fire (Coulter and Carlson, 2007). This results in an extremely sparse neural code (Acsády, Katona, Martínez-Guijarro, Buzsáki, & Freund, 2000).

Information is then passed to the CA3 via mossy fiber connections, where each dentate gyrus granule cell maps on to several CA3 pyramidal neurons (Witter, 2010). These mossy fiber connections are few in number but very strong, allowing a small number of active granule cells to induce action potentials in target CA3 neurons (Henze, Wittner, & Buzsáki, 2002). The CA3 possesses recurrent connections that are believed to perform an auto-associative function, allowing the storage of distributed firing patterns as a coherent memory trace (Marr, 1971). These firing patterns can later be retrieved if all or part of the original pattern

reoccurs. It is believed that this efficient storage and retrieval relies on the ability of the dentate gyrus to form distinct representations of events (Leutgeb et al., 2007).

Finally, the information is passed to the CA1 via Shaffer collaterals, and back to the entorhinal cortex. Only the CA1 subregion projects back to the entorhinal cortex. It is important to note that the perforant path also connects the entorhinal cortex directly with the CA3 and CA1 subregions, by-passing the dentate gyrus. These alternate pathways have been shown to be capable of supporting the formation of certain types of memories (Nakashiba, Young, McHugh, Buhl, & Tonegawa, 2008). It is therefore believed that not all forms of learning and memory require the dentate gyrus and pattern separation.

1.2.2 Electrophysiology

Pattern separation has been proposed to be a function of the granule cell neurons within the dentate gyrus (Leutgeb et al., 2007). Evidence from single cell recordings in the dentate gyrus of rats suggests that while pyramidal place cells in the CA3 region of the hippocampus only remap in response to substantial contextual changes, granule cells only alter their firing pattern in response to subtle contextual changes (Leutgeb et al., 2007). Therefore, it seems that only dentate granule cells are capable of differentially representing highly similar episodes.

1.2.3 Behavioural Data

As mentioned previously, not all forms of learning and memory seem to require the dentate gyrus and therefore pattern separation. Inhibition of the trisy-

naptic pathway in mice revealed that the monosynaptic pathway alone was sufficient for incremental spatial learning whereas the trisynaptic pathway was necessary for other memory tasks, such as contextual learning (Nakashiba et al., 2008). Additionally rats with lesions to the dentate gyrus and CA1 subregion showed no deficit on a paired-associate learning task; however CA3-lesioned rats showed impairments in learning the task (Gilbert & Kesner, 2003). Therefore, pattern separation seems to be required only when a distinction between similar events is necessary.

Recent results from numerous high-resolution functional resonance imaging (fMRI) studies in humans provide evidence to support the role of the dentate gyrus in pattern separation. The typical pattern separation task used in these fMRI studies requires participants to differentiate between previously seen ("old") and similar to previously seen ("similar") stimuli. Although the resolution of such fMRI analyses does not allow for a reliable distinction between the dentate gyrus and the adjacent CA3 subregion, it appears that both activation and volume of the dentate gyrus/CA3 are highly correlated with successful pattern separation in tasks that require a high level of distinction between similar events (Bakker, Kirwan, Miller, & Stark, 2008; Yassa, Stark, et al., 2010; Yassa, Lacy, et al., 2010; Shing et al., 2011). Although these results suggest that the dentate gyrus has a role in pattern separation, they are unable to determine if the dentate gyrus is necessary for pattern separation.

The necessity of the dentate gyrus to perform pattern separation has been reliably demonstrated by lesion studies in rats; selective lesions to the dentate gyrus have resulted in deficits in tasks requiring pattern separation (Hunsaker,

Rosenberg, & Kesner, 2008). For example, Gilbert, Kesner, and Lee (2001) trained rats on a delayed-match-to-sample task that required them to displace an object that covered a food-well. After a short delay, the rats returned to the maze and were rewarded for displacing the same object. However, an identical object (a "lure" object) was placed at varying distances from the original object, creating trials with varying degress of pattern separation required. Rats with lesions to the dentate gyrus were significantly impaired only when the distance between the original object and the lure was small, showing no deficit when this distance was large (Gilbert et al., 2001). Therefore, these results suggest that the dentate gyrus is necessary in tasks that require a distinction between highly similar events and when there is a large potential for interference - the defining features of pattern separation.

Taken together, the results from these studies provide strong evidence to suggest that the dentate gyrus plays a central and critical role in pattern separation. Importantly, not all forms of memory seem to require the dentate gyrus. Indeed, the dentate gyrus seems to only be necessary when it is critical to distinguish between highly similar events, i.e. when pattern separation demands are high. Although the specific mechanisms that allow the dentate gyrus to efficiently reduce the risk of catastrophic interference through pattern separation remain unclear, computational models offer some insight as to how hippocampal neurogenesis may allow the dentate gyrus to form highly distinct memories.

1.3 Neurogenesis

1.3.1   A brief history

Until the 1960s, it was a long-held dogma that the adult mammalian brain possessed no capacity to generate new neurons. However, this changed when Joseph Altman and colleagues provided evidence for the production of new neurons in the dentate gyrus of the hippocampus in adult rats (Altman & Das, 1965). Although these findings initially met with much criticism and hostility, it is now widely accepted that the adult brain of many mammalian species is capable of neurogenesis (Gage, 2000). Since the discovery of adult neurogenesis almost 50 years ago, a vast amount has been learned about the generation of these new neurons. Despite this, many questions still remain unanswered surrounding their functional relevance. In the following section, I will review key research that has shed light on neurogenesis, including the maturation process of new neurons and factors that regulate the proliferation, differentiation, migration and survival of these neurons. Finally, I will present theoretical predictions from computational models that shed light on the functional role of hippocampal neurogenesis in learning and memory, as well as empirical evidence for these hypothesized roles.

Neurogenesis is observed in two regions of the adult brain, the olfactory bulb and the hippocampus. Neurons born in the subventricular zone of the lateral ventricles migrate through the rostral migratory stream and become granule neurons and periglomerular neurons of the olfactory bulb (Doetsch, Caillé, Lim, García-Verdugo, & Alvarez-Buylla, 1999). Neurons born in the subgranular zone of the dentate gyrus differentiate and integrate into the local neural network as granule

cells of the dentate gyrus (Lois & Alvarez-Buylla, 1993). For the purpose of this review, only hippocampal neurogenesis will be considered.

1.3.2 Maturation

The life of adult-born granule cells begins with the proliferation of neural progenitor cells in the subgranular zone of the dentate gyrus (Zhao, Teng, Summers, Ming, & Gage, 2006). Most of these neural progenitor cells differentiate into dentate granule cells, however, a small population become glia cells. Approximately one week after birth, these neural progenitor cells destined to be dentate granule cells migrate a short distance into the inner granule cell layer of the dentate gyrus. Here they extend limited cellular processes, but they do not become synaptically integrated into the network. The dentate granule cells are activated by ambient GABA, which has been shown to be crucial for the establishment of functional GABAergic and glutamatergic synaptic inputs, as well as for regulating dendritic development. These dentate granule cells display high input resistance and low membrane capacity (Espósito et al., 2005; Ge et al., 2006).

After two weeks from birth, the dentate granule cells begin to more closely resemble neurons. They grow polarized processes, with dendrites that extend towards the molecular layer and axons, which will later become mossy fibers, that grow through the hilus towards the CA3 (Hastings, Seth, Tanapat, Rydel, & Gould, 2002). However, at this early stage, these dentate granule cells are still very different than their mature counterparts. They possess higher membrane resistance and different firing properties (Espósito et al., 2005). They also lack glutamatergic input and instead, receive synaptic GABAergic input which results

in neuronal depolarization (Espósito et al., 2005). This depolarization has been suggested to be important for the survival and maturation of these dentate granule cells (Toni et al., 2007).

Three weeks after birth, efferent and afferent synapses of dentate granule cells begin to form (Zhao et al., 2006). Dendritic spines begin to appear, forming synapses with afferent axon fibres in the perforant pathway that come from the entorhinal cortex. At this point, GABA input is no longer excitatory and becomes inhibitory. Instead, the dentate granule cells receive glutamatergic input from the perforant pathway. Although they are even closer to resembling mature neurons, they still possess unique characteristics.

At this point, the dentate granule cells have a high membrane resistance and high resting potentials (Espósito et al., 2005). Furthermore, they exhibit a lower threshold for the induction of glutamatergic long-term potentiation (LTP) when compared to mature neurons (Wang, Scott, & Wojtowicz, 2000; Snyder, Kee, & Wojtowicz, 2001). These characteristics make them more excitable than their mature counterparts and this critical period persists for 4-6 weeks (Ge, Yang, Hsu, Ming, & Song, 2007). These characteristics of hyperexcitability represent important characteristics that may permit the immature neurons to be selectively recruited for encoding. The functional significance of this will be discussed shortly.

After 7 weeks, the granule cells can generate action potentials and show electrophysiological responses very similar to those of surrounding mature cells (Laplagne et al., 2006). After 8 weeks, they reach full maturation and are indistinguishable from mature dentate granule cells.

1.3.3 Regulating neurogenesis

Neural progenitor cells must undergo a lengthy and complex series of transitions before they mature into fully functional dentate granule cells. As such, although it is suspected that a vast amount of neural progenitor cells are generated each day, many experience apoptotic cell death before they can reach maturation. Indeed, in the young rat dentate gyrus, approximately 8000 - 10,000 new neurons are generated each day, with approximately 40% of these neurons surviving and reaching maturity (McDonald & Wojtowicz, 2005).Therefore, it seems that a surplus of neural progenitor cells are generated and only a select subset are chosen to reach full maturation.

There are many factors that affect the proliferation and survival rates of dentate granule cells. For the purpose of this review, I will choose to expand on a select few that are of particular relevance to the research described in this thesis.

Ageing

Ageing has a profound impact on hippocampal neurogenesis. Indeed, evidence suggests that there is a significant reduction in neurogenesis that occurs during the transition from adolescence to adulthood (J. He & Crews, 2007). Although neurogenesis persists throughout adulthood, it has been shown to continue to decrease with age. This age related decline may result from a reduction in cell proliferation (Kuhn, Dickinson-Anson, & Gage, 1996; Kempermann, Kuhn, & Gage, 1998). Although there may be a reduction in the number of generated neurons, evidence from mice suggests that neurons that are generated and do succeed in reaching maturation are functionally equivalent to those generated by younger

mice (Morgenstern, Lombardi, & Schinder, 2008). However, the effects of ageing may vary between species. A reduction in the number of neural progenitor cells has been reported in the dentate gyrus of aged primates, but not in aged mice (Aizawa, Ageyama, Terao, & Hisatsune, 2011). Interestingly, although these neurons showed morphological alterations, there was no observed effect on proliferation rates, suggesting that the decline was due to a reduced maturation rate. Indeed, there is also evidence that supports this notion of a diminished ability for rapid neuronal maturation in rats (Rao, Hattiangady, Abdel-Rahman, Stanley, & Shetty, 2005).

It is important to note that much of the described age-related decline in neurogenesis correlates with additional factors that have been found to regulate neurogenesis. For instance, ageing is associated with increase glucocorticoids, which has also been found to decrease neurogenesis in rodents (Nichols, Zieba, & Bye, 2001). This effect is reversed with the removal of glucocorticoids (Cameron & McKay, 1999). Additionally, exercise, which has been found to increase neurogenesis in rodents, also ameliorates the age-related decline in neurogenesis in mice (van Praag, Shubert, Zhao, & Gage, 2005). These correlations suggest that it is possible that the profound effects of ageing on neurogenesis may be the result of an accumulation of neurogenesis-decreasing factors and a lack of neurogenesis-increasing factors.

Sex Hormones

Recent research has suggested that estrogen increases neurogenesis by increasing both cell proliferation and cell survival of the newly generated dentate gyrus neurons (Galea, Spritzer, Barker, & Pawluski, 2006). Interestingly, this ef-

fect has been shown to vary across the rodent estrus cycle (Tanapat, Hastings, Reeves, & Gould, 1999). Female rats have been found to exhibit increased proliferation rates during the proestrus phase, when estrogen levels are at their peak, when compared with proliferation rates during the estrus and diestrus phases, when estrogen levels are lower. Similarly, estrogen has also been found to normalize deficient proliferation rates in ovariectomized rats (Perez-Martin, Azcoitia, Trejo, Sierra, & Garcia-Segura, 2003). However, the relationship between estrogen and neurogenesis has been found to differ as a result of the time of exposure to, length of exposure to, and amount of estrogen (Galea et al., 2006). This relationship has also been found to be dependent on the presence of other hormones, as progesterone has been found to reduce the enhanced proliferation effect of estrogen (Galea et al., 2006). Although a great amount of research has been conducted to investigate the effect of estrogen on neurogenesis, the effects of testosterone are much less clear. However, there is some evidence to suggest that testosterone also increases neurogenesis through enhanced cell survival, but may have no effect on cell proliferation (Galea et al., 2006).

Although recent research has focused on both the potential cognitive and neuro-protective effects of endogenous hormones, the effects of exogenous hormone treatments on neurogenesis remain less clear. Specifically, while a growing body of research has focused on the potential beneficial effects of hormone replacement therapy in postmenopausal women (Erickson, Voss, Prakash, Chaddock, & Kramer, 2010), little research has investigated the effects of contraceptives, despite their growing popularity.

Recent evidence from a rat study suggests that clinically relevant progestins

may affect neurogenesis when combined with ethinyl estradiol (Liu et al., 2010). Interestingly, the results suggest differential effects of each combination when administered in doses comparable to those used in commonly used contraceptives. Specifically, when combined with ethinyl estradiol, combinations containing Levonorgestrel (Alesse®, Aviane®) and Medroxyprogesterone Acetate (Depo-Provera®) result in increased cell proliferation within the dentate gyrus. However, combinations containing the same progestins, as well as a combination containing Nestorone, also increase apoptosis rates. Unfortunately, this study only included these three clinically relevant combinations, however, the results do suggest that chronic exposure to clinically administered combinations of contraceptives could have long-term implications for neural function, and regenerative capacity of the brain. Therefore, the effects of these combinations need to be further investigated.

Alcohol

The deleterious effects of alcohol on the hippocampus are well documented. However, the mechanisms through which alcohol produces these effects are less understood. Some recent research has focused specifically on the effects of alcohol on dentate gyrus neurogenesis. Evidence suggests that alcohol affects neurogenesis in a manner that is dependent upon duration and pattern of exposure.

For example, Nixon and Crews (2002) exposed adult rats to either an acute or chronic binge exposure paradigm where they received either an individual exposure to ethanol or exposures three times per day for four days. Neural progenitor cell proliferation rates were assessed five hours after treatment and cell survival rates were assessed 28 days after treatment. The results suggest that both acute

and chronic alcohol exposure decrease neural progenitor cell proliferation rates. Additionally, chronic exposure was found to decrease cell survival, a result not seen with acute alcohol exposure. Similar findings have been demonstrated in subsequent research in rats (J. He, Nixon, Shetty, & Crews, 2005; Richardson et al., 2009). These results suggest that alcohol can negatively impact neurogenesis levels even after as little as a single binge-exposure. Furthermore, prolonged binge-exposure can produce significant decreases in neurogenesis levels that can be seen up to one month after last exposure (Nixon & Crews, 2002). These findings have serious implications for todays youth with the increasing prevalence of bingeing.

The adolescent hippocampus has been found to be particularly susceptible to the deleterious effects of alcohol. Indeed, several neuroimaging studies on human adolescents have shown that alcohol consumption during this period of development can result in significant structural atrophy and dysfunction (De Bellis et al., 2000; Medina, Schweinsburg, Cohen-Zion, Nagel, & Tapert, 2007).

Additionally, as in the adult hippocampus, alcohol has also been found to decrease neurogenesis in the adolescent rodent dentate gyrus (Crews, Mdzinarishvili, Kim, He, & Nixon, 2006; Morris, Eaves, Smith, & Nixon, 2010). Interestingly, Crews et al. (2006) found that the severity of this effect was dose-dependent. Acute doses of ethanol were found to significantly decrease neural progenitor cell proliferation rates such that the severity of the reduction significantly grew with increasing doses. Additionally, while only a slight reduction in neural progenitor cell survival rates was observed after low and moderate doses of ethanol, a high dose resulted in a nearly 50% reduction in cell survival rates. Importantly, this reduction was also seen 28 days following exposure. Although a similar study

has not been performed in adults, the results from this study suggest that even an acute bingeing event can result in a long-lasting significant reduction in cell survival rates in the adolescent hippocampus.

Recently, a similar long-lasting reduction in neurogenesis has also been found in adolescent nonhuman primates following chronic binge consumption that resulted in significant reductions in both proliferation and survival rates (Taffe et al., 2010). This decline was observed 2 months after alcohol discontinuation. Importantly, blood alcohol levels reached were equivalent to human blood alcohol levels typically observed during intoxication (Taffe et al., 2010). As previously stated, adolescence is marked by high developmental change and plasticity, including high levels of hippocampal neurogenesis (J. He & Crews, 2007). As such, these results suggest that alcohol consumption may present more severe repercussions in adolescence than adulthood as there may be a greater net "loss" in neurogenesis (Nixon, Morris, Liput, & Kelso, 2010). This may produce severe functional consequences that may extend into adulthood.

Taken together, the evidence from both adult and adolescent populations provide evidence that alcohol has a significant impact on hippocampal neurogenesis. Moreover, the adolescent brain has been found to be particularly susceptible to the adverse effects of alcohol (De Bellis et al., 2000; Medina et al., 2007). As such, it is suggested that alcohol may have a greater impact on adolescent neurogenesis than adult neurogenesis (Nixon et al., 2010). Indeed, a comparison of results from various studies provides support for this hypothesis (Crews et al., 2006; Nixon & Crews, 2002).

While it is clear that alcohol impacts neurogenesis in adolescence, it is unclear as to how long its effects last and whether these effects extend into adulthood, after a prolonged abstenance from bingeing. Although some research has suggested the possibility of long-lasting impacts on neurogenesis, this research has not investigated longitudinal impacts (Crews et al., 2006; Richardson et al., 2009; Taffe et al., 2010). Furthermore, it remains unclear whether these reductions in neurogenesis during this critical period of development result in functional deficits that can be later identified in early adulthood.

Exercise

Exercise has been shown to result in various physical and mental health benefits on the brain and cognition and has also been shown to increase dentate gyrus neurogenesis. In mice, voluntary wheel running has been shown to increase cell proliferation and survival (van Praag, Christie, Sejnowski, & Gage, 1999; van Praag, Kempermann, & Gage, 1999). Exercise has also been shown to ameliorate the age-related decline of neurogenesis. van Praag et al. (2005) introduced young and aged mice to environments without a running wheel or with unlimited access to a running wheel. After one month, both young and aged runners showed significantly greater levels of dentate granule cell proliferation than non-runners. Although this effect was more pronounced in the young runners, running significantly decreased the age-related neurogenic decline. Importantly, the morphology of the newly generated neurons did not differ between young and aged runners, suggesting that the initial maturation of newborn neurons was not affected by ageing.

Furthermore, Pereira et al. (2007) used the established correlation between neurogenesis and angiogenesis (the development of new blood vessels) to demonstrate that a two week-long exercise regime significantly increased MRI measurements of dentate gyrus cerebral blood volume in mice, and that this increase significantly correlated with dentate gyrus neurogenesis. This result suggests that exercise differentially targets the dentate gyrus within the hippocampal formation. It has also been shown that short-term moderate treadmill exercise increases cell proliferation (Ferreira, Real, Rodrigues, Alves, & Britto, 2011). Although all of the above evidence for the link between neurogenesis and exercise was based on research conducted in mice and rats, evidence does suggest that this link appears in humans as well.

In addition to examining the correlation between dentate gyrus cerebral blood volume in mice, Pereira et al. (2007) also used MRI to examine this correlation in humans. Healthy subjects completed a three month aerobic exercise regimen. While using maximum volume oxygen consumption to account for individual difference in degree of exercise, results suggest that exercise had a primary effect on dentate gyrus cerebral blood volume, which in turn correlated with change in maximum volume oxygen consumption. Importantly, this latter correlation was not observed for any other hippocampal region, suggesting that exercise selectively affected dentate gyrus blood volume. These results are analogous to the results found in mice, suggesting that it is possible that neurogenesis in humans may be influenced in the same manner. Additionally, recent research has replicated this correlation between dentate gyrus cerebral blood volume increase and exercise (Dery et al., 2011), and furthermore, has demonstrated a selective cognitive

enhancement on neurogenesis-dependent memory tasks (described in more detail below).

Stress and depression

Perhaps the most potent and extensively researched factor that impacts the generation of new neurons, stress effectively inhibits neurogenesis. In adult rats, acute stress in the forms of predator odour and electric shock have been shown to decrease granule cell proliferation (Tanapat, Hastings, Rydel, Galea, & Gould, 2001; Malberg & Duman, 2003). Additionally, acute social defeat has been shown to decrease survival rates in rats (Yap et al., 2006), and to decrease cell proliferation in tree shrews and marmosets (Gould, McEwen, Tanapat, Galea, & Fuchs, 1997; Gould, Tanapat, McEwen, Flugge, & Fuchs, 1998). More specifically, Lee et al. (2006) investigated the effects of chronic mild stress on proliferation, differentiation, and survival of new-born hippocampal cells. Rats were exposed to chronic mild stress for 19 days. When compared to controls, the stressed rats displayed no differences in proliferation or differentiation of new-born cells. However, the stressed rats did exhibit significantly decreased cell survival. Taken together, these results suggest that both acute and chronic stress seem to inhibit neugogenesis.

The above findings are in line with the well documented effect of stress on the hippocampus. In the event of stress, the hypothalamic-pituitary-adrenal (HPA) axis is activated. This activation is accompanied by the increased release of glucocorticoids (corticosterone in rats and cortisol in humans). The hippocampus has been shown to be negatively impacted by chronic exposure to glucocorticoids (Joëls, 2008). More specifically, glucocorticoids have been suggested to inhibit

hippocampal neurogenesis, as administration of glucocorticoids has been shown to decrease both cell proliferation and survival (Brummelte & Galea, 2010). Conversely, suppression of glucocorticoid release has resulted in increased cell proliferation (Gould, Cameron, Daniels, Woolley, & McEwen, 1992). These results seem to suggest that stress-induced decreases in neurogenesis are causally linked to increased glucocorticoid levels.

The link between neurogenesis and depression relies on a few key lines of evidence. Stress is widely believed to be a major risk factor in the development of depression (e.g. Swaab, Bao, & Lucassen, 2005; de Kloet, Joëls, & Holsboer, 2005), and stress is a potent inhibitor of neurogenesis (see above). Additionally, many factors that aid in treating depression also increase neurogenesis. Both exercise and antidepressant drugs increase neurogenesis and have been shown to be effective in treating depression (Marais, Stein, & Daniels, 2009; Duman, Nakagawa, & Malberg, 2001). Finally, evidence from a recent study provides evidence that suggests that suppressed neurogenesis may increase vulnerability to the development of depression. When compared to controls, neurogenesis-ablated mice exhibited circulating glucocorticoid levels that were slower to recover to baseline levels following both single and repeated exposure to restraint stress (Snyder, Soumier, Brewer, Pickel, & Cameron, 2011). Additionally, neurogenesis-ablated mice displayed abnormal behavioural responses after stress and showed signs of depression, including becoming immobile more quickly and for a greater duration during a forced swim test when compared to controls, as well as displaying anhedonic behaviours. These results strongly suggest that new neurons may play a crucial role in buffering stress responses and preventing the emergence of a depres-

sive phenotype. Taken together, these results suggest that neurogenesis may play a crucial role in both the development and recovery from depression.

Interaction between regulators

The factors that regulate neurogenesis certainly interact, and the action may either be synergistic or they may counteract one another. As previously mentioned, the combination of age and stress results in a significant decrease in cell proliferation that can be ameliorated with the removal of stress (Nichols et al., 2001; Cameron & McKay, 1999). Additionally, exercise has been shown to reliably counteract the effects of age-related neurogenesis decline as well as the negative effects of stress on neurogenesis in both young and aged populations (van Praag et al., 2005; Kannangara et al., 2011; Snyder, Glover, Sanzone, Kamhi, & Cameron, 2009). Exercise has also been shown to reverse the inhibition of neurogenesis that is associated with alcohol consumption (Crews, Nixon, & Wilkie, 2004). These interactive effects suggest that is crucial to keep in mind that neurogenesis levels are the result of a balance between a multitude of factors.

1.3.4 Functional relevance

Since it's discovery in 1965, much has been learned about the development and regulation of hippocampal neurogenesis. However, there is still much debate surrounding the functional relevance of these newborn neurons. As neurogenesis does occur within the adult hippocampus, it is generally believed that these new neurons play a role in the processes of learning and memory. This belief is supported by the fact that hippocampal neurogenesis occurs within the dentate gyrus, a structure that has convincingly been found to play a critical role in the avoid-

ance of interference in memory formation and recall (Bakker et al., 2008; Yassa, Stark, et al., 2010; Yassa, Lacy, et al., 2010; Shing et al., 2011; Hunsaker et al., 2008; Gilbert et al., 2001). Although still debated, computational models suggest mechanisms by which neurogenesis may aid the dentate gyrus in this function.

Evidence from computational models suggests that neurogenesis allows new information to be selectively encoded by newborn neurons, thereby sparing previously encoded information (Becker, 2005; Weisz & Argibay, 2009; Aimone, Wiles, & Gage, 2009). Indeed, simple neural networks have shown that the gradual addition of new neurons into the existing network of the dentate gyrus allows the hippocampus to encode new information without interfering catastrophically with older memories (Wiskott, Rasch, & Kempermann, 2006). Furthermore, a key characteristic of these computational models is that new neurons are predicted to have differential effects on learning and memory at different time scales (Becker, Macqueen, & Wojtowicz, 2009; Weisz & Argibay, 2009; Aimone et al., 2009).

Over long time scales, neurogenesis is proposed to aid in pattern separation. As time elapses, new neurons continue to be generated. As such, the pool of neurons available on a given day differ greatly from that available many days later. Therefore, when similar stimuli are encountered at two different times spaced days apart, the constant supply of new neurons results in very distinctive neuronal representations, as each event is encoded by a separate population of neurons (Figure 2). This results in the avoidance of potential interference between the events (Becker, 2005). Additionally, as they mature, neurons become less excitable and presumably become more selective when responding to input (Ge et al., 2007). As such, neurons that fire in response to one context will not fire in response to

a different context. This, again, ensures a unique neuronal representation of each event.

The hypothesis that neurogenesis is necessary for the reduction of interference predicts that, in the absence of neurogenesis, the ability to generate unique neuronal representations of similar events will be lost. As such, this should result in substantial deficits in distinguishing between highly similar memories. It is important to note that these hypotheses do not predict that a reduction in neurogenesis will result in deficits in all forms of learning and memory. Instead, it is predicted that the behavioural deficits associated with depleted neurogenesis will be specific to tasks that present a high potential for interference between memories. However, within a given context, these new neurons are predicted to possess a different role.

At short time scales, immature neurons have been hypothesized to help link elements of an episode together. As previously mentioned, immature neurons are more plastic than their mature counterparts and possess lower thresholds for LTP and firing activation (Ge et al., 2007; Wang et al., 2000; Snyder et al., 2001). These characteristics are said to make them "hyperexcitable". In this hyperexcitable state, immature neurons may express less discrimination when responding to input. As such, they are more likely to be incorporated in neural representations, adding similarity between encoded events and acting as a "contextual glue" (Aimone et al., 2009; Becker et al., 2009). This process is often referred to as "pattern integration".
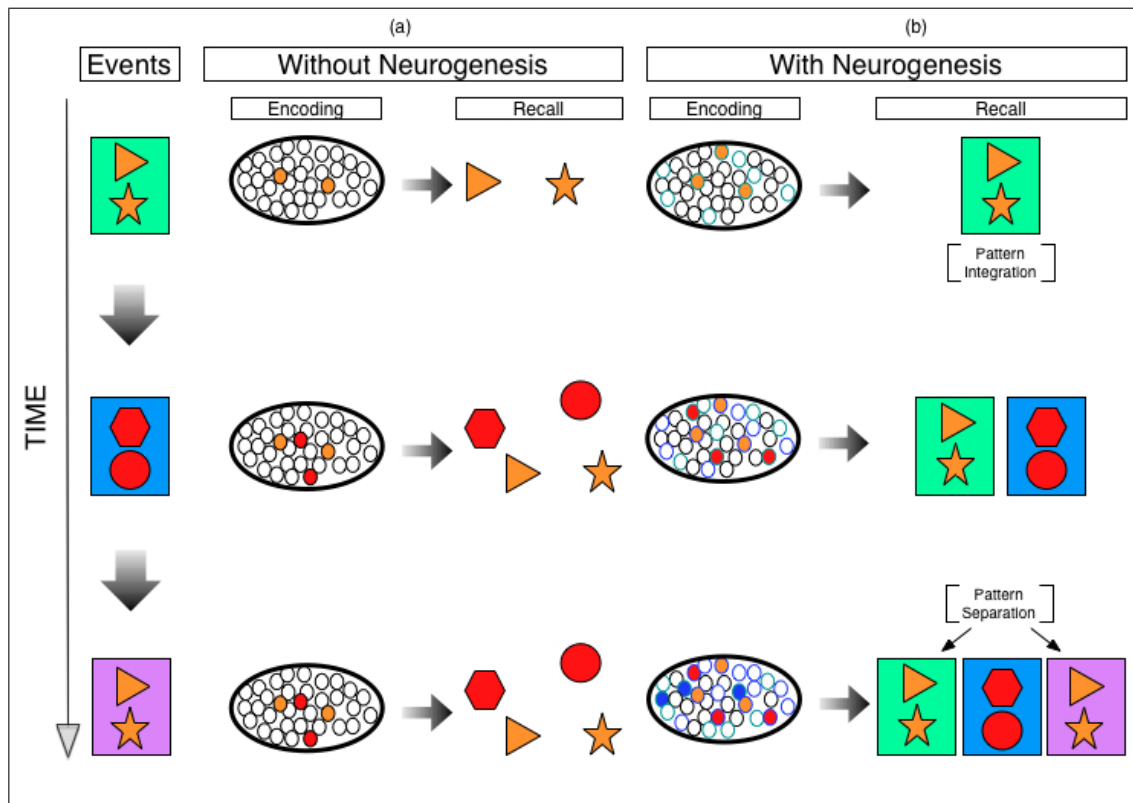
Figure 2: **Hypothesized roles of neurogenesis**. The above diagram displays the encoding of events (left) across time in the dentate gyrus and the output from recollection as a result of this encoding. **(a)** Events encoded without neurogenesis are encoded very separately. Temporal relationships and context are not encoded and highly similar events are not distinguishable from one another. **(b)** Events encoded with neurogenesis are separated yet temporally organized. The addition of new neurons in the dentate gyrus allows temporal information and context to be encoded. Pattern integration binds together the events that share a common context. As time progresses, neurons mature and a new set of neurons are born. Encoding occurs on these new neurons and the encoding of a new context allows the new events to be encoded separately from the previous events. Pattern integration results in a gradually evolving spatiotemporal context. As time continues, neuronal turnover ensures that another set of neurons is used for encoding. The use of the new neurons ensures that even highly similar events are encoded and recalled uniquely, due to the encoding of distinct contexts.

It may seem that pattern integration counteracts or opposes pattern separation. However, it has been suggested that pattern integration may play a role that is complementary to pattern separation. As immature neurons may be thought to act as a "contextual glue", they may function to contribute to a gradually evolving representation of spatiotemporal context that links components of an episode across time (Becker & Wojtowicz, 2007). As proliferation occurs in clusters, hypothetically, one can envision 'waves' of neurons that respond to different aspects of an episode (Becker & Wojtowicz, 2007) . While some neurons respond to more transient aspects of an episode, others may respond to persistent aspects of the environment, such as odours, stationary objects and boundaries. These persistent aspects of the environment form the context of an episode. The neurons that respond to the context will become tuned to this combination of static components and should respond consistently when they experience the same context again (Becker & Wojtowicz, 2007). These neurons may then aid in linking transient events together with the context, to form the representation of a single episode. As such, it is hypothesized that neurogenesis is critical for bridging information across time delays (Becker et al., 2009).

At the same time, while pattern integration may aid in linking together events that share a similar context, it may also function to improve pattern separation, as similar events will be encoded uniquely with their own respective contexts. The hypothesis that immature neurons may function as a contextual glue predicts that an absence of neurogenesis will result in entirely separate neuronal representations of events that share a similar spatiotemporal context. These events will share no representational link and will be encoded uniquely and recalled indepen-

dently. It is easy to conceive how such encoding could be problematic for recollection. However, encoding events without binding them to a common spatiotemporal context could conversely be beneficial in situations in which the context could be misleading.

For example, Saxe et al. (2007) presented mice with a radial arm maze task. In the sample phase of the task, the animals were required to learn the location of a sample arm. In the test phase, the sample arm and an additional arm (the testing arm) were made accessible and the mice were required to learn to retrieve food pellets from the test arm. Interestingly, neurogenesis-ablated mice performed better than mice with normal neurogenesis rates if the sample arms and test arms were employed repeatedly and interchangeably in different trials (Saxe et al., 2007). Successful performance on these high interference trials required that each trial was approached individually, without any bias due to previous experience in a similar situation. These results suggest that the absence of immature neurons, and therefore the absence of pattern integration or the contextual glue, results in a unique approach to each trial without regard or influence of previous experience with a similar event in the same context. Therefore, these results are in line with the predicted role of immature neurons at short time scales and within a context.

Thus, at both short and long time scales, computational models suggest that the role of hippocampal neurogenesis is to allow the unique encoding of highly similar events. Evidence from animal research, and more recently human research, provides support for this role.

Support for the role of neurogenesis in interference reduction stems from research on animals with depleted neurogenesis by means of irradation. Clelland et al. (2009) used irradiation to focally ablate neurogenesis in the hippocampus of 8-week old adult female mice. Two months after irradiation, the mice were tested in a delayed nonmatching to place (DNMP) radial arm maze task. During the sample phase, the mice were permitted to visit a sample arm and retrieve a food pellet reward. During the choice phase, the sample arm and an additional, novel arm were opened and mice received a reward for selecting the novel (nonmatching) arm. Importantly, the distance between the novel arm and sample arm varied. Results from the study suggest that, while irradiated mice performed similarly to controls at high separations distances, they were significantly impaired at the low arm separation distance. Similar results were obtained from a task that utilized a touchscreen device for spatial discrimination (Clelland et al., 2009). These results suggest that neurogenesis is necessary for successful spatial discrimination only when there is a high degree of similarity between events. Interestingly, object recognition memory remained unaffected after neurogenesis irradiation, suggesting that neurogenesis is not required for successful object recognition.

Further support for the necessity of neurogenesis for successful pattern separation has been demonstrated in studies of contextual fear memory (Saxe et al., 2006; Winocur, Wojtowicz, Sekeres, Snyder, & Wang, 2006; Wojtowicz, Askew, & Winocur, 2008), and trace conditioning (Shors et al., 2001). Here, again, results suggest that not all types of memory require neurogenesis as irradiation was found to have no effect on cued fear conditioning (Saxe et al., 2006; Winocur et al., 2006) and delay conditioning (Shors et al., 2001). Furthermore, recent evidence

from rats with suppressed hippocampal neurogenesis suggests that neurogenesis is necessary for the differentiation between conflicting, context-dependent memories (Winocur, Becker, Luu, Rosenzweig, & Wojtowicz, 2012). This deficit was eliminated in irradiated animals that exercised using a running wheel, suggesting that voluntary exercise, which has been shown to increase neurogenesis in the dentate gyrus, may sufficiently restore normal neurogenesis levels and functioning on hippocampally-dependent tasks (Winocur et al., 2012).

Interestingly, additional research suggests that other factors that have been shown to negatively impact neurogenesis levels may also have impacts on cognition. Factors such as alcohol, stress, and ageing, have also been demonstrated to impact performance specifically in hippocampally-dependent tasks associated with interference (Hunt, Levillain, Spector, & Kostelnik, 2009; McCormick, Nixon, Thomas, Lowie, & Dyck, 2010; Yassa, Lacy, et al., 2010).

Taken together, these results provide strong support for the hypothesized role of neurogenesis in interference avoidance and suggest that hippocampal neurogenesis is necessary for the ability to discriminate between highly similar events to protect older memories from interference. However, support for the involvement of neurogenesis in pattern separation in human behaviour is much more scarce.

To date, few studies have provided support for the role of neurogenesis in interference avoidance in humans. This has largely been due to the lack of a direct means of non-invasively measuring neurogenesis in humans; however recent methods have been developed (see above; Pereira et al., 2007). Although exercise can be administered, it is unethical to subject humans to neurogenesis-depleting

treatments. Instead, it is possible to assess the performance of individuals that are likely to have reduced neurogenesis levels such as those that are highly depressed/stressed, partake in little to no physical exercise, and/or have high alcohol intake.

In one study, undergraduate students were administered neuropsychological inventories for stress, depression and anxiety. Among other cognitive tasks, they were administered a delayed match to sample (DMS) task that resembles the DNMP task found to be dependent upon neurogenesis in animals (Clelland et al., 2009). In the DMS task, participants were shown an abstract target image. After a varying delay, participants were required to identify the target image among three highly similar images. Interestingly, after controlling for baseline performance, when compared to non-depressed peers, participants with depression scores that indicated severe depression performed significantly worse at the longest (12 second) delay (Becker et al., 2009). As depression has been shown to negatively impact neurogenesis levels (Tanapat et al., 2001; Malberg & Duman, 2003), these results suggest that those likely to possess reduced neurogenesis levels perform significantly worse when there is a great potential for interference. This deficit is hypothesized to result from a failure in the ability to discriminate between highly similar stimuli. This result provides support to the hypothesis that neurogenesis is critical on tasks that require bridging information across time delays.

Additionally, exercise, which has also been shown to impact neurogenesis (van Praag, Christie, et al., 1999; van Praag, Kempermann, & Gage, 1999; Pereira et al., 2007; Winocur et al., 2012), has been demonstrated to affect performance on putatively neurogenesis-dependent tasks (Dery et al., 2011). In particular, in-

dividuals that displayed a fitness benefit from an intense 6-week exercise program displayed superior performance on a high interference condition of a common visual pattern separation task that has been hypothesized to require dentate gyrus pattern separation (Kirwan & Stark, 2007; Yassa, Lacy, et al., 2010; Dery et al., 2011). In accordance with the findings of Becker et al. (2009), these participants also demonstrated superior performance on the DMS task at the longest delay.

Therefore, although limited, evidence from humans seems to suggest that neurogenesis may play a vital role in the avoidance of interference between memories. Furthermore, these studies suggest that it is viable to effectively gauge neurogenesis levels and performance on putatively neurogenesis-dependent tasks via neurogenesis correlates such as stress/depression levels and other lifestyle factors.

Together, the hypothesized roles of neurogenesis at both long and short time scales provide an interesting area of investigation. The results from the Saxe et al. (2007) study suggest that the ablation of neurogenesis results in enhanced performance on a working memory task in situations where is it beneficial to avoid pattern integration. However, the effect of neurogenesis in humans in such a situation remains to be investigated.

Computational model suggests that, at short delays and within a context, neurogenesis should help create a continuously evolving spatio-temporal context (Becker & Wojtowicz, 2007; Aimone et al., 2009). Here, a reduction in neurogenesis should result in a lack of binding between related events and their context. Additionally, at longer delays and between contexts, neurogenesis should aid in the differential preservation of overlapping memories, via interference avoidance,

and a reduction of neurogenesis in such situations should result in increased susceptibility to interference.

Therefore, a comparison between the functional role of neurogenesis in a situation where it may aid and a situation where it may hinder performance needs to be conducted. More specifically, an investigation needs to be conducted on the effects of normal versus depleted neurogenesis on a task that provides both neurogenesis-aiding and hindering situations. Such a task should, at times, require the successful avoidance of interference between highly similar events and the utilization of pattern integration and separation in order to allow contextual-dependent memory to inform behaviour. At others times, however, this task should require that highly similar events be approached uniquely, as acting based upon prior experience in a similar situation will hinder performance.

In order to test performance on such a task, we have created the Concentration Memory Task (CMT), which is a variation of the classic card game known as "Concentration" or "Memory". In the CMT, we have instilled variations that present situations in which it is beneficial to utilize context-dependent memory to successfully avoid interference between highly similar events. We have also instilled variations that present situations in which is it beneficial to ignore context-dependent memory, as acting according to previous experience with a similar situation will hinder performance.

Therefore, in accordance with the roles of neurogenesis suggested by computational theories and previous research, we hypothesize that, compared to individuals expected to possess normal neurogenesis levels, individuals that are expected

to possess reduced neurogenesis levels will perform worse in situations where the ability to ability to avoid interference between similar events and utilize context-dependent memory to guide behaviour will aid performance. Conversely, we hypothesize that these individuals will perform better than individuals expected to possess normal neurogenesis levels in situations where it is beneficial to ignore or avoid utilizing context-dependent memory to guide behaviour and approach individual trials uniquely.

Methods and Materials

2.1 Participants

The protocol for this study was approved by the McMaster Research Ethics Review Board (MREB). Participant were 109 female McMaster University undergraduates (mean age = 19.01, SD = 1.92) with normal or corrected-to-normal vision. Of these participants, 70 participants completed the CMT task and 38 completed the visual pattern separation task. All participants had never been diagnosed with or treated for any psychiatric disorder to avoid any potential influence of treatment on neurogenesis levels. Data from an additional eight participants was excluded from data analysis. Eight participants were excluded for having been previously diagnosed with a mood disorder (7 with Major Depressive Disorder, 1 with Bipolar Disorder), and one participant was excluded on the basis of low performance/compliance on the CMT. Participants received course credit as compensation for their participation and informed consent was given prior to study initiation.

2.2 Inventories

2.2.1 Stress, depression, and anxiety inventories

Perceived Stress Scale (PSS)

Participants were administered Cohen's Perceived Stress Scale (PSS) to assess stress levels. The PSS consists of 10 multiple-choice questions that ask participants to rate perceived stress during the past month on various measures on a 5-point scale where a response of 0 indicates "never" and a response of 4 indicates "very often" (e.g., "In the last month, how often have you felt nervous and stressed?"). A stress score is obtained by reversing responses to positively stated items and then summing across all scale items. A higher score indicates a higher level of perceived stress.

Beck Depression Inventory (BDI)

Participants were also administered the Beck Depression Inventory (BDI). The BDI consists of 21 multiple-choice questions that assess participant mood during the past month and each question has a set of at least four possible answer choices, ranging in intensity (e.g., "Sadness: 0 = I do not feel sad, 1 = I feel sad, 2 = I am sad all the time and I can't snap out of it, 3 = I am so sad and unhappy that I can't stand it"). A depression score is obtained by summing across the values associated with each selected response. The depression score ranges from 0 - 63, where a score from 0 - 13 is associated with minimal depression, 14 - 19 associated with mild depression, 20 - 28 associated with moderate depression, and 29 - 63 associated with severe depression.

Beck Anxiety Scale (BAI)

Finally, participants were administered the Beck Anxiety Scale (BAI). Like the BDI, the BAI consists of 21 multiple-choice questions that possess at least four possible answer choices and measures the severity of an individuals anxiety during the past month (e.g., "Unable to relax: 0 = Not at all, 1 = Mildly but it didn't bother me much, 2 = Moderately - it wasn't pleasant at times, 3 = Severely - it bothered me a lot"). An anxiety score is obtained by summing across the values associated with each selected response. The anxiety score ranges from 0 - 63, where a score from 0 - 7 indicates a minimal level of anxiety, 8 - 15 indicates mild anxiety, 16 - 25 indicates moderate anxiety, and 26 - 63 indicates severe anxiety.

2.2.2 Lifestyle Questionnaire

To assess the effects of the various neurogenesis-effecting lifestyle factors discussed earlier, a lifestyle questionnaire was administered to all participants (See Appendix A). The lifestyle questionnaire assessed alcohol consumption patterns and history at various time-points, including within the two weeks, three months, and the year prior to testing. More specifically, participants were asked about average daily consumption, and the most alcohol consumed within a single episode at the each of the various time points. The lifestyle questionnaire also assessed current and previous exercise regime, specifically pertaining to aerobic, cardiovascular, and resistance training. The questionnaire also assessed hormonal factors, including contraceptive use history. Participants were asked if they were currently using or previously used any form of contraceptive (i.e., oral-contraceptive, intrauterine device, etc.). Furthermore, participants with exposure to a contraceptive were asked

to indicate the brand name of the contraceptive and length of time that they used each contraceptive. To assess menstrual cycle phase, participants were also asked the start date of their last menstrual period and the expected start date of their next menstrual period, and were also asked to gauge cycle regularity on a likert scale.

2.3 Cognitive Tasks

2.3.1   Concentration Memory Task

In order to investigate the relationship between neurogenesis, pattern separation, and working memory, we created the Concentration Memory Task (CMT) (Figure 3). The CMT is a visual-spatial task with a high potential for interference that heavily relies on spatial working memory. Participants were presented with a grid of facedown cards and the objective of the game was to locate matching cards. At the beginning of each trial, a card briefly "flipped-over", revealing its underlying object. This object represented the "target" object.

The participant was then asked to classify this object as either "new", "old", or "similar". If the participant believed that they had not previously seen this object, or an object like it, within the context of the CMT, they were instructed to identify it as "new". If they believed that they had been presented with this exact object previously in the CMT, they were instructed to identify it as "old". Finally, if they believed that they had not seen this exact object in the CMT, but believed that they had been presented with an object that resembled it, they were instructed to identify it as "similar".

Importantly, no feedback was given once object classification was made. After classifying the target object, the card flipped back over and the participant was presented with the face-down side of every card. They then attempted to locate the matching card, which possessed an object identical to that of the target object, selecting as few cards as possible to locate it. Selections were made by "touching" the cards displayed on a touchscreen device. Once a participant selected a card, if they failed to locate the matching card, they were notified that they had selected an incorrect card and were prompted to make a different selection. After this notification, they were once again displayed the grid of face-down cards and continued to select cards until they located the matching card.

Once the participant had located the matching card, this card flipped-over and revealed the target image once again. The next trial then commenced, at which point a new target object was revealed and the participant attempted to locate the matching card for that target object. This cycle continued until the participant had successfully completed all trials by locating all of the matching cards in the game. Once the participant had completed a game, they were notified that they had made all of the matches and that they were going to begin a new game. The task consisted of a total of four games and each game possessed 12 trials (12 pairs of cards, for a total 24 cards in each game). Each game was associated with a unique card colour that made it easy for the participant to identify when they had began a new game.

At this point, it is important to make a few notes. The target object was randomly selected at the beginning of each trial and it was only revealed to the participant once. The participant was not able to view it again for the remainder
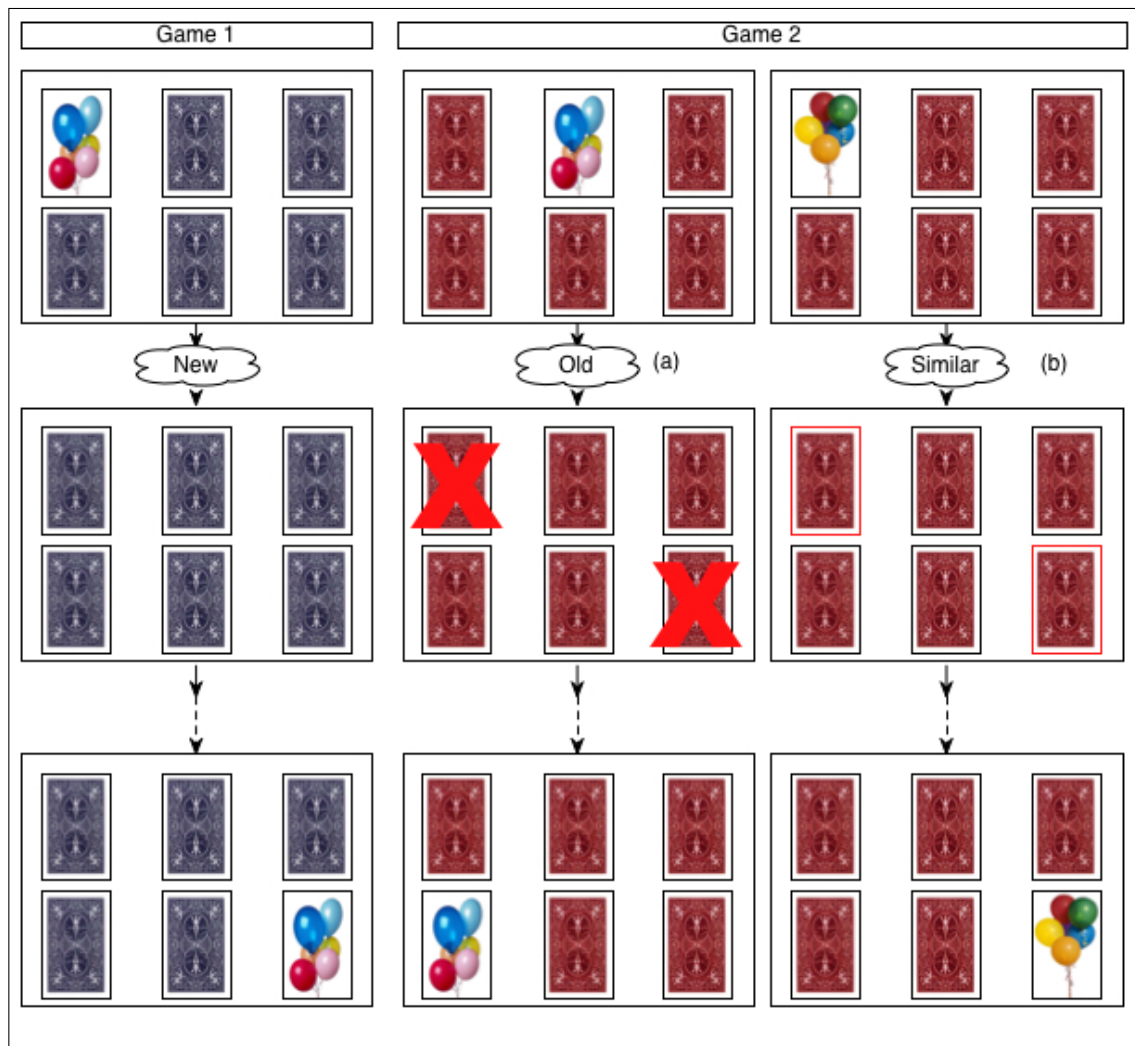
Figure 3: **Concentration Memory Task**

of the trial.

It is also important to note that, once selected, an incorrect card would not reveal its underlying object. This manipulation ensured that the level of difficulty was constant for all participants. Previous piloting suggested that if incorrect selections revealed underlying objects, it became beneficial to make numerous errors at the beginning of a game, as doing so allowed a participant to view the locations of various objects. Once these objects were subsequently presented as target objects, the participant possessed prior knowledge about the location of the matching card, decreasing the difficulty of the task. By ensuring that incorrect selections did not reveal the underlying object, the only knowledge gained from an incorrect trial was that the target object was not located in the selected position. As such, there was no benefit to committing numerous errors.

Finally, all of the cards and their respective objects remained in their location and on the screen, face-down, for the entirety of each game. This manipulation ensured a high spatial working memory demand as successful performance required the utilization of both the location of matches made in previous trials, as well as search attempts within a current trial.

As the participant progressed throughout the task, subsequent games contained repetitions of previously encountered ("old") objects (Figure 3a). Participants were instructed that when they encountered an "old" object, none of the images of that pair would be located in their previous locations. In relation to the Saxe et al. (2007) study, "old" trials represented a situation in which it was beneficial to uniquely approach a previously encountered, highly similar event. If

neurogenesis did hinder performance in such a situation, it was predicted that reduced levels of neurogenesis would enhance performance on "old" trials.

In addition to "old" objects, subsequent games also contained instances of "similar" objects (Figure 3b). Participants were instructed that "similar" objects were always located in the previous positions of the images that they resembled. For example, in Game 1 a participant may have been presented with a set of balloons in the top-left and bottom-right corner positions. In game 2, if they may have encountered a similar set of balloons in the top-left corner. If they correctly determined that the second set of balloons was similar to the first set of balloons and recalled the previous locations of the first set, they could utilize this information to correctly guess that the match for the second set of balloons was located in the bottom-right corner position. Therefore, "similar" trials represented a situation in which it was beneficial to utilize neurogenesis' role in interference avoidance to be able to distinguish between the two presentations of balloons. As such, in the "similar" trials, it was predicted that reduced levels of neurogenesis would significantly hinder performance. This prediction is in line with the hypothesized role of neurogenesis in dissociating between highly similar events.

The remaining objects in each game were novel ("new") objects. New objects were located in random locations in each game and functioned as a control measure of performance on trials with no possibility of interference. There were an equal number of "old", "similar", and "new" trials in each game (i.e., 4 of each trial in each game).

Numerous measures of performance on the CMT were collected and, importantly, all of the following measures were recorded and calculated as a function of the participant's classification or misclassification of each target item. That is, performance on all measures pertaining to "old" items was recorded and calculated when an "old" item was correctly identified as "old" (old given old, or Old | Old), an "old" item was misidentified as "similar" (similar given old, or Similar | Old), and when an "old" item was misidentified as "new" (new given old, or New | Old). All measures pertaining to "similar" and "new" items were calculated in the same manner (i.e., Similar | Similar, Old | Similar, New | Similar, New | New, Similar | New, and Old | New).

The number of card selections required before locating the matching card was recorded and averaged for each trial. A measure of average search distance from the correct card location was also recorded. This distance was defined as the smallest number of cards between the correct card and the participant's search locations, including horizontal, vertical, and diagonal translations (see Appendix B). Finally, as previously mentioned, all "old" items possessed two previous locations and participants were instructed that "old" items would never be presented in these same locations in a subsequent trial. As such, returns to these locations suggest a susceptibility to interference such that a failure of pattern separation resulted in an inability to distinguish between contexts (i.e., games). Therefore, returns to previous locations of an "old" item when the "old" item was the target item, were recorded.

2.3.2 Delayed Match to Sample

Taken from the Cambridge Neuropsychological Test Automated Battery (CANTAB), the Delayed Matching to Sample (DMS) task (Figure 4a) is a forced choice recognition memory task that utilizes complex non-verbalizable patterns. The participant was shown a target pattern and then, either simultaneously or after a 0, 4, or 12 second delay, presented with four highly similar patterns. The participant was required to identify the target pattern among the very similar lures, which presented a high potential for interference. This potential for interference was at its highest at the 12 second delay. In line with the findings of Becker et al. (2009), it was predicted that a deficit in neurogenesis would hinder performance at the longest delay.

The number of selections necessary to locate the correct pattern was recorded and averaged across trials for all delays. The amount of time (in milliseconds) that elapsed before each selection was also recorded. Finally, total number of errors made was also recorded.

2.3.3 Visual Pattern Separation

The pattern separation task (Figure 4b) was adapted from Kirwan and Stark (2007), whereby, in the study phase, participants were shown a series of 16 images of familiar everyday objects for 2500 ms each with a 500 ms inter-trial interval. Following a brief delay, in the test phase, a series of images was presented and the participants were asked classify each object as new, old, or similar to an object presented in the previous set. Each study and test phase formed a block and
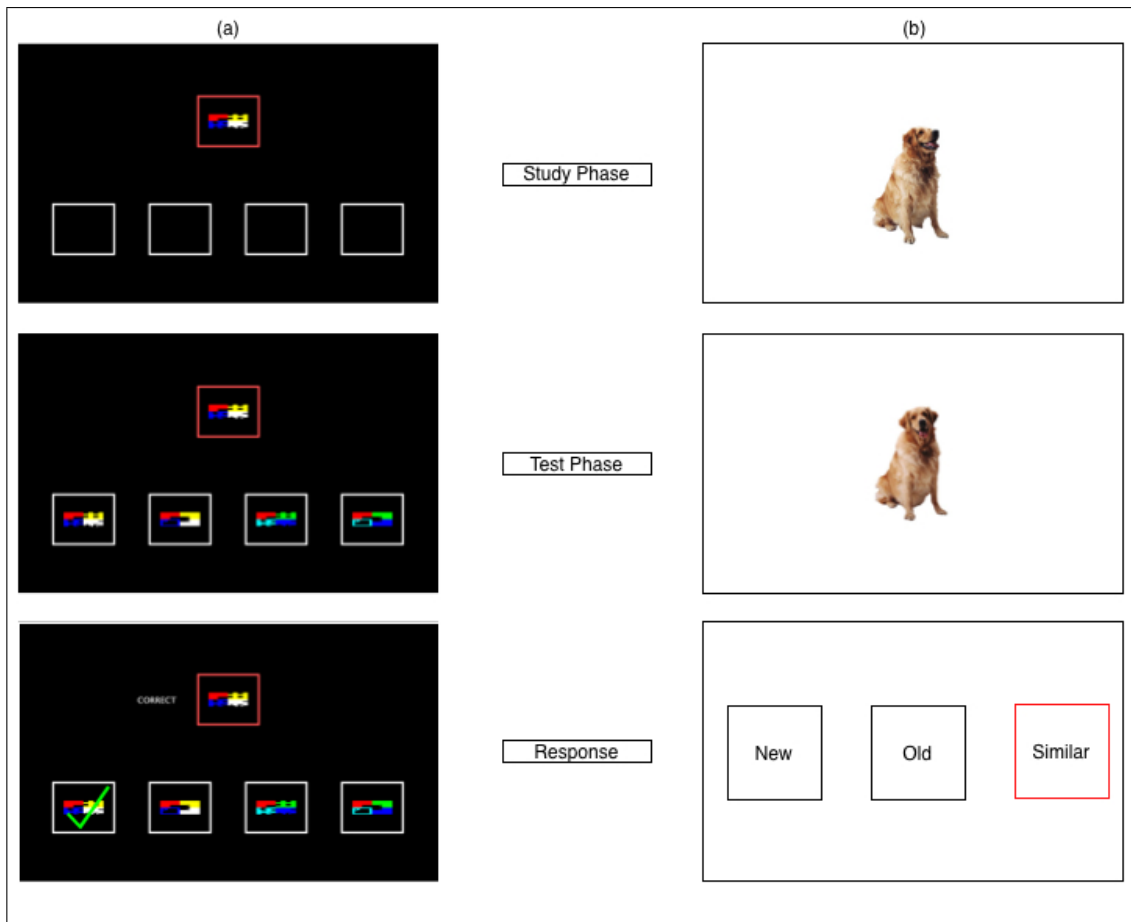
Figure 4: **Putative neurogenesis-dependent tasks** a) Delayed Match to Sample (DMS) b) Visual Pattern Separation

the task consisted of total of 8 blocks. Much like the DMS, the visual pattern separation task involved discrimination between previously learned patterns and highly similar lures, providing a potential source of interference. Furthermore, recent research suggests that performance on these high interference items may be neurogenesis-dependent (Dery et al., 2011). In line with the findings of Dery et al. (2011), it was predicted that an increase in neurogenesis would aid performance on high interference items and that a decrease in neurogenesis would hinder performance on high interference items.

2.3.4 Rapid Visual Processing

Another task taken from CANTAB, the Rapid Visual Processing (RVP) task (Figure 5a) is a test of sustained attention. In this task, a white box appeared in the centre of the laptop screen, inside which digits, from 2 to 9, appeared in a pseudo-random order, at the rate of 100 digits per minute. Participants were instructed to detect target sequences of digits (for example, 2-4-6, 3-5-7, 4-6-8) and to respond only to the last digit in each of the target sequences. The right trackpad mouse button was used to register responses. The probability of a hit and false alarm were calculated. This task was administered to control for any attentional deficits.

2.3.5 Paired Associate Learning

Also taken from CANTAB, the Paired Associate Learning (PAL) task (Figure 5b) is a hippocampally dependent, putatively non-neurogenesis-dependent, visual memory task. In this task, boxes were displayed on the touchscreen device and were opened in a randomized order. One or more of the boxes would contain an abstract pattern. Once all of the boxes had been opened, the revealed patterns were displayed in the middle of the screen, one at a time. The participant was then required to touch the box where each pattern was originally located. After placing all of the patterns, if the participant incorrectly placed at least one of the patterns, the patterns were represented to remind the participant of their locations. Therefore, correct placement of all of the presented patterns was required for progression through the task. The difficulty increased with progression through the task, with the number of displayed patterns increasing from 1 to 8 patterns. The

total number of presentations necessary to complete each trial and total number of errors made were recorded.

As mentioned, paired associate learning has been found to be hippocampally dependent, but does not seem to rely on the dentate gyrus or pattern separation for successful completion (Gilbert & Kesner, 2003; Nakashiba et al., 2008). As such, it was hypothesized that this task was not dependent on dentate gyrus neurogenesis and was administered to ensure that any correlations revealed between the putative neurogenesis-dependent tasks and measures of neurogenesis regulating factors was not due to global hippocampal memory deficits or variations between participants.

2.3.6   Backward Digit Span

The Backward Digit Span task was adapted from the protocol of Waters and Caplan (2003). In this task, participants were presented with a series of digits. Following presentation, they were required to repeat the series in the reverse order of presentation. Responses were entered using a keyboard. Digits were drawn from the digits 1 to 9 and presented pseudo-randomly. The span size of each sequence increased from 2 digits to 8 digits and there were five trials at each span size. Participants were instructed to insert a space, using the spacebar on the keyboard, if they knew that an item had been presented in a particular serial position but could not remember what the item was. The participants span was defined as the longest span size for which they correctly recalled all of items in the correct serial order on three out of five trials. An additional 0.5 was added if the participant was correct on two out of five trials at the next span size.
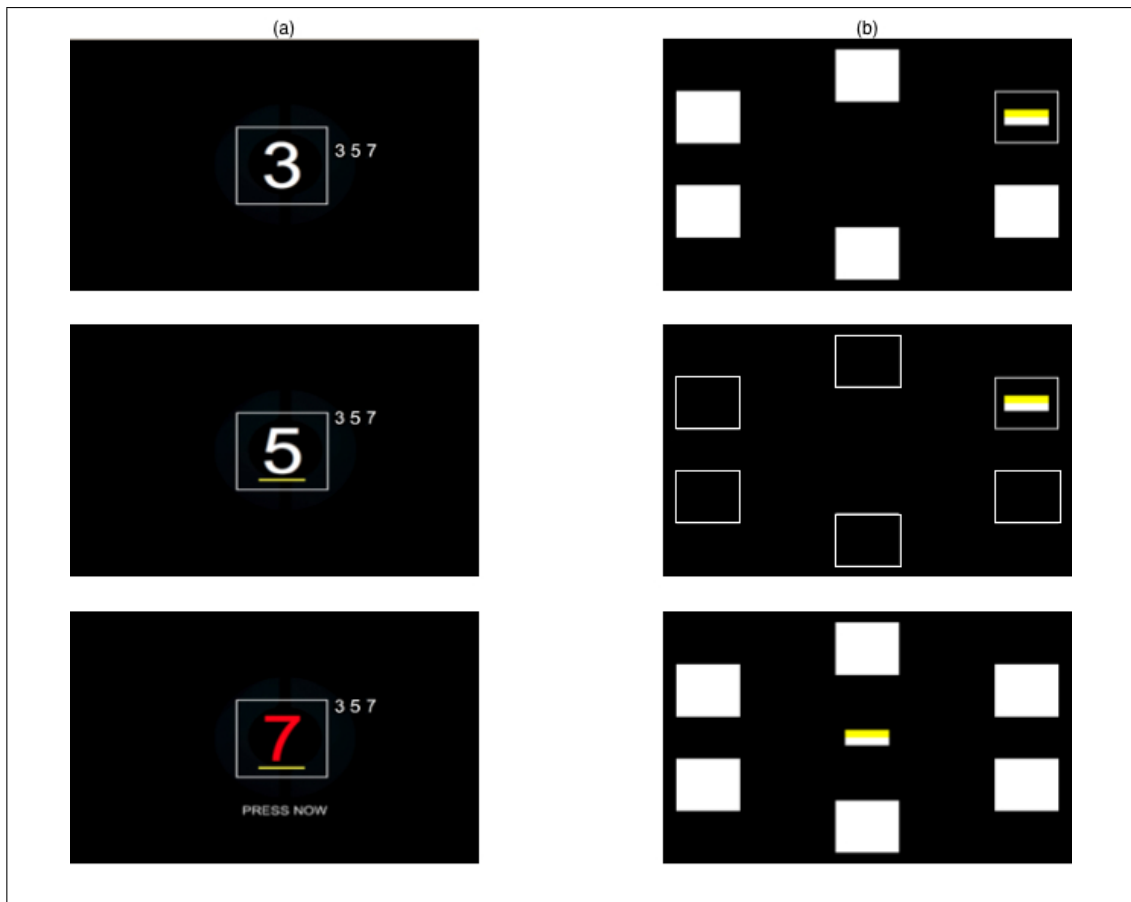
Figure 5: **Control tasks** a) Rapid Visual Processing (RVP) b) Paired Associate Learning (PAL)

2.4 Protocol

All of the questionnaires and tasks were completed on a Sony Vaio laptop and a touchscreen device when appropriate. Once consent was received, participants completed the BDI, BAI, PSS, and the lifestyle questionnaire.

Following completion of the questionnaires, participants completed either the CMT and DMS or the visual pattern separation task, followed by the PAL, RVP, and backward digit span tasks. Testing was carried out in a private testing room

that provided complete privacy.

Data for all participants was collected and stored anonymously. Each participant was given a random ID and participant number. As responses on the BDI could indicate potential for severe mental and physical health concerns, each participant's BDI score, along with their name and contact information, was submitted to the undergraduate advisor who would submit this information to a clinical psychologist at the McMaster Centre for Student Development (CSD) if warranted. The clinical psychologist could them offer them the opportunity for counselling. Indication of high risk was determined by their BDI score and a score within the range of "severely depressed" (a score of 29 or higher) was submitted for referral. Two participants had a BDI score within this range.

Results

3.1 CMT Performance

In order to correct for potential response bias, identification performance was corrected according to Yassa, Lacy, et al. (2010). As seen in Figure 6, participants most accurately identified New items, correctly identifying approximately 97% (SD = .052) of New items. Furthermore, participants correctly identified 73% (SD = .252) of Old items, while misclassifying 17% (SD = .179) of Old items as Similar. Finally, participants correctly identified only 57% (SD = .233) of Similar items, while misclassifying nearly 32% (SD = .212) of Similar items as Old.

A repeated measures ANOVA was conducted to evaluate differences on identification accuracy between the three conditions on items that were correctly iden-
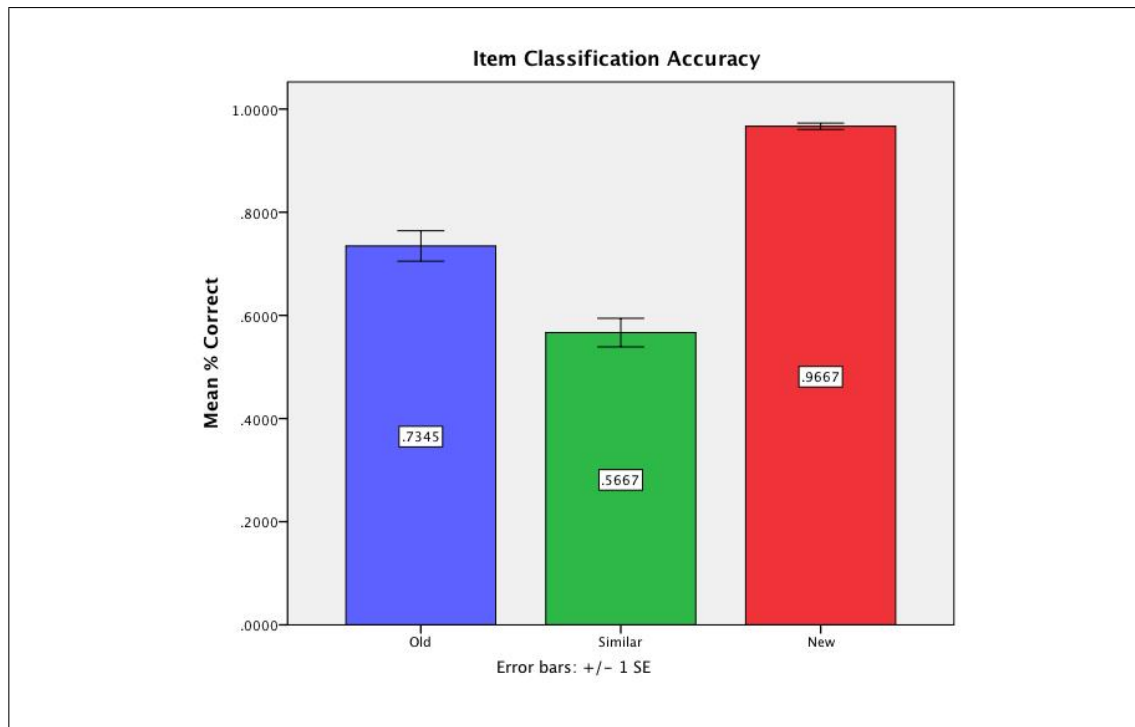
Figure 6: **Percent of items correctly identified**

tified (i.e., New|New, Old|Old, Similar|Similar). Mauchly's test of sphericity indicated that the assumption of sphericity had been violated, χ^2 (2) = 12.06, $p < .05$, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\epsilon = .86$). The repeated measures ANOVA revealed a significant difference in classification accuracy between the three conditions, F (2,138) = 77.81, $p < .001$. Post hoc analysis using a Bonferroni correction revealed that all pairwise comparisons were significant, suggesting that participants were more accurate at identifying New items ($M = .967$, $SD = .006$) than both Old ($M = .735$, $SD = .029$) and Similar items ($M = .567$, $SD = .028$). Additionally, participants more accurately identified Old items when compared to Similar items.

The mean number of selections necessary to find the matching card for correctly identified items (i.e., Old|Old, Similar|Similar, New|New) for each of the conditions are shown in Figure 7. Participants required the least number of selections for Similar items, requiring more for New items, and required the most selections for Old items. A repeated measures ANOVA was conducted to evaluate differences in the number of selections among the three conditions (Old, Similar, and New). A within-subject effect of condition was found, $F(2, 138) = 16.40$, $p < .001$. Post hoc analysis using a Bonferroni correction revealed that participants located Similar items ($M = 9.62$, $SD = .479$) more quickly than both Old ($M = 13.24$, $SD = .54$) and New ($M = 11.58$, $SD = .318$) items. Additionally, participants were quicker in locating New items than Old items.

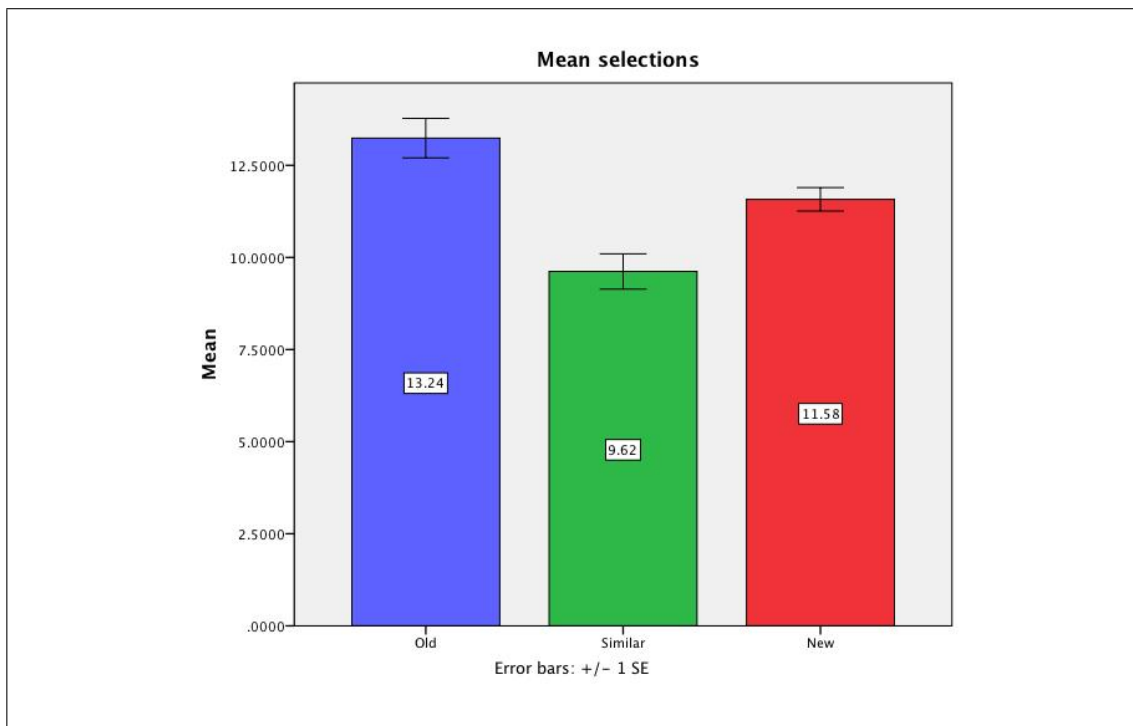Figure 7: **Mean selections to success**

3.2 DMS & Visual Pattern Separation

To provide support for the hypothesis that CMT performance was sensitive to changes in neurogenesis levels, we aimed to use the data collected from the lifestyle questionnaire and mood inventories to generate a linear regression model to predict scores on DMS and visual pattern separation; two tasks previously hypothesized to be sensitive to changes in neurogenesis (Becker et al., 2009; Dery et al., 2011). Using the coefficients generated by the model, it would then be possible to generate a hypothetical "neurogenesis score" by simply entering participant data for each of the independent variables into the linear equation. A correlational analysis could then be performed between this hypothetical "neurogenesis score" and measures on the CMT that are hypothesized to be neurogenesis-dependent.

To analyze DMS and visual pattern separation performance, data from percent correct at the longest delay of the DMS (12 seconds) and percent of high interference items (lure items) correctly identified was collected and converted into z scores. These z scores were then combined to form a single potentially-neurogenesis sensitive variable. Data obtained from the lifestyle questionnaire and mood inventories was then used to predict scores on the potentially-neurogenesis sensitive variable.

In an attempt to reduce the number of independent variables in the regression model, we computed standardized mood, exercise, and alcohol use index scores. First, z scores were calculated for the 3 mood inventories (BDI, BAI, PSS). Z scores were also calculated for the 9 questions on the lifestyle questionnaire pertaining to exercise regime, namely, hours spent running, taking part in other aerobic exercise,

and taking part in resistance training during the two weeks prior to testing, the 3 months prior to testing, and the year prior to testing. Finally, z scores were also calculated for 6 alcohol use questions on the lifestyle questionnaire, including average daily consumption of alcohol and the most alcohol consumed on a single occasion during the two weeks prior to testing, the 3 months prior to testing, and the year prior to testing. Subsequently, the mood, exercise, and alcohol use indices were computed by taking the means of the respective z scores.

A linear regression model for the putatively neurogenesis sensitive variable was used. We entered the mood, exercise, and alcohol use indices as independent variables, along with data on oral contraceptive use. Oral contraceptives were grouped according to the synthetic progestin used. As such "BC1" represented oral contraceptives containing levonorgestrel (i.e., Alesse®, Aviane®), "BC2" represented oral contraceptives containing norgestimate (i.e., Tricyclen®, Ortho-tricyclen®), and "BC3" represented oral contraceptives containing drosperinone (i.e., Yaz®, Yasmin®). Use was encoded dichotomously.

It was hypothesized that the lifestyle and mood indices would successfully predict performance on the putative neurogenesis-dependent tasks. Furthermore, it was hypothesized that a positive relationship would be displayed between performance and exercise scores, while a negative relationship was predicted between performance and mood and alcohol consumption scores. No predictions were made as to the direction of the relationship between performance and use of the individual contraceptive categories.

The resulting regression model is displayed in Figure 8. Although accounting for approximately 12% of the total variance, the model failed to reach significance (Adjusted R^2 = .050, $F(6,75)$ = 1.72, p = .129. Although not significant, both the alcohol use index (β = -.233, p = .060) and BC1 (β = .217, p = .061) nearly predicted scores on the putatively neurogenesis sensitive variable.
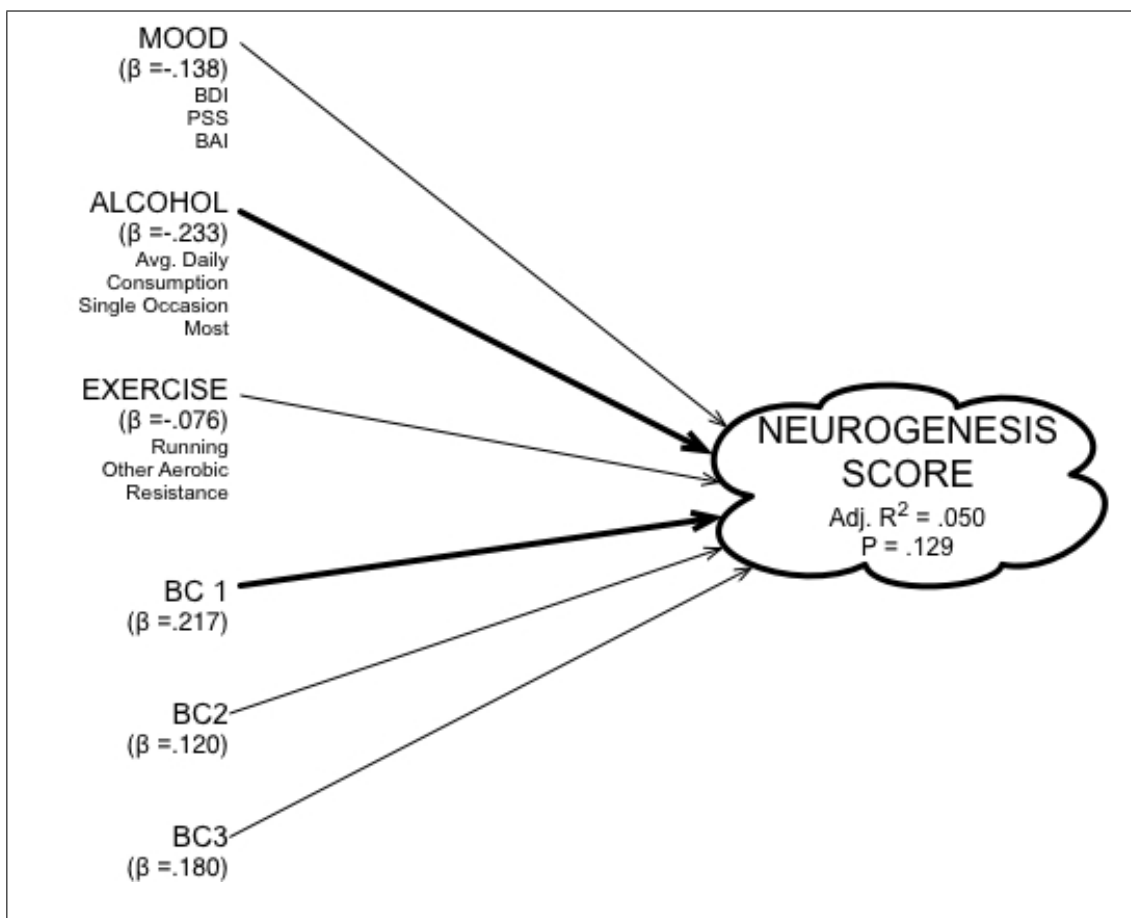

Figure 8: **Neurogenesis Score**

When correlated with measures on the CMT thought to be neurogenesis-dependent, the alcohol use index significantly correlated with both the search time and distance from the correct location of similar items mistakenly classified

as old (Old|Similar Selections, $r = .312$, $p < .05$; Old|Similar Distance, $r = .304$, $p < .05$). Additionally, those taking a BC1 oral contraceptive displayed a significantly greater search distance from the correct location on similar items mistakenly classified as old when compared to those not taking a BC1 oral contraceptive (Old|Similar Distance), $t(65) = 1.994$, $p < .05$.

3.3 DMS Correlates

As participant data from the lifestyle questionnaire and mood inventories failed to predict performance on the DMS and visual pattern separation measure, in line with Becker et al. (2009), a baseline-corrected score of DMS performance was calculated by subtracting the percent correct performance at the longest (12 second) delay from percent correct performance at the zero second delay. Correlational analysis was performed between this measure of DMS performance and measures on the CMT that were hypothesized to be putatively neurogenesis dependent, which included performance on similar items correctly identified as similar (Similar|Similar), similar items incorrectly identified as old (Old|Similar), and old items incorrectly identified as similar (Similar|Old).

It was predicted that performance on the DMS at the longest delay would be positively correlated with measures of performance on all three of these conditions as they were hypothesized to be neurogenesis-dependent. Furthermore, it was predicted that neither performance on DMS at the longest delay nor performance on the CMT measures would correlate with performance on any of the control tasks (PAL, RVP, backward digit span).

DMS performance was significantly correlated with number of erroneous returns to the previous locations (i.e., number of interferences) of old items when incorrectly classified as similar (Old|Similar interferences, $r = .395$, $p < .01$). Additionally, DMS performance was almost significantly correlated with distance from the correct location of similar items correctly identified as similar (Similar|Similar distance, $r = .242$, $p = .09$) and distance from the correct location of similar items incorrectly identified as old (Old|Similar distance, $r = .271$, $p = .059$). Importantly, this measure of DMS performance was not correlated with performance on the PAL, RVP, or backward digit span tasks, suggesting that the observed variance in performance did not reflect differences in global hippocampal, attentional, or working memory functioning.

In line with Saxe et al. (2007), an additional correlational analysis was performed between DMS performance and measures on the CMT that were hypothesized to present a situation in which neurogenesis could hinder performance. As such, correlational analysis was performed between DMS performance and performance on Old items correctly identified as Old (Old|Old Selections and Distance). In contrast with our hypotheses, there were no correlations between DMS performance and performance on Old|Old.

Multiple regression models for DMS performance were evaluated on the basis of the results of the bivariate analyses; only the variables that were (borderline) significantly correlated with DMS performance were included in the multiple regression and only these measures were considered for further analysis. The resulting model is displayed in Figure 9. The model significantly predicted DMS performance, explaining 25% of the total variance (Adjusted $R^2 = .184$, $F(3,37) =$

3.998, p < .05). The results from the regression indicated that Similar|Old inter-ferences significantly predicted DMS performance ($\beta = .361$, $p < .05$). A trimmed model was obtained by including only Similar|Old interferences. The trimmed model explained 16% of the variance in DMS performance (Adjusted $R^2 = .135$, $F(1,40) = 7.40$, $p < .01$).

Figure 9: **CMT predicting DMS performance**
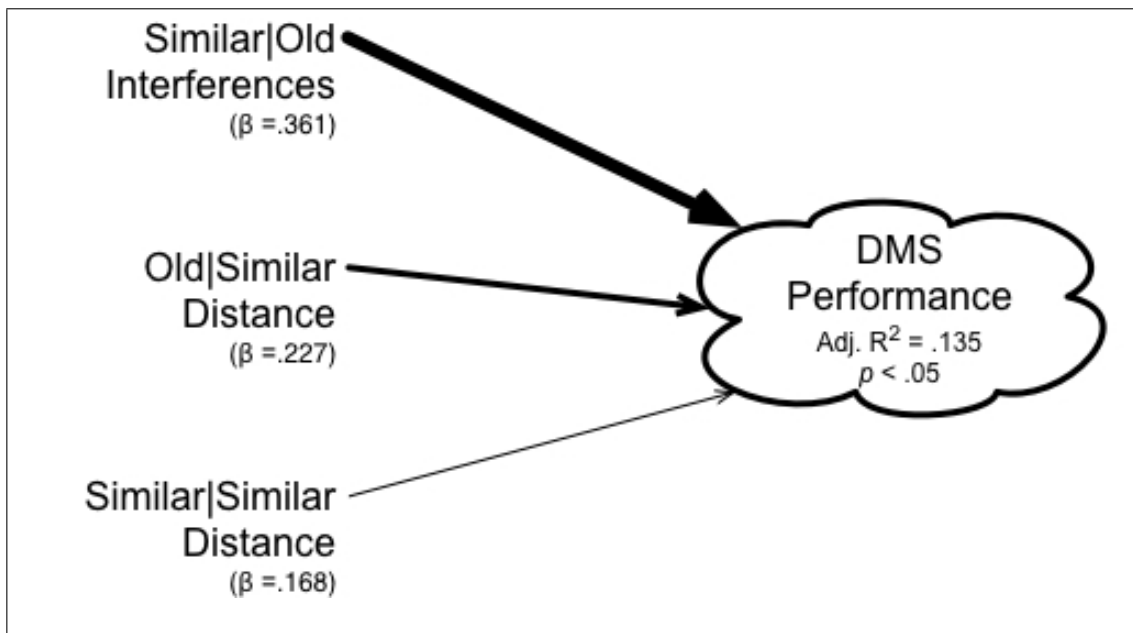
3.4 CMT Correlates

3.4.1   Similar|Old Interferences

A correlational analysis was performed between Old|Similar Interferences and the mood and lifestyle factors assessed by the inventories and lifestyle ques-tionnaire. The analysis revealed a significant correlation between Old|Similar in-terferences and stress ($r = .271$, $p < .05$). Additionally, Old|Similar interferences

was almost significantly correlated with the average amount of daily alcohol consumption within the two weeks prior to testing (r = .238, p = .072), and the most alcohol consumed on a single occasion within the two weeks prior to testing (r = .245, p = .062). A linear regression model consisting of these three variables accounted for 15% of the variance of Old|Similar interferences (Adjusted R^2 = .099, $F(3,53)$ = 3.05, $p < .05$) . In this model, stress was the sole significant predictor of Old|Similar interferences (β = .29, $p < .05$). A trimmed model with stress as the only independent variable accounted for 8% of the total variance (Adjust R^2 = .06, $F(1,55)$ = 4.44, $p < .05$).

3.4.2 Old|Similar Distance

Correlational analysis revealed that Old|Similar distance was significantly correlated with the average amount of daily alcohol consumption within the two weeks prior to testing (r = .311, $p < .05$). It was also significantly correlated with the most alcohol consumed on a single occasion within the two weeks prior to testing (r = .251. $p < .05$), the most alcohol consumed on a single occasion within the three months prior to testing (r = .276, $p < .05$), and the most alcohol consumed on a single occasion within the year prior to testing (r = .254, $p < .05$). A linear regression model consisting of these variables failed to reach significance (Adjusted R^2 = .056, $F(4,61)$ = 1.96, p = .113) and none of the independent variables significantly predicted Old|Similar distance.

3.4.3 Similar|Similar Distance

Correlational analysis revealed that Similar|Similar distance was not significantly correlated with any of the measures from the mood inventories or the

lifestyle questionnaire. Further correlational analysis revealed that Similar|Similar distance was significantly correlated with performance on the PAL task, as it significantly correlated with mean errors committed before successful placement of a target (i.e., Mean errors to success, $r = .358$, $p < .01$). When placed in a regression model, PAL performance significantly predicted Similar|Similar distance, accounting for 13% of the total variance in Similar|Similar distance (Adjusted R^2 = .115, F(1,67) = 9.85, $p < .01$).

3.5 Lifestyle Correlates

Correlations were performed between the data collected from the lifestyle questionnaire and mood inventories and the measures on the CMT hypothesized to be neurogenesis-sensitive, namely, performance on Similar|Similar, Old|Similar, and Similar|Old items. A summary of the significant correlations can be found in Table 1. The results suggest that average number of selections necessary on Old|Similar items significantly correlated with recent average daily alcohol consumption ($p < .05$), and most alcohol consumed recently ($p < .05$), within the past 3 months ($p < .05$), and within the past year ($p < .01$). PSS was significantly correlated with Similar|Old Interferences, and negatively correlated with Old|Similar classification errors ($p < .05$).

An independent samples t-test on a median split of BDI scores ($M = 9$) revealed that participants with lower BDI scores ($N = 38$, $M = 1.40$, $SD = .58$) searched closer to the correct location than those with high BDI scores ($N = 32$, $M = 1.68$, $SD = .46$) on Old|Similar items, t(65) = -2.143, $p < .05$.

An additional independent samples t-tests revealed that those taking BC1 oral contraceptives searched farther from the correct location on Old|Similar items than those not taking any oral contraceptive, $t(65) = 1.994$, $p < .05$. Finally, a last independent samples t-test revealed that those taking BC2 oral contraceptives required significantly less selections to locate Old|Similar items, $t(65) = -2.060$, $p < .05$.

Table 1: CMT Lifestyle Correlations

Lifestyle Variable	Old|Similar Selections	Old|Similar Distance	Old|Similar Classification	Similar|Old Interferences
PSS			-.291*	.271*
Recent Alcohol	.271*	.311*		
Recent Alcohol Most	.303*	.251*		
Past 3 Alcohol Most	.291*	.276*		
Past Year Alcohol Most	.328**	.254*		

* p < 0.05, ** p < 0.01

3.6 Working Memory Correlates

The results from Saxe et al. (2007) suggest that a decrease in neurogenesis may have resulted in superior working memory performance. As such, correlational analysis were performed between backward digit span and measures on the DMS and CMT thought to be working memory dependent, namely performance on DMS at no delay and the shortest delay (simultaneous and 0 second delay) and performance on correctly identified New and Old items on the CMT (New|New Selections, New|New Distance), Old|Old Selections, New|New Distance). A negative trend was visible between backward digit span and percent correct at the

simultaneous presentation on the DMS but failed to reach significance (r = -.278, p = .053). Backward digit span did not correlate with performance on DMS at the 0 second delay, or CMT performance on both New or Old items.

Discussion

4.1 Neurogenesis Score

As the CMT is a novel task, a key component of this study was to confirm that measures on the CMT hypothesized to be neurogenesis dependent were influenced by potential changes in neurogenesis levels. We aimed to accomplish this by utilizing data on various lifestyle and mood factors, which have been suggested to influence hippocampal neurogenesis, to predict, via linear regression, performance on putatively neurogenesis-dependent tasks. The equation obtained from the linear regression model would then be utilized to generate a neurogenesis score for participants by entering lifestyle and mood data into the equation. Subsequently, any measures on the CMT that were found to correlate with the neurogenesis score could then also be said to represent a measure that is influenced by potential changes in hippocampal neurogenesis.

Contrary to our hypotheses, the generated linear regression model failed to reach significance and, although the alcohol use index and use of an oral contraceptives containing levonorgestrel neared significance, none of the predictors succeeded in significantly predicting performance on the putatively neurogenesis-dependent tasks. A potential interpretation of this result is that lifestyle and mood factors are not reliable predictors of performance on DMS items at the longest de-

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lay and performance on high interference items on the visual pattern separation task. However, evidence from Becker et al. (2009) and Dery et al. (2011) suggests that this is not the case. Alternatively, it is more plausible that the model simply did not contain enough power to significantly predict performance due to the limited sample. In the following section, I will discuss the components of the regression model and address potential limitations.

4.1.1 Mood Index

The mean BDI score of the participants whose data was used for this analysis was 9.78 ($N = 88$); a score associated with minimal depression. Furthermore, only 8 participants within this data set possessed a score associated with moderate depression (20-28), and only a single participant possessed a score associate with severe depression (29-63). It is plausible that this lack of variance in depression scores resulted in the mood index (consisting of the BDI, PSS, and BAI scores) failing to predict performance on the putatively neurogenesis-dependent tasks.

It is important to note that Becker et al. (2009), who found that DMS performance at the longest delay was significantly hindered in potentially depressed individuals, utilized data from nearly twice the number of undergraduate students utilized in the current analysis ($N = 154$). Furthermore, this deficit was displayed when healthy individuals were compared to potentially depressed individuals (BDI score of 29 or higher). In the current analysis, only 1 participant possessed a BDI score within the potentially depressed range. Therefore, such a comparison was not possible in the current analysis. However, the relationship between mood index performance on the putatively neurogenesis-dependent measures displayed

a trend in the predicted positive direction. As such, further research is necessary to further investigate the effects of mood variables on performance on putatively neurogenesis-dependent tasks.

4.1.2 Exercise Index

The results from the present study suggest that the questions pertaining to exercise in the lifestyle questionnaire were not sensitive to the effects of exercise on cognitive function. It is well established that exercise promotes both physical and mental benefits (e.g. Yeung & Hemsley, 1997; Magnusson et al., 1998; Adler et al., 2000; Babyak et al., 2000; X. Z. He & Baker, 2004; Weuve et al., 2004). Importantly, these benefits have been shown to also include increasing hippocampal neurogenesis (Pereira et al., 2007; van Praag, Christie, et al., 1999; van Praag, Kempermann, & Gage, 1999; Ferreira et al., 2011). Furthermore, this enhancement of neurogenesis has been shown to result in increased performance on putative neurogenesis-dependent tasks in animals and humans (van Praag, Christie, et al., 1999; van Praag, Kempermann, & Gage, 1999; Winocur et al., 2012; Dery et al., 2011).

Dery et al. (2011) found that an intense 6-week aerobic exercise training program was sufficient to produce a significant increase in cerebral blood volume (CBV) in the dentate gyrus of healthy sedentary undergraduate students. Furthermore, this increase in CBV was accompanied by enhanced performance on DMS at the longest delay and performance on the high interference items on the visual pattern separation; two putatively neurogenesis-dependent tasks.

A key component to note in this study is that the exercise program that was utilized was specifically designed to improve maximal aerobic capacity (VO_2peak). When compared to both continuous moderate- and continuous vigorous-intensity exercise, near-maximal-intensity exercise has been found to produce a significantly greater improvement in VO_2peak (Gormley et al., 2008). As such, the exercise program utilized in Dery et al. (2011) involved high-intensity interval training at levels near maximal-intensity. Therefore, it is possible that such high intensity interval training is necessary to produce an enhancement in dentate gyrus CBV, a biological measure shown to increase hippocampal neurogenesis (Pereira et al., 2007), that results in an enhancement on putatively neurogenesis-dependent tasks.

Conversely, the data collected pertaining to aerobic exercise in the lifestyle questionnaire did not assess the type of training regime utilized by participants. As such, it is not possible to assess the type of exercise regime that participants engaged in. Further analysis is necessary to assess the effects of exercise regime on putatively neurogenesis-dependent tasks. A future study should include measures on the lifestyle questionnaire to assess the effects of varying exercise regimes.

4.1.3 Alcohol Use Index

Results from the linear regression model also suggest that the alcohol use index nearly predicted performance on the putatively neurogenesis-dependent tasks. It is well established that alcohol has a negative impact on neurogenesis (Nixon & Crews, 2002; J. He et al., 2005; Crews et al., 2006; Richardson et al., 2009; Taffe et al., 2010). As such, it was predicted that increased consumption of alcohol would be associated with decreased neurogenesis and deficits in performance on

putatively neurogenesis-dependent tasks. Therefore, the observed trend provides warrant for the continued investigation of the effects of alcohol on neurogenesis-dependent tasks.

4.1.4 Hormones

The results from the linear regression model suggest that the use of oral contraceptives that contain levonorgestrel (i.e., Alesse®, Aviane®) was nearly a significant predictor of performance on the putatively neurogenesis-dependent tasks. This trend is of particular interest as previous research on rats suggests that the combination of levonorgestrel and synthetic estrogen may produce significant effects on dentate gyrus neurogenesis (Liu et al., 2010). The behavioural implications of such effects remain unclear and further research is necessary to investigate the effects of oral contraceptives on neurogenesis. As such, the current trend of results warrants future research.

To date, very little research has focused on the cognitive effects of oral contraceptives. Still, the limited research that has been performed provides evidence that oral contraceptives can produce significant impacts on cognition. Furthermore, evidence suggests that the effects of oral contraceptive use on cognition may vary between oral contraceptives.

Wharton et al. (2008) investigated the effects of various clinically relevant oral contraceptives on a visuospatial task (mental rotation task, MRT). In this study, oral contraceptives were grouped according to their androgenicity; the property of producing physiological reactions similar to those produced by androgens

(i.e., testosterone). It has been established that androgens are associated with enhanced visuospatial ability (Aleman, Bronk, Kessels, Koppeschaar, & van Honk, 2004) and, accordingly, results from this study suggested that enhanced performance on the MRT was displayed in users of oral contraceptives with greater androgenic activity when compared to users of oral contraceptives with less androgenic activity, anti-androgenic activity, and nonusers. Furthermore, users of oral contraceptives with anti-androgenic properties displayed a significant deficit when compared to nonusers.

Additional research has also shown that oral contraceptive use may be associated with enhanced performance on verbal memory tasks (California Verbal Learning Test), and conditioned eyeblink acquisition (Mordecai, Rubin, & Maki, 2008; Holloway, Beck, & Servatius, 2011).The results from previous research provide convincing evidence that oral contraceptives can have a significant impact on cognition and that this effect may vary between oral contraceptives.

Furthermore, previous research also implicates the use of oral contraceptives in affecting hippocampal neurogenesis (Liu et al., 2010). As such, it is possible that the use of oral contraceptives may produce significant impacts on performance on putatively neurogenesis-dependent tasks. Indeed, the results from the linear regression model suggest that such a trend may exist. Interestingly, of the three classes of oral contraceptives considered in this analysis, only those that contain levonorgestrel neared significance. Levonorgestrel has been found to possess highly androgenic activity (Kumar, Koide, Tsong, & Sundaram, 2000). As androgens are typically associated with increase spatial memory, it could stand to reason that the introduction of an androgenic oral contraceptive would enhance spatial abilities.

However, it is important to note that only 22 participants admitted to using an oral contraceptive; 11 of which reported using an oral contraceptive that contains levonorgestrel and only 6 and 5 participants reported to using an oral contraceptive associated with norgestimate and drosperinone, accordingly. Therefore, further research is necessary to investigate the relationship between oral contraceptives and spatial memory.

4.2 DMS & CMT

In addition to generating a neurogenesis score, an alternative approach to validating measures on the CMT as potentially neurogenesis-dependent measures was to directly correlate performance on a putatively neurogenesis-dependent task with measures on the CMT hypothesized to be neurogenesis-dependent. This analysis revealed that performance on the DMS at the longest delay was significantly correlated with performance on Old items misidentified as Similar (Similar|Old) and Similar items misidentified as Old (Old|Similar). More specifically, performance on DMS at the longest delay was significantly correlated with the number of interferences committed on Similar|Old items, as well as search time and search distance from the correct location on Old|Similar items.

Furthermore, a linear regression model containing these three CMT measures as independent variables significantly predicted DMS performance. As such, measures on the CMT thought to be neurogenesis-dependent significantly predicted performance on a putatively neurogenesis-dependent task. These results provide encouraging evidence that the CMT may serve as an effective tool for assessing the effects of fluctuations in hippocampal neurogenesis. As such, further analysis

was completed to investigate the relationships between these measures and factors that have been suggested to influence neurogenesis (i.e., lifestyle and mood factors). These analyses are discussed in the following section.

Conversely, item classification accuracy, a measure on the CMT that was hypothesized to be sensitive to putatively neurogenesis-dependent measures, was not correlated with performance on DMS. Additionally, there were no correlations between performance on correctly identified Old items with either performance on the DMS, backward digit span, or lifestyle and mood factors. The implications and possible interpretations of these null findings will be discussed shortly.

4.3 CMT Search Performance

An effect of search time by condition was observed. Overall, participants performed optimally on Similar|Similar items, requiring significantly less search time (i.e., less selections) for correctly identified Similar items (Similar|Similar) than both correctly identified New items (New|New) and correctly identified Old items (Old|Old items). Additionally, participants required the most search time for Old|Old items, utilizing significantly more selections to locate Old|Old matches than both Similar|Similar and New|New items.

4.3.1   Similar|Similar

The observed pattern of results suggests that participants successfully utilized the rules of the CMT to aid performance on correctly identified similar items (Similar|Similar). That is, when participants correctly identified a similar item, they successfully recalled the locations of the previously encountered analogous

item (i.e., the Old item) and utilized this information to quickly locate the correct card. Furthermore, it was hypothesized that this measure would correlate with performance on putative neurogenesis-dependent tasks. Evidence from correlational analysis and a linear regression model provided support for this hypothesis.

However, further analysis revealed that performance on Similar|Similar items did not correlate with any of the potentially neurogenesis-influencing variables assessed in the lifestyle questionnaire and mood inventories. Although this null result could be interpreted as a failure of the lifestyle questionnaire and mood variables to detect potential changes in hippocampal neurogenesis, further analysis revealed that performance on Similar|Similar items was significantly correlated with PAL performance. More specifically, mean errors committed on PAL predicted the mean search distance from the correct location on Similar|Similar items. These results suggests that performance on Similar|Similar items may not be solely dependent on neurogenesis in the dentate gyrus; instead performance on Similar|Similar items may also be dependent on global hippocampal functioning.

As previously mentioned, previous research suggests that performance on PAL is hippocampally dependent, but not dentate gyrus dependent (Gilbert & Kesner, 2003; Nakashiba et al., 2008). Successful performance on PAL requires that the participant learn the location of a target item and, following subsequent learning, successfully recall the previous location of that item. The task demands of PAL are analogous to the task demands associated with locating the matching card of Similar|Similar items on the CMT. Indeed, successful performance on Similar|Similar items requires that the participant learn the locations of an item (i.e., placement of the associated Old item) and, following subsequent learn-

ing, recall these locations in order to successfully locate the matching Similar item. Therefore, it is possible that object placement on PAL and performance on Similar|Similar items on the CMT possess the same cognitive demands. As such, it would stand to reason that increased errors on PAL, due to poor spatial recollection, could be predictive of greater average search distance from the correct location on Similar|Similar items. The results from the correlational analysis and linear regression provide support for this interpretation.

It is important to note that performance on DMS did not correlate with performance on PAL. Although successful performance on DMS is associated with learning and, following a delay, recalling an item to perform a recognition task, there is no spatial demand in DMS. As such, PAL and performance on Similar|Similar items require the recollection of a spatial location, while DMS requires the recognition of an abstract image.

Furthermore, unlike PAL, the DMS task requires the participant to successful avoid interference from highly similar lures, which is hypothesized to necessitate adequate levels of neurogenesis (Becker et al., 2009). Similarly, performance on the Similar|Similar also requires successful interference avoidance, as the participant must successfully recall and identify the spatial location of the correct card among many identical and spatially adjacent cards.

Therefore, it is plausible that successful performance on Similar|Similar items may require the same cognitive functions as both PAL and DMS. However, the relative importance of these cognitive functions for successful performance remains unclear. As such, further research is necessary to investigate the cognitive func-

tions involved in performance on Similar|Similar items. This research may be able to shed light as to why performance on Similar|Similar items did not appear to correlate with lifestyle and mood variables.

4.3.2 Old|Similar

A trend between performance on DMS at the longest delay and average search distance on Old|Similar items neared significance ($p = .059$). This trend is in line with our hypotheses and suggests that those with reduced performance on DMS at the longest also displayed further search distances from the correct locations on Old|Similar items.

It was hypothesized that performance on Similar items misclassified as Old (Old|Similar) items would be sensitive to changes in neurogenesis. More specifically, it was hypothesized that individuals with potentially insufficient neurogenesis would misclassify Similar items as Old more frequently than those with sufficient neurogenesis. Although the results suggest that those with potentially reduced neurogenesis did not commit more Old|Similar classification errors, the results do suggest that these individuals experienced longer search times and farther search distances than those with sufficient neurogenesis when they did misclassify a Similar item as Old. Indeed, both Old|Similar search time and distance were significantly correlated with scores on the alcohol use index. Additionally, those with BDI scores above the median displayed significantly greater search distance from the correct location on Old|Similar items than those below the median.

The observed results suggest that performance on Old|Similar items was also influenced by hormonal factors. More specifically, those taking an oral contraceptive containing norgestimate (i.e., Tricyclen®, Ortho-tricyclen®) required longer search times than participants not taking an oral contraceptive. Additionally, those taking an oral contraceptive containing levonorgestrel (i.e., Alesse®, Aviane®) displayed significantly farther search distances on Old|Similar items than those not taking an oral contraceptive.

Taken together, these results suggest that potentially reduced neurogenesis in the dentate gyrus resulted in reduced performance on Old|Similar items. Furthermore, individuals hypothesized to possess reduced levels of neurogenesis searched farther from the correct location and subsequently required longer search times (i.e., more selections) than individuals hypothesized to possess healthy levels of neurogenesis. This pattern of results suggest that when these participants failed to successfully distinguish Similar items from Old items, they subsequently failed to recall having any previous experience with an item that resembles the target item and, as such, can be interpreted as a failure of pattern integration.

A hypothesized role of neurogenesis in pattern integration suggests that the short-term function of immature neurons is to retain a relationship between events across time that are encoded by the same new neurons (Becker et al., 2009; Aimone et al., 2009). As such, this integration permits the hippocampus to retain a link between events in the hippocampus. Subsequently, this associative link could help to retrieve all related events for comparison when a discrimination decision needs to be made (Aimone, Deng, & Gage, 2010). Indeed, evidence from animal research provides support for the involvement of neurogenesis in such a role (Clelland et

al., 2009). Furthermore, it is this hypothesized role that has been suggested to cause neurogenesis to hinder performance on the working memory task utilized by Saxe et al. (2007); in which it was advantageous to disregard prior experience. Therefore, it is reasonable to conceive that a lack of pattern integration, as a result of a reduced production of immature neurons, could hinder performance on Similar items in the CMT.

In terms of performance on Similar items in the CMT, this pattern integration role would enable neurogenesis to aid in efficient localization of the matching card. As the population of available neurons would not have been expected to change throughout the duration of the study, when participants were presented with a Similar item in a subsequent game, immature neurons in the dentate gyrus could have cued the retrieval of the locations of the appropriate previously experienced item (the Old item) and allowed the subject to quickly navigate toward and locate the correct matching card. Importantly, this cued retrieval could occur regardless of the participants' classification of the Similar object via implicit functioning (Chun & Jiang, 2003; Park, Quinlan, Thornton, & Reder, 2004; Greene, Gross, Elsinger, & Rao, 2007). Therefore, it is plausible that when individuals with healthy levels of neurogenesis explicitly misclassified a Similar item as Old, implicit memory functioning continued to cue the locations of the previously encountered Old item; resulting in the efficient discovery of the correct card. Conversely, such implicit memory functioning could have been impaired in individuals with potentially reduced levels of neurogenesis due to a lack of pattern integration. As such, such implicit retrieval would not aid in the search for the correct card, resulting in further search distance and increased search times.

Therefore, the observed pattern of results provides support for the hypothesized importance of neurogenesis for successful performance on Old|Similar items as individuals with potentially reduced levels of neurogenesis require significantly longer search times and tend to search further away from the correct location than individuals hypothesized to possess healthy levels of neurogenesis. As this measure nearly correlates with performance on DMS performance at the longest delay, these results provides encouraging support for the development of the CMT as a measure of neurogenesis-dependent functioning.

4.3.3 Similar|Old Interferences

It was hypothesized that the number of returns to the previous locations of Old items when they were misidentified as Similar (i.e., Similar|Old Interferences) would be sensitive to performance on neurogenesis-dependent tasks. Furthermore, it was hypothesized that increased interference errors would be displayed by participants hypothesized to possess reduced levels of neurogenesis.

Interestingly, the average number of Similar|Old interferences was the sole significant predictor of performance on the DMS task at the longest delay. As such, this result suggests that increased returns to these interference locations correlated with and predicted reduced performance on DMS at the 12 second delay. These significant results provide strong evidence to support the notion that performance on Similar|Old items is sensitive to performance on a putatively neurogenesis-dependent task.

This interpretation is further supported by the results of the subsequent analysis between Similar|Old interferences and the variables assessed on the lifestyle and mood inventories. Similar|Old interferences was positively correlated with stress scores on the PSS. Additionally, positive trends between Similar|Old interferences and alcohol consumption within the two weeks prior to testing neared significance. Together, stress and recent alcohol consumption significantly predicted the number of Similar|Old interference errors committed on the CMT. These results suggest that those with high stress scores and those with high alcohol consumption within the two weeks prior to testing committed the most Old|Similar interferences.

These results may be caused by a lack of pattern separation in individuals with potentially reduced neurogenesis. Efficient performance on Similar|Old items requires the participant to recognize that the target item is identical to a previously encountered item and avoid the previous locations in which it was located in the original presentation. Furthermore, it requires the participant to discriminate between a previously encountered highly similar trial and the current trial.

As such, these task demands mirror those of a delayed non-match to sample (DNMS) task used by Winocur et al. (2006); a task in which the suppression of neurogenesis was shown to result in significant deficits. In the sample phase of this task, rats were presented with either a black or white cylinder that cued the location of a submerged platform in a pool. Following a delay, the rats were then placed in the pool and allowed to swim to the platform, which had been moved and was now cued by the previously unused coloured cylinder. As such, successful performance on this DNMS task required the rats to form conditional rules

and memories for specific events (i.e., pattern separation). The results suggested that while rats with irradiate neurogenesis performed adequately at short delays, significant deficits were displayed at long delays (greater than 60 seconds).

Similar to the DNMS task used by (Winocur et al., 2006), successful performance on Similar|Old items in the CMT requires that participants recall the location of the previously presented stimulus and then choose another location. Furthermore, consistent with the findings of Winocur et al. (2006), individuals hypothesized to possess suppressed neurogenesis seemed to be unable to adequately utilize contextual associations to avoid the previous locations. Therefore, these results suggest that while participants believed to possess healthy levels of neurogenesis were able to perform this interference avoidance, participants believed to possess reduced neurogenesis did not. As such, this inability to avoid interference resulted in increased returns to the previous locations of Old objects. These results provide encouraging evidence to support the hypothesis that performance on Similar|Old items is significantly hindered with reduced neurogenesis.

4.4 CMT Classification Accuracy

Classification accuracy on the CMT was in line with previous research. As revealed by a repeated measures ANOVA, participants most accurately classified New items (96% correct) and least accurately classified Similar items (57% correct). Furthermore, participants mistakenly classified 32% of Similar items as Old (Old|Similar), suggesting that classification of Similar items successfully presented a high potential for interference. This pattern of results replicates the findings of previous research on tasks requiring a similar object recognition task (Kirwan

& Stark, 2007; Yassa, Lacy, et al., 2010; Dery et al., 2011). Previous research on a similar pattern separation task suggests that this type of object recognition is heavily dependent on the dentate gyrus (Kirwan & Stark, 2007; Yassa, Lacy, et al., 2010) and has been suggested to be sensitive to changes in hippocampal neurogenesis (Dery et al., 2011).

However, correlational analysis failed to reveal any significant correlations between classification of Similar items and both lifestyle and mood variables in favour of these hypotheses. In fact, a sole negative correlation was found between proportion of Similar items mistakenly classified as Old and PSS scores. This correlation suggested that increased stress levels resulted in reduced misclassification of Similar items as Old; a finding contrary to our original hypotheses.

However, previous research has suggested that the effects of stress on learning and memory may be influenced by gonadal hormones. Luine (2002) exposed male and female rats to 21 days of restraint-stress. Following the stress period, the rats were administered an object recognition memory task. Interestingly, while stressed males displayed a significant deficit when compared to controls, stressed female rats displayed no impairment on the object recognition memory task when compared to sex-matched controls. Through further analysis, the authors deduced that estrogen in the female rats may have played a neuroprotective role and influenced the biological and behavioural effects of the stress response.

Furthermore, as previously reviewed, evidence from previous research suggests that the neuroprotective effects of estrogen may extend to hippocampal neurogenesis (Perez-Martin et al., 2003; Galea et al., 2006). Therefore, a possible

explanation of the observed correlation between Old|Similar classification errors and stress is that estrogen levels buffered the inhibitory effects of stress on neurogenesis. As such, it is possible that increased levels of estrogen during the time of testing resulted in a superior performance on Similar items that resulted in fewer misclassifications of Similar items as Old.

Although we attempted to estimate gonadal hormone levels through estimates of menstrual cycle phase and oral contraceptive use, a more invasive and controlled investigation is necessary to adequately assess the effects of gonadal hormones on CMT object classification performance. Therefore, subsequent research is necessary to further investigate the role of estrogen and stress on CMT object classification.

4.5 Working Memory

A key research component of this study and in the design of the CMT was to attempt to investigate the effects of neurogenesis on working memory. More specifically, we aimed to shed light on the paradoxical results of Saxe et al. (2007).

Saxe et al. (2007) introduced mice to a radial arm maze task in which the sample and test arms were employed repeatedly and interchangeably between trials. Interestingly, enhanced performance was observed in mice with ablated neurogenesis when compared to healthy controls.

Theoretically, this result is in line with the hypothesized role of hippocampal neurogenesis in pattern integration within a short-time span. Within a short-time span, neurogenesis is hypothesized to function as a contextual glue; binding

multiple elements of an episode together such that partial retrieval of the event cues retrieval of the entire event (Becker et al., 2009; Aimone et al., 2009). As such, it is hypothesized that the removal of neurogenesis results in the unique encoding of events that would normally be encoded with an associative link. Therefore, task performance is hypothesized to benefit from suppressed neurogenesis on such tasks as the task used by Saxe et al. (2007), whereby successful performance required unique encoding of all events, allowing the rat to approach subsequently related trials free of bias from previous experience.

In an attempt to mirror such a task, repeated items (i.e., Old items) on the CMT were never located in the same locations as initial presentation. As such, it was hypothesized that optimal performance on such items would require that the participant approach subsequent related trials in a unique manner that was free of bias from previous experience. Therefore, we hypothesized that optimal performance on Old|Old items would be seen in participants with potentially reduced levels of neurogenesis. However, we failed to uncover any evidence in support of this hypothesis.

One plausible interpretation of this null result is that a verbal working memory task is not sufficient to detect changes in spatial working memory performance. Indeed, evidence from previous research suggests that verbal and spatial working memory are implemented by different neural structures (Smith, Jonides, & Koeppe, 1996; Lycke, Specht, Ersland, & Hugdahl, 2008). As such, it is possible that a spatial working memory task may have been better suited to detect variance in performance on Old|Old items in the CMT.

Although troubling, an alternative, and more plausible interpretation of the observed null result is that we failed to adequately create a situation within the CMT in which it is actually beneficial to forget previous experience with related events.

In the current study, participants were instructed that when they encountered an Old object, none of the images of that pair would be located in their previous locations. Therefore, participants knew that an Old object was guaranteed not to be located in any of it's previous locations. As such, participants could have effectively reduced the number of viable search locations by simply recalling the previous locations of Old objects. Furthermore, the inclusion of Similar items, and the rule that they are always located in the initial locations of the objects that they resembled, could have also made it beneficial to always learn and recall the previous locations of Old objects. Consequently, on Old items, there was always a performance enhancement to be gained by learning and later recalling the previous locations of those objects.

With this limitation in sight, a critical difference is highlighted between the working memory task utilized by Saxe et al. (2007) and the task demands that accompany Old items in the CMT. Within the working memory task utilized by Saxe et al. (2007), maximum interference was obtained by repeatedly and interchangeably employing the sample and test arms. Furthermore, all learning and testing occurred within a single context. As such, neither previous experience nor contextual information could be utilized to enhance task performance and it was never beneficial to recall previous experience.

As such, a future version of the CMT should be made to include a working memory measure that is more analogous to the task administered by Saxe et al. (2007). Potential revisions to the CMT that may address this limitation are discussed in the following section.

Limitations & Future Directions

The current study has provided encouraging evidence as to the efficacy of the CMT as a potential tool for highlighting the behavioural effects of altered neurogenesis in humans. At the same time, however, the current protocol and task design are subject to many limitations. The following identifies areas of concern that will need to be addressed in the continued development of this research.

5.1 CMT

One of the most critical limitations of the current study was the failure to truly recreate a condition that was analogous to the working memory task used by Saxe et al. (2007) in which successful performance required the avoidance of pattern integration and the disregarding of prior experience. To address this limitation, a revised version of the CMT is proposed (Figure 10) and the following revisions should be considered.

5.1.1 Condition Separation and Instructions

Saxe et al. (2007) found enhanced performance with the suppression of neurogenesis when the sequence of trial presentation resembled the following: In the sample phase of the first trial, mice entered an arm to gain a reward (Arm 1).

After a delay, they were given the choice between Arm 1 and an adjacent novel arm (Arm 2). Successful performance required them to enter Arm 2. In the next trial, the arm adjacent to Arm 2 was entered in the sample phase (Arm 3). As such, successful performance in this subsequent choice phase required the avoidance of Arm 3 and a reward was given for entering Arm 4. Later in the task, mice entered Arm 2 during a sample phase. In the subsequent choice phase, mice were then given the choice between Arm 2, which had previously led to a reward in the first choice phase, and Arm 3, which had led to no reward in the second choice phase. Therefore, successful performance required that the mice disregard previous experience with both arms and simply avoid the arm that was presented in the immediately previous sample phase (Arm 2) in favour of the novel arm (Arm 3). Importantly, subsequent analysis revealed that the suppression of neurogenesis led to enhanced performance only in the event of such inter-trial memory interference.

Conversely, the current protocol of the CMT focused on intra-trial interference between Similar and Old items, as participants were presented with both conditions within a single game. For example, Figure 3 presented the example of a set of balloons. In the second game, both the exact set and a analogous set of balloons were utilized for both the Old and Similar conditions (Figure 3a and 3b, respectively). As such, it was always beneficial to recall the original locations of the balloons, as doing so could narrow the viable search options when the exact set (Old item) was presented again in a subsequent trial and could substantially decrease search time when an analogous set of balloons (Similar item) was presented in a subsequent trial.
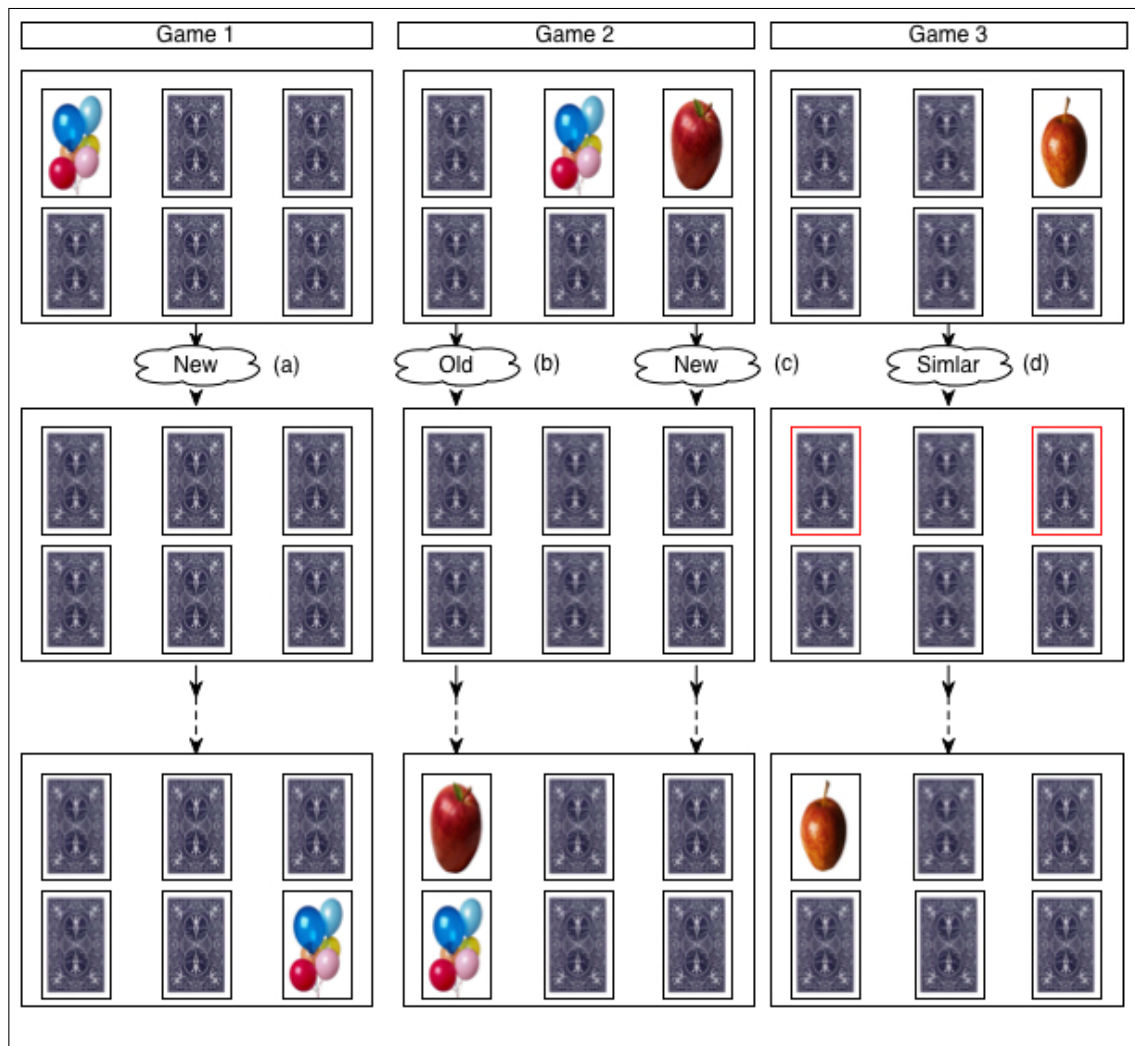
Figure 10: **Revised Concentration Memory Task**

It is therefore proposed that in a revised CMT protocol, participants not be told that Old items are never located in the same location or that Similar items are located in the previous locations of the images that they resemble. Furthermore, Old and Similar items should be separated by ensuring that New items become either Old items or Similar items in subsequent trials and that Old and Similar items do not utilize the same objects. Additionally, Similar items and Old items should not be presented in the same game.

In Figure 10a, a participant is presented with a set of balloons. In Game 2, the participant is presented with the same set of balloons (Figure 10b). Contrary to the current version of the CMT, the participant does not possess any information that could enhance search performance on the old set of balloons. As such, it is beneficial to disregard all previous experience with a related event and search for the matching card as if it was a New item.

Additionally, in Game 2, the participant is then presented with a novel image of an apple (Figure 10c). In Game 3, they are then presented with a similar apple (Figure 10d). As in the current version of the CMT, if the participant is able to distinguish between the similar images, they can utilize previous experience to quickly locate the matching image for this trial. Therefore, with these revisions, Old items present a condition in which it is beneficial to disregard previous experience and Similar items present a condition in which it is beneficial to act based on prior experience in an analogous situation.

5.1.2 Context removal

In the present study, participants were notified when they had successfully made all of the matches within an game and that they were going to begin the next game. Furthermore, each game was associated with a unique card colour. Hence, participants were always aware when the context had changed. This could have resulted in priming effects that lead to improved accuracy and performance on Old items. Conversely, there was no contextual shifts or alterations in the working memory task administered by Saxe et al. (2007). As such, a future version of the CMT should remove all indications of a contextual shift. This revision would involve the removing the notification when a participant completes a game and begins a new one, as well as implementing a consistent card throughout the entirety of the task.

5.2 Recruitment

An obvious limitation that heavily influenced the ability to effectively assess the effects of lifestyle and mood factors on putatively neurogenesis-dependent tasks was the relatively small sample size. More specifically, the sample population recruited for this study lacked variance in a few critical lifestyle and mood variables; namely, we lacked an adequate sample of participants that scored within the potentially depressed range on the BDI and we also lacked an adequate sample of participants on a variety of clinically relevant oral contraceptives. It is believed that this lack of adequate population sampling resulted in the failure to generate a linear regression model that could significantly predict performance on putative neurogenesis-dependent tasks.

Recruitment for the current study did not involve any pre-screening methods; participants simply signed-up for participation using an online experiment scheduling system (Experimetrix). As such, we randomly sampled from the undergraduate student population.

Future research should utilize more controlled methods of recruitment to ensure that an adequate sample size on all critical measures is obtained. By utilizing online survey software applications (e.g., FluidSurveys®), key pre-screening questions and responses to these questions can be assessed before the participant is scheduled for testing. If a potential participant does meet inclusion criteria, by indicating pre-determined responses to critical questions (i.e., use of an oral contraceptive, increased likelihood of risk for developing depression, or high alcohol consumption), they can be provided with the appropriate information necessary to sign-up for study participation. However, if a potential participant does not meet inclusion criteria, they can be invited to participant in the study as a control. Importantly, potential control participants can be recruited as necessary. Therefore, these revised recruitment methods will allow for more efficient participant recruitment and the recruitment of more participants likely to possess lifestyle and mood factors of critical interest.

5.3 Lifestyle Questionnaire

Surprisingly, we failed to identify any performance enhancements related to exercise regime. As exercise has convincingly been shown to enhance both mental and cognitive functioning, this null result provides a potentially major limitation to the power and implications of the current study. As such, the questions on

the lifestyle questionnaire pertaining to exercise habits need to be revised. Furthermore, additional questions should be included to assess the effects of type of exercise regime (i.e., continuous vs interval training).

Conclusions

We developed a novel and versatile task to assess the roles of hippocampal neurogenesis in memory performance in various cognitive scenarios. While still in the early-development stages, the results from the present study provide strong encouragement for the continued development of the CMT as a task for evaluating the effects of lifestyle and mood variables on putatively-neurogenesis dependent memory tasks.

Furthermore, performance on several key measures on the CMT that were hypothesized to be neurogenesis-dependent were significantly- or nearly significantly-sensitive to changes in many lifestyle and mood variables that have previously been shown to influence neurogenesis levels in the dentate gyrus. As such, the current study is a tremendous first step toward the ultimate goal of understanding the role of neurogenesis in human behaviour. However, the current study was also fraught with some extensive limitations. As such, the recruitment method, lifestyle questionnaire, and CMT should be revised to improve the efficiency and analytical power of this research.

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Appendices
A: Lifestyle Questionnaire

1. Please indicate your sex.
   1) Male
   2) Female

2. Please indicate your age: _____

3. Please indicate your height:_____

4. Please indicate your weight:_____

5. From grade 1 onwards, how many years of schooling have you completed? ____

***Hormone Module*:**

6. When was the date of your last menstrual period? _____ (mm/dd/yy)

7. How confident are you in this response?
   Not very   1   2   3   4   5   6   7 Very

8. What is the anticipated date of your next menstrual period? ____ (mm/dd/yy)

9. How regular are your cycles?
   Not very   1   2   3   4   5   6   7   Very

10. How confident are you in this response?
    Not very   1   2   3   4   5   6   7 Very

11. Do you currently use a form of contraception?
    1. Yes
    2. No

If yes,

12. Please select which type:
    3. An oral contraceptive (birth control pill) (E.g., Alesse, YAZ, Yasmin, Ortho-tri-cyclen, etc.)
    4. An intra-uterine device (IUD) (E.g., Mirena)
    5. A hormonal injection of progestin (E.g., Depo-Provera)
    6. A vaginal ring (E.g., NuvaRing)
    7. A birth control patch (E.g., Ortho Evra)

If yes to question 3, these follow-up questions were asked:

Please name the brand of birth control you are taking: _____

How long have you been using THIS TYPE of contraceptive? _____

Have you ever taken a different type of contraceptive?   Y / N
If yes**, **question 3 will be repeated with follow-up questions being presented in past tense.

13. Have you used the morning after pill as a form of contraception? (E.g., Plan B) 1 = Yes, 2 = No
    If yes,
    How many days ago did you take the morning after pill?
    How many times have you used the morning after pill?

Recent/Past/Distant History:
The following questions were each asked at 3 different time points and prefaced with the following:
- Recent History: In the last weeks, on average…
- Past History: In the past 3 months, on average…
- Distant History: In the last 1 year, on average…

Alcohol Module
14. What was your daily alcohol consumption?
    8. 0 drinks
    9. 1-2 drinks
    10. 3-4 drinks
    11. 5-6 drinks
    12. More than 6 drinks

15. What was the most alcohol you consumed on a single occasion?
    13. N/A
    14. 1-2 drinks
    15. 3-4 drinks
    16. 5-6 drinks
    17. More than 6 drinks

16. How many days ago did this consumption event occur?
    18. N/A
    19. 1 day ago
    20. 2 days ago
    21. 3 days ago
    22. 4 days ago
    23. 5 days ago
    24. 7+ days ago

Exercise Module
17. How many hours per week did you run?
    25. 0 hours
    26. 1-2 hours
    27. 3-4 hours
    28. 5-6 hours
    29. 7+ hours
18. How intense was each running session?
    Not very   1   2   3   4   5   6   7   Very

19. How many hours per week did you take part in other aerobic exercise? (E.g., swimming, elliptical, cycling, Aquafit)
    30. 0 hours
    31. 1-2 hours
    32. 3-4 hours
    33. 5-6 hours
    34. 7+ hours

20. How intense was each aerobic exercise session?
    Not very   1   2   3   4   5   6   7   Very

21. How many hours per week did you take part in resistance training? (E.g., weight lifting)
    35. 0 hours
    36. 1-2 hours
    37. 3-4 hours
    38. 5-6 hours
    39. 7+ hours

22. How intense was each weightlifting session?
    Not very   1   2   3   4   5   6   7   Very

B: Distance Measure

5	4	3	3	3	3	3	3	3	4	5
5	4	3	2	2	2	2	2	3	4	5
5	4	3	2	1	1	1	2	3	4	5
5	4	3	2	1	Correct Location	1	2	3	4	5
5	4	3	2	1	1	1	2	3	4	5
5	4	3	2	2	2	2	2	3	4	5
5	4	3	3	3	3	3	3	3	4	5

This measure represents the distance between the card selection and the correct
card; defined as the smallest number of cards between the correct card and the par-
ticipant's search location, including horizontal, vertical, and diagonal placements.
All cards immediately surrounding the card are said to be 1 card away from the
correct card, and all cards immediately surrounding those cards are said to be 2
cards away from the correct card, etc. Note: The above diagram is centred on the
correct card location and the surrounding cards represent all possible surrounding
card placements for all potential placements of the "Correct Location".