Determining the contribution of neurogenesis to learning and memory by investigating the effects of depression and alcohol consumption on spatial pattern separation using high interference memory tasks

DETERMINING THE CONTRIBUTION OF NEUROGENESIS TO LEARNING AND MEMORY BY INVESTIGATING THE EFFECTS OF DEPRESSION AND ALCOHOL CONSUMPTION ON SPATIAL PATTERN SEPARATION USING HIGH INTERFERENCE MEMORY TASKS

BY

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Abstract

Many young adult university students engage in frequent alcohol bingeing and have high depression scores, both of which are factors that can reduce hippocampal neurogenesis in rodents. Rodents with depleted neurogenesis exhibit selective deficits on high interference memory tasks including visual and spatial pattern separation. We predicted that young adult humans with high bingeing and depression scores would exhibit similarly impaired spatial pattern separation as a result of neurogenesis reductions. The relationships between alcohol bingeing, depression, and spatial pattern separation have, to this point, not been investigated in humans. We developed a novel computerized memory task for assessing spatial pattern separation in humans, loosely based on the "Concentration" memory card game. To further identify how sensitive this pattern separation function is to spatial separation between two stimuli, we developed the spatial separation recognition task (SSRT). We found that young adults with elevated depression and alcohol consumption scores exhibited impaired spatial pattern separation, in spite of intact performance on control tasks, consistent with a selective neurogenesis reduction. Further, this difference in performance seemed to be driven by performance at relatively larger separations.

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Notation and abbreviations

DG	Dentate Gyrus
CA3	Cornu Ammonis Layer 3
CA1	Cornu Ammonis Layer 1
EC	Entorhinal Cortex
LTP	Long-Term Potentiation
AHN	Adult Hippocampal Neurogenesis
SGZ	Subgranular Zone
SVZ	Subventricular Zone
OB	Olfactory Bulb
GABA	gamma-Aminobutyric acid
HPA	Hypothalamus-Pituitary-Adrenal (axis)
BrdU	Bromodeoxyuridine
M	Mean
SD	Standard Deviation
SE	Standard Error
CMT	Concentration Memory Task
SSRT	Spatial Separation Recognition Task

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Chapter 1

Introduction

1.1 Hippocampus in Episodic Memory

The hippocampus has long been implicated in learning and memory, most notably episodic memory formation (Scoville & Milner, 1957). Episodic memories are our internal representations of everyday events, often involving complex associations between time, objects, and places. The importance of this region for episodic memory was famously demonstrated through study of H.M., a hippocampectomy patient. Removal of the hippocampus and surrounding regions resulted in severe anterograde amnesia, preventing H.M. from generating new episodic memories. Since this time, additional study has supported the role of the hippocampus in episodic memory (Squire & Zola-Morgan, 1991; Tulving & Markowitsch, 1998; Zola-Morgan, Squire, & Amaral, 1986) and has further elucidated its role in a number of specific learning and memory capacities such as contextual and spatial memory (Burgess, Maguire, &

O'Keefe, 2002; Kim & Fanselow, 1992; Phillips & LeDoux, 1992; Winocur, Wojtowicz, Sekeres, Snyder, & Wang, 2006)(also see review by Moscovitch et al. (2005)).

1.2 Hippocampal Formation

The hippocampal formation consists of the entorhinal cortex (EC), dentate gyrus (DG), and cornu ammonis sub-layers CA1 and CA3. The EC is located at the rostral end of the temporal lobe in humans and receives information from the perirhinal-, parahippocampal-, and prefrontal cortices. As a result, this regions receives information from every cortical sensory system before relaying this information to the dentate gyrus, CA3 sublayer, and CA1 sublayers via the perforant pathway (Amaral 2007). It is these extensive connections between high level association areas and the EC that allow the hippocampus to collect, for binding and integration, the type of information contained within highly contextual and associative episodic memories. The EC is also a major output region from the hippocampal CA1 sublayer, back projecting to the aforementioned cortical regions.

Once information reaches the DG from the EC, it is projected onto the CA3 via mossy fiber connections. Activation of a small number of these synapses, which are few in number but quite large in size, was initially thought by modellers to be sufficient to trigger activation in a single CA3 pyramidal cell (Treves & Rolls, 1992, 1994). As a result, they were termed detonator synapses. More recent evidence suggests that a single synapse is capable of triggering a number CA3 pyramidal cells (Henze,

McMahon, Harris, & Barrionuevo, 2002). In this way, sparse activation of DG granule cells is sufficient to cause strong activation in the CA3 layer during encoding that is relatively independent of auto associative activity in the CA3 (McNaughton & Morris, 1987; Treves & Rolls, 1992).

From the CA3, information is passed to the CA1, which can also receive direct input from the EC via the perforant path. Physiological studies involving the deletion of the NMDAR1 gene in CA1 pyramidal cells suggest that the CA1 sublayer is a significant contributor to spatial learning (Tsien, Huerta, & Tonegawa, 1996). Adult mice with the deletion are unable to undergo long term potentiation (LTP) and fail to demonstrate activity dependent synaptic modification. Interestingly, these mice demonstrate impaired spatial memory but intact non-spatial learning (Tsien et al., 1996). More recent investigation involving temperoammonic lesion of the CA1 sublayer suggests that the region is also critical for long-term memory consolidation (Remondes & Schuman, 2004). While the contributions of the EC and CA1 are no doubt important for the formation of episodic memory, a significant amount of more recent work has focused on the contributions of the DG and CA3 regions to the somewhat opposing computational processes of pattern separation and pattern completion respectively.

1.3 Role of the Dentate Gyrus & CA3 in Pattern Separation & Completion

Pattern completion was first proposed as a function of the CA3 sublayer by David Marr (1971). He proposed that this pattern completion function could be made possible by the many recurrent collateral connections between pyramidal cells in the CA3 sublayer, which act to create an auto-associative network (Marr 1971). Recurrent collateral connections are neuronal connections between the axons of one neuron and the dendrites of neighbouring neurons and allow for the activity of a single neuron to trigger similar activation in neighbouring neurons as well. In this way, exposure to fractional elements of a previous episode can trigger activation of the entire representation of that previous episode (McNaughton & Morris, 1987). Such a function is beneficial as we are often required to retrieve a memory representation without being exposed to a previous experience in its entirety.

Early computational modellers initially proposed that very strong input to the CA3 sublayer would be required to initially form an encoding or memory representation that is relatively independent of firing associated with recurrent collaterals (Treves & Rolls, 1992, 1994). This strong input is thought to reach the CA3 via the mossy fiber pathway from the DG. During retrieval processes, it is thought that the auto associative mechanism must dominate and therefore requires the input from a separate pathway. The perforant pathway connecting the EC and CA3 is believed to perform this function (Treves & Rolls, 1992, 1994). This auto associative memory

function has been an integral mechanism in many models of CA3 pattern completion since Marr's time (Hasselmo, Schnell, & Barkai, 1995; Hasselmo & Wyble, 1997; Norman & O'Reilly, 2003; Treves & Rolls, 1992, 1994).

As the principle region involved in episodic memory, an important requirement of the hippocampus is the ability to encode new, potentially overlapping experiences as distinct memory representations, a computational process known as pattern separation. David Marr (1971) was the first modeller to suggest such a role for the hippocampus, whereby two highly similar inputs are orthogonalized such that the output is more distinct. This would reduce the potential for interference between memories of highly similar experiences. Under circumstances where separation fails, these encoded experiences can interfere with each other making it difficult to recall specific features of the affected memories. In the worst case scenario, the memory representations of these highly similar experiences may become completely overwritten by one another resulting in catastrophic interference (McClelland, McNaughton, & O'Reilly, 1995; Norman & O'Reilly, 2003). The physiology and positioning of the DG, between the EC and CA3, within the trisynaptic circuit make this region the ideal candidate to perform this pattern separation function within the hippocampus.

One physiological feature of the DG that is important for its pattern separation function is the relatively large number of neurons that comprise this region compared to both the EC and CA3 (Amaral, Ishizuka, & Claiborne, 1990). While there are many more neurons in this region, the overall neuronal activity in the DG is much lower than both the EC and CA3, partly due to activity of inhibitory neurons in both the DG and neighbouring regions (Acsády, Katona, Martínez-Guijarro, Buzsáki, &

Freund, 2000; Jung & McNaughton, 1993). This results in the production of a very sparse neural code, important for the creation of distinct representations of highly similar EC inputs. By orthogonalizing and reducing redundancy between similar inputs through sparse coding, the DG can act as a pattern separator (Marr, 1971; McClelland et al., 1995; Norman & O'Reilly, 2003). This pattern separation reduces the potential for interference between similar stored patterns in the CA3. A simple example is sufficient to illustrate the importance of pattern separation and completion in everyday life. For example, both processes would be required to remember where in the shed you hid your spare house key yesterday, when you have hidden the key in many different locations many times in the past. One must be able to create distinct, orthogonalized representations of one's experiences with the key and shed in order to avoid memory interference when you must remember where the key is hidden today.

In addition to computational models, the role of the DG in pattern separation has been supported by more recent empirical studies in non-human animals, including lesion studies. One such study involved the use of a cheese-board maze to train rats on a delayed-match-to-sample task that required rats to find and displace an object to obtain a food reward. Later trials required the rats to re-enter the maze and displace the exact same object in the presence of a similar "lure" object, which varied in proximity to the original object. Rats with lesions to the DG exhibited impaired spatial discrimination of objects and this impairment was dependent on spatial separation. That is, the rats were impaired when the original and lure objects were presented with small separations, with high potential for pattern overlap,

but exhibited intact performance at large separations with lower potential for pattern overlap (Gilbert, Kesner, & Lee, 2001). Furthermore, Gilbert and Kesner (2003) examined the effects of neuro-toxic induced lesions (in either the rat DG, CA3, or CA1 region) on performance on object-place and odour-place paired associate learning. Performance in rats with DG lesions was on par with normal controls while the CA3 lesioned rats performed significantly worse. This seems to suggest that the DG primarily contributes to learning and memory under conditions of high interference or pattern overlap, a task characteristic notably absent in paired associate learning. The CA3, therefore, is implicated in memory function in the absence of interference. Further research suggests that the dorsal DG contributes to metric spatial information processing through orthogonalization of sensory input patterns, contributing critically to spatial learning and subsequent recall (Goodrich-Hunsaker, Hunsaker, & Kesner, 2008).

The role of the DG in pattern separation has been supported by electrophysiological investigation as well. Using single cell recording techniques, Leutgeb, Leutgeb, Moser, and Moser (2007), have shown that granule cell activity in rats changes most in response to subtle changes in the environment. As environments becomes more and more distinct, changes in DG activity taper off. In the CA3, no discernible changes in activity occur in response to subtle environmental changes. However, as environments becomes more distinct, new CA3 cell assemblies are recruited. This suggests that altered DG granule cell firing is associated with pattern separation under conditions of high interference while recruitment of new CA3 cell assemblies, via the perforant path, is associated with conditions of low interference.

More recently, researchers have begun to show evidence for the role of the DG in pattern separation using a combination of functional Magnetic Resonance Imaging (fMRI) and high interference memory tasks in humans. For example, Kirwan and Stark (2007) used a modified continuous recognition memory task and were able to show hippocampal activity indicative of pattern separation in the DG/CA3 region. The task itself consists of a continuous presentation of images of objects, one at a time, on a computer screen and requires participants to identify whether each image is "new", "old", or "similar" based on the images they have seen previously. Performance on the "similar" trials is of particular interest as these images are thought to generate the most interference based on their overlapping visual features, and as a result, require successful pattern separation. It was initially found that the DG/CA3 region exhibited similar activation in response to "similar" and "new" images, but exhibited less activation in response to "old" images (Bakker, Kirwan, Miller, & Stark, 2008; Lacy, Yassa, Stark, Muftuler, & Stark, 2011). While this neuroimaging study is limited by the inability to distinguish DG activity from that of the CA3, it is nonetheless informative and suggests a role for the DG in human pattern separation. Interestingly, this pattern of DG/CA3 activity seems to be somewhat diminished in older participants. That is, older participants tend to require greater dissimilarity between images before the same type of DG/CA3 activity can be evoked (Yassa & Stark, 2011). Behavioural performance differences also manifest in much the same manner, as older participants require a greater amount of dissimilarity in order to correctly identify an image as "similar" compared to younger individuals. These activation and performance differences between young and old participants could

potentially be due to perforant path degradation, which can lead to hyperactivity in both the DG and CA3 (Yassa, Mattfeld, Stark, & Stark, 2011; Yassa & Stark, 2011).

While it seems that the DG is capable of supporting pattern separation in the hippocampus, the specific mechanism remains somewhat less clear. Computational modelling first suggested that the sparse coding of the DG was sufficient to support pattern separation but there is also evidence to suggest that adult hippocampal neurogenesis (AHN) is an important contributor as well.

1.4 Adult Hippocampal Neurogenesis

Since the discovery of AHN almost 50 years ago (Altman & Das, 1966), a considerable amount of research has been conducted to better understand the associated processes of cell proliferation, maturation, and survival as well as the functional significance of neurogenesis itself. Neurogenesis refers to the creation of new neurons and occurs in two regions of the adult brain: the subgranular zone (SGZ) of the DG and the sub ventricular zone (SVZ) of the lateral ventricles. In the SVZ, neural stem cells differentiate into neuroblasts which migrate along the rostral migratory stream, with the aid of specialized astrocytes, toward the olfactory bulb (OB) (Doetsch, Caillé, Lim, García-Verdugo, & Alvarez-Buylla, 1999; Lois & Alvarez-Buylla, 1994; Luskin, 1993). In the OB, approximately 60% of neuroblasts die while the majority of the remaining 40% develop and mature into inhibitory interneurons (Petreanu & Alvarez-Buylla, 2002; Winner, Cooper-Kuhn, Aigner, Winkler, & Kuhn, 2002).

Similarly, in the SGZ, astrocytes are believed to divide into neural progenitor

cells, some of which are able to divide quickly and proliferate, while others become glia cells (Doetsch et al., 1999). In the rat dentate gyrus, approximately 10000 new neurons are generated each day. While not every cell survives the initial development period, 40% of these neurons do reach cellular maturity (McDonald & Wojtowicz, 2005). In contrast to those neurons produced in the SVZ, neurons produced in the SGZ travel only a short distance within the dentate gyrus of the hippocampus, after the first developmental week, to the granule layer (Ming & Song, 2005). At this stage of development these new granule cells are not fully integrated into the functional neuronal network of the DG and remain GABAergic in nature, a characteristic that is thought to promote cell survival at this stage (Espósito et al., 2005; Toni et al., 2008, 2007). Typically, the binding of gamma-Aminobutyric acid (GABA) to GABA receptors has an inhibitory effect on granule cell activity but, early in cell development, these receptors are excitatory.

After the second developmental week, the new neurons produce polarized processes, the dendrites of which extend toward the molecular layer where they will eventually receive excitatory input from various cortical areas through projections from the EC. Axons are also developing at this time toward the CA3 layer and will eventually form the mossy fiber connections capable of triggering strong activation in the CA3. After the third developmental week, the newborn neurons develop functional synapses at both the efferent and afferent ends along with dendritic spines to facilitate communication with other more mature fibers of the perforant path (Markakis & Gage, 1999; Stanfield & Trice, 1988; Zhao, Teng, Summers, Ming, &

Gage, 2006). A major functional change at this point is the switch from GABAergic activity to glutamatergic activity whereby GABA serves an inhibitory function (similar to fully mature neurons) and glutamate serves an excitatory function. Adult born neurons at this stage of development are referred to as immature granule cells and, while they possess some characteristics of mature adult born neurons, are not fully developed. In comparison to mature adult born neurons, immature cells exhibit higher resting potentials, lower thresholds for the induction of LTP, and weaker perisomatic inhibition (Espósito et al., 2005; Schmidt-Hieber, Jonas, & Bischofberger, 2004; Snyder, Kee, & Wojtowicz, 2001; Wang, Scott, & Wojtowicz, 2000). In combination, these characteristics contribute to the hyper excitable nature of immature granule cells in the DG. Within 8 weeks, the physiological response of these neurons changes to become more similar to the mature population of DG granule cells (Laplagne et al., 2006).

1.5 Contribution of Adult Hippocampal Neurogenesis to Pattern Separation

Given that AHN occurs in many species, including humans (Eriksson et al., 1998; Manganas et al., 2007; Pereira et al., 2007), there has been substantial interest in determining the functional significance of this process. Early on, neurogenesis was

proposed as a mechanism that facilitated the clearance of memories following consolidation (Feng et al., 2001). This was supported by computational work using neural network models that incorporated neural turnover and which demonstrated improved memory acquisition through neurogenesis-related memory clearance (Chambers, Potenza, Hoffman, & Miranker, 2004; Deisseroth et al., 2004). Rather than clearing new memories, it has also been hypothesized that immature or new adullt-born neurons are preferentially recruited to encode new memory representations to avoid interference with previously stored memories (Nottebohm, 2002). This too has been supported by a neural network model with neuronal turnover, which proved resistant to catastrophic interference (Wiskott, Rasch, & Kempermann, 2006).

Becker (2005) further suggested that the role of AHN in episodic memory formation is to reduce memory interference by facilitating the distinct encoding of complex associations that possess overlapping information. When incorporated into a computational model of hippocampal function, neural turnover facilitated cued recall of highly confusable and overlapping patterns but provided no such advantage for the cued recall of random patterns or paired associates. Through neuronal turnover in the DG, AHN has been proposed as a mechanism that contributes to the distinct encoding of similar events across time owing to changes in the DG granule cell population present at the time of encoding (Becker, Macqueen, & Wojtowicz, 2009). At shorter timescales, AHN has also been proposed as a mechanism which facilitates the linking of similar episodes across time due to the hyper excitable nature of immature granule cells. Activity in these cells may be less selective and may serve as a sort of contextual glue linking features of episodes together by responding consistently

across episodes that may share this temporal context (Becker et al., 2009; Becker & Wojtowicz, 2007), a process refereed to as pattern integration (Aimone, Wiles, & Gage, 2009). Recent empirical study suggests that it is possible that AHN may facilitate pattern separation by influencing the ratio of immature to mature DG granule cells present in the DG at any given time.

Using a transgenic mouse line, researchers were able to selectively inhibit the activity of mature granule cells in the DG. They found that transgenic mice exhibited impaired performance on a rapid pattern completion task but exhibited enhanced performance when distinguishing between highly similar context pairs. Upon irradiation and subsequent immature granule cell ablation, transgenic mice became impaired at distinguishing between highly similar context pairs. This suggests that immature DG neurons support pattern separation while more mature DG neurons support the somewhat opposing pattern completion process (Nakashiba et al., 2012). The researchers propose that a greater ratio of immature to mature neurons may facilitate the distinct encoding of overlapping input due to the seeming propensity of this population to detect subtle changes in input (Nakashiba et al., 2012). By responding to static aspects of an episode consistently across time, mature granule cells have contributed to the encoding of many episodes in the past. These episodes, or at least the elements of these episodes encoded by mature granule cells, would therefore be highly overlapping in the CA3. In this way, mature granule cells drive the CA3 network toward previously existing attractor states, facilitating pattern completion. On the other hand, immature granule cells have only recently been functionally integrated into the DG. The potential of these neurons to drive the CA3 network toward

previous attractor states is much lower. In fact, it may instead be more likely that activity in these cells will drive the CA3 toward new attractor states. Despite the fewer number of immature (5%) compared to mature (95%) neurons in the DG, the hyper excitable nature of the immature granule cells may help offset the population size differences (Nakashiba et al., 2012).

In order to better understand the functional significance of AHN to pattern separation, researchers have begun to turn to empirical research in non-human animals. Clelland et al. (2009) used focal irradiation to ablate AHN in-8-week old mice and examined performance on two high interference spatial memory tasks requiring successful spatial pattern separation. The first was an 8-arm radial maze that required mice to perform a delayed non-match to sample task whereby rats must learn to avoid the previously rewarded arm when presented with a choice between a previously rewarded arm and a new arm. The spatial separation of the arms was manipulated to create trials of varying potential for memory interference. Irradiated mice with ablated AHN performed significantly worse than controls but only on the low separations, suggesting that AHN was required to avoid the interference between the spatially similar maze arms. The second task utilized a touchscreen whereby mice learned to perform a similar delayed non-match to sample 2-choice spatial discrimination task involving varying levels of spatial separation. Again, irradiated mice with reduced neurogenesis performed significantly worse than controls on the low separation/high interference trials. Critically, object recognition memory of irradiated mice was no different than controls suggesting that pattern separation is only required under conditions of high interference or pattern overlap.

Other researchers have demonstrated spatial pattern separation deficits in animals with reduced neurogenesis. Using a transgenic line of mice to ablate AHN, Dupret et al. (2008) trained animals on a variable start-location version of the Morris Water Maze. In this relational version, mice must encode spatial relationships in order to locate the platform from various views within the maze environment. This relational encoding or allocentric search strategy requires the use of previously learned spatial information to infer where the platform is located. As a result, the task has a high potential for interference as mice must encode a series of highly similar experiences within the maze and distinguish between them in order to develop a search strategy. Mice with ablated neurogenesis exhibited impairments specific to the relational memory version of the maze task. Similar research involving differential learning strategies for the Morris Water Maze found that mice with ablated neurogenesis via temozolomide administration failed to learn a spatially precise strategy and exhibited impairments in reversal learning (Garthe, Behr, & Kempermann, 2009). Others have also shown that while initial learning of the traditional Morris Water Maze task is unaffected by AHN ablation, long-term retention of spatial location memory is inhibited (Snyder, Hong, McDonald, & Wojtowicz, 2005).

In addition to these studies of spatial pattern separation, animals with reduced neurogenesis demonstrate impairments on other high interference memory tasks including trace conditioning (Shors et al., 2001), contextual fear conditioning (Drew, Denny, & Hen, 2010; Saxe et al., 2006; Wojtowicz, Askew, & Winocur, 2008), learning overlapping odour pair discriminations Luu et al. (2012) and long-term retention

of a learned visual discrimination when an interfering visual task is performed subsequently (Winocur, Becker, Luu, Rosenzweig, & Wojtowicz, 2012). Conversely, genetic and exercise manipulations that up-regulate neurogenesis enhance performance on many of the same tasks in rodents (Creer, Romberg, Saksida, van Praag, & Bussey, 2010; Sahay et al., 2011; van Praag, Shubert, Zhao, & Gage, 2005; Winocur et al., 2012; Wojtowicz et al., 2008). Outside of the lab, neurogenesis levels can be affected by a variety of factors that include, but are not necessarily limited to, alcohol consumption, stress, and depression.

1.6 Regulation of Adult Hippocampal Neurogenesis

The effects of environmental factors on AHN were first described in a study by Kempermann, Kuhn, and Gage (1997) in which they show enhanced DG neuronal survival in mice exposed to enriched environments. Similar work in non-human animals extended these findings, demonstrating increased AHN in response to environmental enrichment (Fowler, Liu, Ouimet, & Wang, 2002; Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999). In addition to environmental enrichment, exercise can also up-regulate AHN (Fabel et al., 2009; Olson, Eadie, Ernst, & Christie, 2006; Pereira et al., 2007; van Praag, Kempermann, & Gage, 1999) and is associated with various cognitive benefits as well (Creer et al., 2010; Sahay et al., 2011; van Praag et al., 2005; Winocur et al., 2012; Wojtowicz et al., 2008). While factors like environmental enrichment and exercise can up regulate AHN, many lifestyle variables can reduce

AHN.

Chronic stress is a potent inhibitor of AHN (Cameron & Gould, 1994; Malberg, Eisch, Nestler, & Duman, 2000; McEwen, 2001) and is believed to act on AHN through the release of corticosterone in non-human animals via the hypothalamus-pituitary-adrenal (HPA) axis (Cameron & Gould, 1994; Cameron & McKay, 1999). While stress is generally accepted to be a major causal factor in the pathogenesis of depression, depression itself seems to related to reduced AHN. For example, neurogenesis has been demonstrated as being required for the therapeutic effects of anti-depressant medications in rodents (Santarelli et al., 2003) and non-human primates (Perera et al., 2011). Further, anti-depressant medications, electroconvulsive therapy, and running/exercise therapies that are used to treat the behavioural symptoms of depression, have been shown to up-regulate hippocampal neurogenesis (Encinas, Vaahtokari, & Enikolopov, 2006; Madsen et al., 2000; Malberg et al., 2000; van Praag et al., 1999, 2005). Moreover, selective serotonin reuptake inhibitors (SSRI's) have been shown upon autopsy to increase DG cell proliferation and angiogenesis in humans with major depressive disorder (Boldrini et al., 2012, 2009). Similar to stress and depression, alcohol bingeing is associated with AHN reductions as well.

Animal studies have shown that alcohol bingeing can inhibit neurogenesis in the SGZ and SVZ and that these effects are much more severe in adolescents than adults. Some of this work investigated the effects of chronic and acute alcohol bingeing on neural progenitor cell proliferation and survival in adolescent and adult rats using various concentrations of ethanol (Crews, Mdzinarishvili, Kim, He, & Nixon,

2006; Nixon & Crews, 2002). In an acute condition, rats were gavaged once with a 5g/kg dose of ethanol, injected with Bromodeoxyuridine (BrdU) to stain active proliferating cells and were then killed either 5 hours or 28 days later. In a chronic condition, rats were infused with ethanol (mean dose of 9.3g/kg/day) 3 times per day for a four-day period. These rats were also injected with BrdU once on each of the four days and killed either after the last day of treatment or 28 days later. Experimenters found reduced neural progenitor cell proliferation in the SGZ and SVZ in both conditions suggesting that even acute alcohol bingeing in adult rats impairs neurogenesis in the SGZ. Furthermore, the effects of alcohol bingeing have been demonstrated to be even more severe in adolescent rats than adults. Rat studies have found that alcohol potently and dose dependently reduces neurogenesis in the SGZ and SVZ in adolescent rats to a greater degree than adult rats (Crews et al., 2006). Additionally, researchers demonstrated that even an acute alcohol binge of 2.5g/kg weight resulted in immediate reductions in neurogenesis (5 hours later) as evidenced by significant reductions in BrdU/Neuronal N labeled cells compared to controls. Experimenters also provided evidence for long-term reductions in neurogenesis (28 days later), documenting sustained decreases in BrdU labeled cells 28 days after the last binge treatment. Considering the significant amount of change being undergone in the adolescent brain, it is not surprising to find that adolescents seem to be more susceptible to alcoholic insult. Studies in non-human adolescent primates have also demonstrated that alcohol consumption leads to long lasting reductions in hippocampal neurogenesis (Taffe et al., 2010). This is a powerful finding as non-human primates are more genetically and physiologically similar to humans

than rats, exhibit many similar cognitive capabilities, and readily consume alcohol to the point of intoxication like humans (Taffe et al., 2010).

1.7 Aim of Experiment 1 and 2

Due to the difficulty of assaying neurogenesis levels non-invasively, relatively little attention has been paid to studying neurogenesis-dependent memory functions in humans. Pereira et al. (2007) showed in mice that exercise-induced increases in neurogenesis were associated with increased DG blood volume (as measured by contrast-enhanced MR imaging), indicative of enhanced DG angiogenesis, and furthermore, they showed a similar increase in DG blood volume in humans who took part in several weeks of exercise coupled with enhanced performance on a test of verbal learning. Our lab has begun to analyze performance on human analogs of tasks found to be neurogenesis sensitive in rodents. We have shown that a controlled exercise intervention enhances performance on a task with high visual pattern interference, Kirwan and Stark's (2007) Behavioural Pattern Separation task - Object version (BPS-O) (Déry et al., 2013). Conversely, high depression scores are associated with impaired performance on the CANTAB delayed match to sample (DMS) task, which tests delayed recognition of unfamiliar complex visual targets amongst highly similar lures (Becker et al., 2009), and other visual pattern separation tasks as well (Déry et al., 2013; Shelton & Kirwan, 2013). It is important to note that these effects appeared to be highly selective, as exercise and depression levels did not correlate with performance on any other measure in our large battery of control

tasks (Becker et al., 2009; Déry et al., 2013).

In the current study, we sought to extend these findings to two spatial pattern separation tasks, and to examine the effects of depression and alcohol bingeing on this potentially neurogenesis-dependent form of memory. The first task was designed as a fun and novel game for potential diagnostic use while the second was designed to investigate spatial pattern separation performance more systematically and at a finer scale. The current study is the first to investigate the relationships between depression, alcohol bingeing and neurogenesis through performance on high-interference spatial memory tasks in humans.

Chapter 2

Experiment 1

2.1 Method and Materials

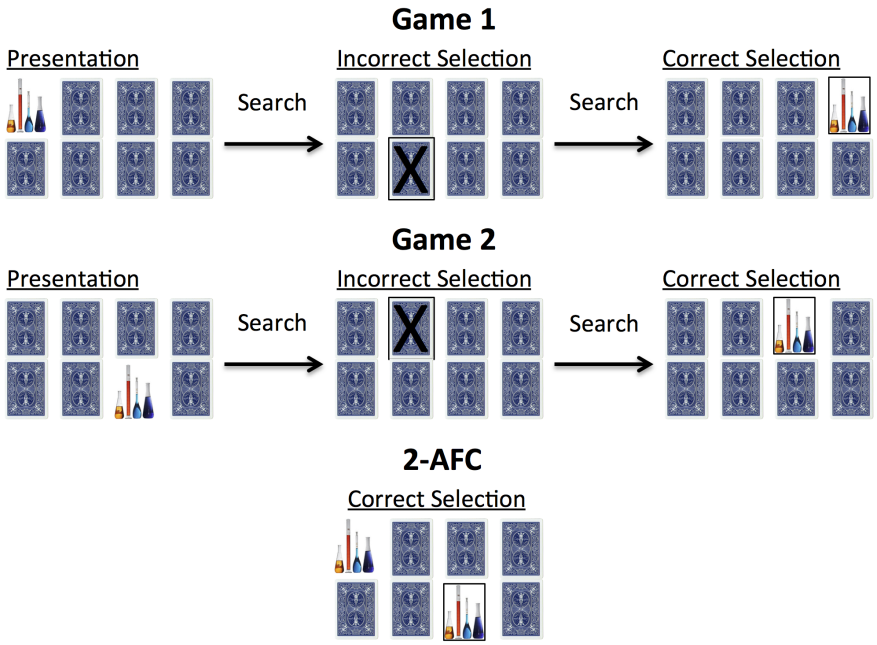
Outlier detection was used (Hoaglin, Iglewicz, & Tukey, 1986) to identify participants that may have misunderstood the instructions or did not attend to the task. On this basis, one person's data were removed from the analysis of Experiment 1 resulting in 72 participants' data included in the final analysis (26 males, 46 females; mean age=18.6 years, SD=1.43). Participants were McMaster University students enrolled in Introductory Psychology with normal or corrected to normal vision and no history or previous diagnosis of major depression or other psychiatric disorders. All participants were recruited through "www.experimetrix.com/mac", an online recruitment programs used by McMaster University. For participation in research, participants received a credit counting towards their final grade in Introductory Psychology. The McMaster Research Ethics Board (MREB) approved all aspects of our study.

To obtain measures of depression and alcohol consumption respectively, we administered the Beck Depression Inventory-II (BDI) (psychological corporation) and a lab designed questionnaire probing a number of lifestyle variables including drinking habits. The BDI is a widely used standardized, commercially available test consisting of 21 multiple-choice questions, each on a 4-point scale, about the individual's mood during the past week. Measures of alcohol consumption included in the lifestyle questionnaire were number of drinks consumed on a typical drinking occasion (typical alcohol consumption) and a series of questions probing frequency of bingeing at ages 13-22. To quantify typical consumption, we asked participants to identify how many drinks they typically consume when they drink. A drink is defined as a 12 fluid ounce (can) of beer or other 5% alcoholic beverage, 5 fluid ounce glass of wine, and 1.5 fluid ounce shot or glass of "hard liquor" (whiskey, gin, rum, vodka, tequila etc.). A binge is defined by the United States National Institute on Alcohol Abuse and Alcoholism to be 4 drinks per 2 hours for a female and 5 drinks per 2 hours for a male.

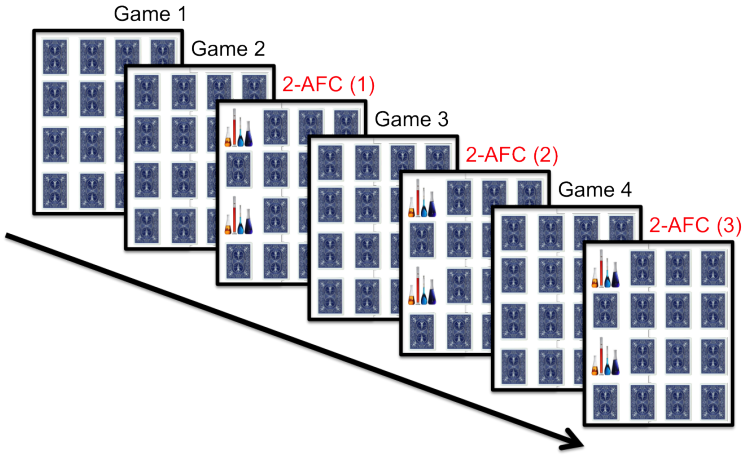
The first of the two control tasks administered in both experiments was CANTAB's paired associate learning task (PAL), a well-established visuo-spatial associative learning task sensitive to hippocampal pathology, but lacking a high-interference component that may rely heavily on DG neurogenesis (See Appendix). Indeed, we have previously shown that performance on this task does not fluctuate as a function of BDI score (Becker et al., 2009; Déry et al., 2013). The task involves the presentation of a series of patterns that are unique in shape and colour. During a study trial, six white boxes are distributed around the screen and are opened, one

at a time, in a random order to reveal concealed pattern. Once all of the white boxes have revealed what was concealed behind them, the test trial begins. In a test trial, patterns are presented one at a time in the middle of the screen with the white boxes still distributed around the screen as in the study trial. The participants must select the white box where the pattern was originally located in the study trial. If an error is made, the participant is allowed to finish the test trial before the patterns are presented again to remind the participant of their locations. The test becomes progressively more difficult by increasing the number of patterns hidden behind the white boxes on a particular study trial. The second control task used was a computerized reverse digit span task to assess working memory. Participants are shown a series of digits and are required to remember this series and then input the digits in reverse order using a keyboard (See Appendix). All statistical analyses were performed using SPSS version 18 (SPSS Inc.).

We developed a novel spatial pattern separation task, the Concentration Memory Task (CMT), which may require neurogenesis by incorporating a high interference component (Figure 2.1). The task was designed to mimic spatial pattern separation problems encountered in everyday life, such as remembering where one parked one's car today within a parking lot in which one has parked numerous times before. Using a touchscreen computer, participants play a game in which they performed an exhaustive search through a grid of 16 face down playing cards to find matching image pairs. After completion of Game 1, three more challenging games are played in which some images from the previous game are repeated at new locations. These repeated images appear in a total of 4 different locations within 2 consecutive games. After



(a)



(b)

Figure 2.1: (a) A target is briefly revealed at the start of a trial and then hidden. Participants must search the grid until they find the correct match. Importantly, some images are repeated between games so that these images are experienced in different spatial locations. (b) Participants complete 4 games in which they search for 8 image pair matches within a 4x4 grid of playing cards. Following games 2, 3 and 4, participants complete 2-alternative forced choice tasks consisting of 4 trials each for a total of 12 trials (Game 1 - Game 2 - 2AFC(1) - Game 3 - 2AFC(2) - Game 4 - 2AFC(3)).

each game, using a 2-alternative forced choice (2-AFC) paradigm, participants are then tested on whether they can identify in which of two locations they have seen an image most recently, with 1 image having been presented in the most recent game and the other presented in the game immediately prior. Optimal performance on this task requires the avoidance of interference from multiple similar memory representations, requiring successful spatial pattern separation to segregate the memories of identical objects experienced in more than one location. We predict that the high potential for memory interference associated with multiple object presentations places a high demand on neurogenesis, consistent with the rodent literature (Clelland et al., 2009; Creer et al., 2010; Luu et al., 2012; Winocur et al., 2012, 2006). Participants played a total of 4 games for a total of 32 image pair searches (8 per game) and completed three, 2-alternative forced choice tasks appearing after games 2, 3 and 4. Each 2-AFC consisted of 4 trials for a total of 12 2-AFC trials. It was predicted that those with elevated depression and alcohol consumption scores would have suppressed neurogenesis and exhibit selective performance deficits on the neurogenesis sensitive CMT while maintaining normal performance on the two control tasks predicted to be neurogenesis-independent.

2.2 Results

To obtain an estimate of neurogenesis-sensitive performance, percent correct on the CMT 2-AFC was analyzed. During the 2-AFC, participants select the location where they thought they had seen an image most recently. This proved to be relatively

difficult, as evidenced by mean performance of 71.1% (SD=15.1%). Correlation analysis revealed a significant, negative relationship between performance on the CMT and BDI ($r_s(72)$=-.308, p=.008). A correlation was also found between CMT performance and typical alcohol consumption ($r_s(52)$=-.280, p=.04). No correlations were found between CMT and any other measures of alcohol consumption. These relationships can be seen in Figure 2.2. Confidence intervals for the bootstrapped correlations can be found in Table 2.1.

Task	**Variable**	**Coefficient**	**p-value**	**95% CI**
CMT	BDI	-.308	.008	[-.595, -.036]
CMT	Typical Alcohol Consumption	-.280	.04	[-.451, -.120]
PAL	BDI	.011	.930	[-.241, .258]
PAL	Typical Alcohol Consumption	-.027	.850	[-.274, .232]
Digit Span	BDI	.060	.621	[-.196, .314]
Digit Span	Typical Alcohol Consumption	-.181	.199	[-.448, .106]

Table 2.1: Spearmans's rank correlation coefficient used for correlation analysis involving BDI and typical alcohol consumption scores as they did not follow a normal distribution.

As a means of assessing the reliability of the neurogenesis dependent 2-AFC task, split-half reliability was used. The trials were split into odd and even trial groups. There was a significant positive correlation between performance on the even and odd trials (r(72)=.409, $p < .001$). This reliability estimate was then adjusted for full test length using the Spearman-Brown prediction formula resulting in a predicted reliability (P^*xx') of 0.581.

To further examine the relationship between depression, alcohol consumption, and CMT performance, we separated individuals into those scoring either low or high on the aforementioned lifestyle variables based on a median split. Significant group

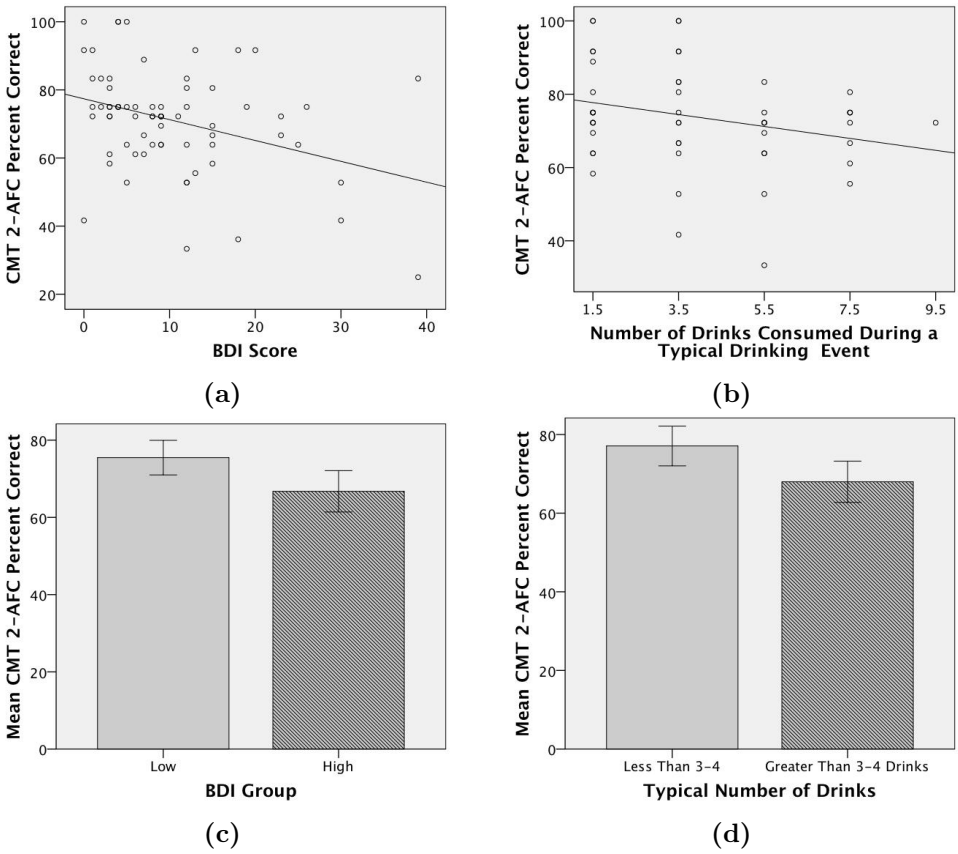


Figure 2.2: a) Correlation between CMT performance and depression scores (BDI), (b) Correlation between CMT performance and typical alcohol consumption. (c) Median split analysis of CMT performance between those scoring at or below the median on the BDI (Low) and those scoring above (High)(± 2 standard error). (d) CMT performance of those scoring at or below the median on typical alcohol consumption and those scoring above (± 2 standard error). Median value is right at the definition of a binge used by the United States National Institute of Alcohol Abuse and Alcoholism.

differences in percent correct on the CMT were found between the above-median (M=66.7%, SD=15.8%) and below-median (M=75.2%, SD=13.4%) BDI groups using the Mann-Whitney U test (U(69) = 430.5, z = -2.311, p = .021). Cohen's effect size value (d=.58) suggests a moderate to high effect of depression on performance. Significant group differences in percent correct on the CMT were also found between above-median (M=67.8%, SD=11.2%) and below-median (M=77.1%, SD=14.3%) alcohol consumption groups (U(49) = 196.5, z = -2.111, p = .035). Cohen's effect size value (d=.7) suggested a moderate to high effect of alcohol on performance. No such group differences were found between above and below-median BDI groups on either control task including the paired associates learning (U(69) = 551, z = .-.929, p = .353) and reverse digit span task (U(68) = 579, z = -.373, p = .709). The same is true of above and below-median alcohol consumption groups (U(51) = 310, z = .-.382, p = .703); (U(50) = 242, z = -1.371, p = .170) on PAL and reverse digit span respectively.

Finally, linear regression was used to identify variables that would best predict performance on the CMT and, as a result, give an indication of the state of hippocampal neurogenesis. Variables were entered into a regression model. The model that accounted for the greatest amount of variance in CMT performance was that which included both BDI score (Beta=-.397, p=.003) and typical alcohol consumption (Beta=-.274, p=.033) and accounted for 20.7% (adjusted r-squared=.207) of observed variance in CMT performance (F(2,49)=7.655, p=.001). The low degrees of freedom in this model can be attributed to only 50 of the 72 participants reporting ever consuming any alcohol at all. To further identify the individual contributions

of each predictor variable to CMT performance, semi-partial correlations were calculated. These correlations have the potential to give a truer indication of the relationship between a predictor and dependent variable, by removing the variance in one predictor that is accounted for by another. The semi-partial correlation between BDI score and CMT performance was found to be slightly higher than that of the traditional spearman correlation (sr = -.396). The same was true of typical alcohol consumption (sr = -.320), potentially indicating the true relationship between these variables and task performance. However, these correlations were performed on non-normally distributed data and should be interpreted with that in mind.

2.3 Discussion

Although these relationships are noisy, individuals who score higher on scales of depression and typical alcohol consumption tend to score more poorly on the CMT as evidenced by a lower percent correct score when determining the location in which an image was seen most recently. The lack of relationship between BDI, alcohol consumption, and control task performance suggests that high depression scores and high alcohol consumption levels are associated with a selective deficit on spatial pattern separation. The lack of relationship between these variables and control tasks suggests that the impairments observed on the CMT are not the result of a working memory impairment, a general hippocampal impairment or a general memory impairment. The selective deficit in CMT performance found in those with elevated BDI and alcohol consumptions scores, in spite of intact performance on both control

tasks, in conjunction with previous research suggests that the observed impairments may be due to a pattern separation deficit, which may be caused by a reduction in neurogenesis.

Interestingly, when participants were divided into light and heavy drinkers based on a median split of typical alcohol consumption scores, the median value of typical consumption (3.5 drinks) nicely paralleled the definition of a binge used by the United States National Institute of Alcohol Abuse and Alcoholism, which is 4 drinks for a woman and 5 for a man. Participants in Experiment 1 that reported consuming 4 or more drinks per session performed significantly worse on the CMT compared to those consuming less than 4 drinks. It is important to note that none of our participants in either experiment had a clinical diagnosis of depression. Thus, participants with higher scores should be considered as being only relatively, or sub-clinically, depressed.

Chapter 3

Experiment 2

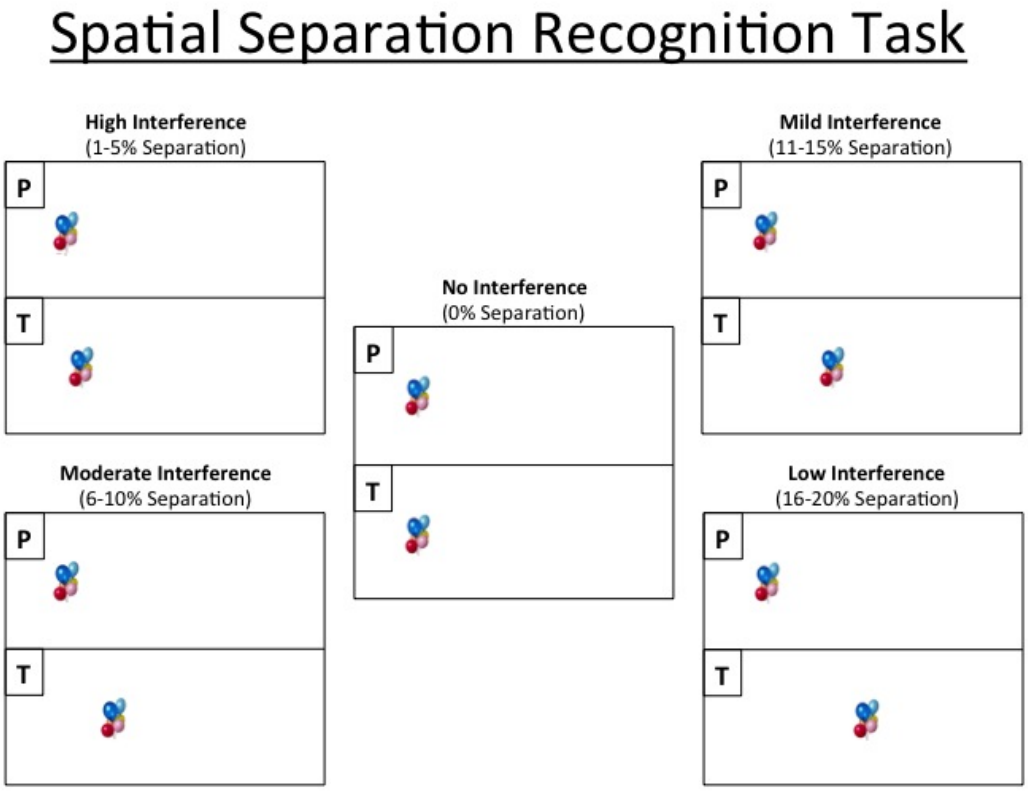
3.1 Methods and Materials

Outlier detection was used (Hoaglin et al., 1986) to identify participants that may have misunderstood the instructions or did not attend to the task. On this basis, the data from 5 participants were removed from the analysis of Experiment 2 resulting in 120 participants' data included in the final analysis (33 males, 87 females; mean age=18.8 years, SD=1.64). Participants were McMaster University students enrolled in Introductory Psychology with normal or corrected to normal vision and no history or previous diagnosis of major depression or other psychiatric disorders. All participants were recruited through "http://mcmaster.sona-systems.com", an online recruitment programs used by McMaster University. For participation in research, participants received a credit counting towards their final grade in Introductory Psychology. The McMaster Research Ethics Board (MREB) approved all aspects of our

study.

To obtain measures of depression and alcohol consumption respectively, we administered the Beck Depression Inventory-II (BDI) (psychological corporation) and a lab designed questionnaire probing a number of lifestyle variables including drinking habits. The BDI is a widely used standardized, commercially available test consisting of 21 multiple-choice questions, each on a 4-point scale, about the individual's mood during the past week. Measures of alcohol consumption included in the lifestyle questionnaire were number of drinks consumed on a typical drinking occasion (typical alcohol consumption) and a series of questions probing frequency of bingeing at ages 13-22. A binge is defined by the United States National Institute on Alcohol Abuse and Alcoholism to be 4 drinks per 2 hours for a female and 5 drinks per 2 hours for a male. The first of the two control tasks administered in both experiments was CANTAB's paired associate learning task, a well-established visuo-spatial associative learning task sensitive to hippocampal pathology, but lacking a high-interference component that may rely heavily on DG neurogenesis (See Appendix). The second control task used was a computerized reverse digit span task to assess working memory. Participants are shown a series of digits and are required to remember this series and then input the digits in reverse order using a keyboard (See Appendix). All statistical analyses were performed using SPSS version 18 (SPSS Inc.).

To assess spatial pattern separation ability at finer separations, we developed the Spatial Separation Recognition Task (SSRT). During the presentation phase, participants view images of objects, the locations of which vary along the horizontal axis of the computer screen while the vertical axis is held constant at 50 percent of



(a)

Figure 3.2: The two trial types, **same** (separation of 0%) and **different** (separations of 1-5%, 6-10%, 11-15%, 16-20%), for the Spatial Separation Recognition Task. "**P**" represents the presentation phase. "**T**" represents the test phase.

the screen. During the testing phase, participants are shown the same images, one at a time, in either the exact same location as the presentation trial or a different location. The "different" trials are divided into 4 groups consisting of 5 separation each which ranged from 1-20% of the screen. Separations 16-20% were grouped as the large-, 11-15% as the moderate-, 6-10% as the medium-, and 1-5% as the low-separation conditions, each with a different potential for memory interference. Despite being labelled as the high separation trials (therefore trials with relatively weakest potential for interference), the 16-20% trials are still challenging and have a high potential for interference. It is only relative to the low separation trials that these trials are characterized as low-interference. Participants respond by pressing the 1-key if the image is in the same location or the 2-key if the image is in a different location. Optimal performance on this task requires the participant to create distinct memory representations of each image location during the presentation phase so as to avoid interference when presented with the same image during the testing phase. We predicted that those with higher depression and alcohol scores would perform more poorly than those with lower scores on this spatial pattern separation task, with our separations elucidating where these differences were largest. The experiment consists of 24 blocks, each with 7 presentation and 7 testing trials for a total of 168 trials.

3.2 Results

As a means of assessing the reliability of the SSRT, split-half reliability was used. The separations were grouped into odd and even groups (2,4,6, etc. & 1,3,5, etc).

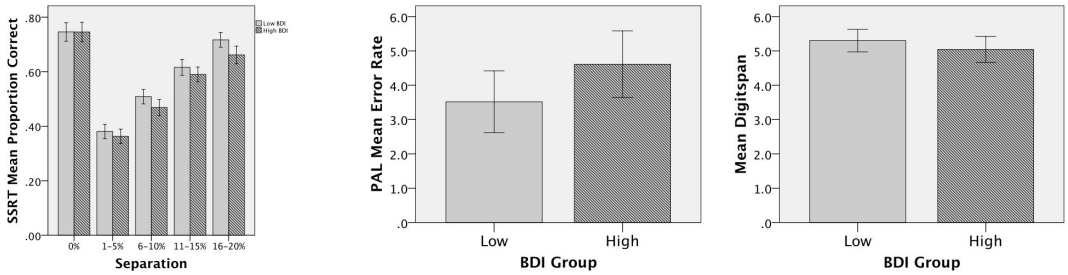
There was a significant positive correlation between performance on the even and odd trials (r(120)=.712, $p < .001$). This reliability estimate was then adjusted for full test length using the Spearman-Brown prediction formula resulting in a predicted reliability (P^*xx') of 0.832.

A repeated measures analysis of variance (ANOVA) was used to identify whether there was an effect of spatial similarity (separation) on SSRT performance. The assumption of sphericity was found to be violated ($\chi^2(209) = 340.823, p < .001$) therefore degrees of freedom were corrected using Huynh-Feldt estimates of sphericity ($\epsilon = .854$). A main effect of spatial separation was found $F(17.076, 2032.001) = 106.922, p < .001$ as well as a significant linear trend, $F(1, 119) = 766.23, p < .001$, indicating that as separation increased, performance increased proportionately. Previous work by Dery et al. (2013) indicated that performance differences on the behavioural pattern separation task for objects between high and low BDI groups was restricted to visual stimuli pairs that were relatively less similar in terms of their visual characteristics. These findings are similar to those of Stark, Yassa, and Stark (2010) looking at performance differences between young and aged participants on a spatial pattern separation task. In the study by Stark et al. (2010), aged participants were further broken down into aged-normal and aged-impaired groups. While the aged-impaired group was not clinically impaired, these individuals scored 1 standard deviation below the expected range of performance for young individuals on the Rey Auditory Verbal Learning task. The aged-normal group scored on par with the expected performance range of young individuals. Significant performance

differences between the young and aged-impaired group on the spatial pattern separation task were restricted to the largest separations. As a result, we expected to find performance differences between individuals with high and low depression scores, particularly on the relatively less similar trials, those with relatively greater separation. Correlation analysis revealed a significant, negative relationship between performance on the SSRT and BDI ($r_s(120)$=-.182, p=.046), but only at the large separations. Participants were also separated into high (BDI above 8, N = 57, M = 16.16) and low (BDI at or below 8, N = 63, M = 4.67) BDI groups using a median split in order to perform group comparisons. Levene's test indicated unequal variances ($F = 24.147, p < .001$) so degrees of freedom were adjusted from 118 to 68.539. These groups differed significantly in BDI score, $t(68.539) = -11.045, p < .001$. Overall, the low BDI group (M = 55.3%, SD = 8.27%) was significantly better at identifying the correct location on "different" trials compared to the high BDI group (M = 51.9%, SD = 8.18%), (U(118) = 1402.5, z = -2.066, p = .039). This difference was found to be the result of performance on the large separation trials (16-20% shift) where the low BDI group (M = 71.7%, SD = 10.8%) significantly outperformed the high BDI group (M = 66.2%, SD = 12.2%), (U(118) = 1293.5, z = -2.640, p = .008). The same performance differences between high and low BDI groups were not found on PAL (U(110) = 1448.5, z = -.713, p = .476) and digit span control tasks (U(96) = 1053, z = -1.056, p = .291).

Of the 120 participants, 68 reported binge drinking with some regularity between the age of 13 and 22. Interestingly, typical alcohol consumption was not found to be correlated with SSRT performance. Instead, the number of years of reported

bingeing was correlated with SSRT performance but only at the large separations, $r_s(68) = -.248, p = .041$, and not the smaller separations. However, number of years bingeing was not significantly correlated with either PAL or digit span performance. Linear regression was used to quantify the amount of variance in SSRT performance that could be accounted for by BDI grouping and bingeing history. These variables were entered into a stepwise regression model. Together, BDI score (Beta = -.315, p = .006) and number of years bingeing (Beta = -.316, p = .006) accounted for 16.5% (adjusted $r^2 = .165$) of observed variance in SSRT performance, $F(2, 65) = 7.621, p = .001$. Further analysis using this same regression model produced semi-partial correlations between BDI score and SSRT performance (sr=-.315) and number of years bingeing and SSRT performance (sr=-.316). As mentioned previously, it is important to note that the semi-partial correlations were run on non-normally distributed data.



(a) **(b)** **(c)**

Figure 3.3: (a) Median split analysis of performance between low and high BDI groups on all separations of the SSRT (± 2 standard error). (b) Median split analysis of mean number of errors on neurogenesis-independent paired associate learning task (± 2 standard error). (c) Median split analysis of mean digit span (± 2 standard error).

3.3 Discussion

In Experiment 2, we found that those with higher depression scores performed significantly worse than those with lower scores on the pattern separation SSRT. Interestingly, this performance difference was restricted to the large separations. When analyzed as a continuous variable, binge years was negatively correlated with performance (but only at the largest separations), suggesting that as the number of years bingeing increases, spatial pattern separation performance decreases in a linear fashion. The lack of relationship between BDI, binge years, and control task performance suggests that high depression scores and high alcohol consumption levels are associated with a selective deficit on spatial pattern separation. It is important to note that none of our participants in either experiment had a clinical diagnosis of depression. Thus, participants with higher scores should be considered as being only relatively, or sub-clinically, depressed.

Chapter 4

Conclusion

4.1 General Discussion

The data presented here are consistent with our hypothesized spatial pattern separation deficit, a result of reduced DG neurogenesis, in those with higher depression scores and those who tend to binge drink. The linkage between stress, depression and neurogenesis has been the subject of considerable speculation (see e.g. Becker & Wojtwoicz, TiCS). Chronic stress not only suppresses neurogenesis (Cameron & Gould, 1994; McEwen, 2001), but is widely believed to be a major causal factor in the pathogenesis of major depression in humans (Brown, Rush, & McEwen, 1999). Furthermore, neurogenesis reductions in combination with stress, have been shown to lead to depressive-like symptoms in mice suggesting that neurogenesis may help to buffer the stress response in intact animals (Snyder, Soumier, Brewer, Pickel, & Cameron, 2011). Through the use of functional imaging, it has been shown that those

with higher BDI scores exhibit reduced DG/CA3 activity upon exposure to highly similar visual stimuli (Fujii, Saito, Yanaka, Kosaka, & Okazawa, 2014), potentially supporting the proposed role of neurogenesis in pattern separation and its relationship with depression. This negative correlation between activity and BDI score can potentially be understood when we consider the firing properties of immature neurons, which are thought to be important for their contribution to pattern separation in the DG. Their plastic and hyper-excitable nature (Marín-Burgin, Mongiat, Pardi, & Schinder, 2012; Schmidt-Hieber et al., 2004; Wang et al., 2000) could make this population the ideal cellular units for responding to subtle changes in cortical input. It is possible that these neurons are tuned to specific features of an episode and fire much more strongly in response to these features than mature granule cells. In other words, these cells are much more sensitive to the features they respond to than more mature granule cells. In this way, activity of immature granule cells is the major determining factor in successful pattern separation. In fact, it has been shown that more immature DG neurons support pattern separation while more mature DG neurons support the somewhat opposing pattern completion process (Nakashiba et al., 2012). Higher BDI, indicative of greater depression and reduced neurogenesis, could result in a shift of the neuronal makeup of the DG such that at any given time, this population of neurons would consist of far fewer immature granule cells. In such a state, an individual would be expected to exhibit impaired performance on a task that stresses pattern separation ability. In the current study we show that those with higher depression scores exhibit impaired performance on two spatial pattern separation tasks, a behavioural result that is in line with previous findings regarding the

physiological nature of immature granule cells (Marín-Burgin et al., 2012; Nakashiba et al., 2012; Schmidt-Hieber et al., 2004; Wang et al., 2000) as well as documented relationships between AHN and depression (Boldrini et al., 2012, 2009; Encinas et al., 2006; Madsen et al., 2000; Malberg et al., 2000; Santarelli et al., 2003; van Praag et al., 1999, 2005).

From a clinical standpoint, trouble with everyday pattern separation problems, in combination with high stress or depression scores, may be an early sign of a shift toward major depression and could be important for diagnostic purposes in the future. Currently, functional imaging is an expensive diagnostic procedure for the identification of potential biomarkers of subclinical depression (Fujii et al., 2014). The CMT presents itself as a cheaper, potential early cognitive marker that could be used as the basis for pre-clinical interventions as well as to quantify the effect of a pro-neurogenic therapy, like exercise or pharmaceutical treatment.

As mentioned previously, spatial pattern separation deficits were also found in those who tend to binge drink. Adolescence is a period of important neuronal maturation as well as vulnerability. Among other areas, the brain undergoes changes in neurotransmission and plasticity in both the hippocampus and prefrontal cortex (Adriani et al., 2004; Lewis, Cruz, Eggan, & Erickson, 2004)(See also: Crews, He, and Hodge (2007)). It is not surprising then that during this period of heightened plasticity, the adolescent rat brain is more susceptible to alcoholic insult than that of an adult. Previous work in rodents has shown that binge ethanol exposure more potently depletes neurogenesis in adolescent rats compared to adults (Crews et al., 2006). Similar to what was described previously with depression, this type of alcohol

consumption has the potential to drastically alter the neuronal makeup of the DG by reducing the ratio of immature to mature neurons. Such a shift would result in the DG/CA3 being less apt to detecting subtle changes in stimulus presentation essential for successful pattern separation. As the sample used in the current study consisted of adolescent university students, it was expected that any effects of binge drinking on neurogenesis would be especially prevalent and that this effect would manifest as performance impairments on our selection of spatial pattern separation tasks.

In both experiments we observed alcohol-related deficits in spatial pattern separation. However, the specific measures of alcohol consumption that correlated with task performance differed across the two experiments. In Experiment 1, current alcohol bingeing patterns predicted a deficit on the CMT, whereas in Experiment 2, years of bingeing / age of onset of bingeing correlated negatively with performance on the SSRT. We would have expected the same measures of alcohol consumption to relate to performance in both Experiment 1 and 2. One reason for this discrepancy may be that the participant pools were slightly different. That is, in Experiment 1, only 30% of drinkers reported bingeing for more than 3 years whereas 43% reported bingeing for more than 3 years in Experiment 2. Under these circumstances, it was not entirely surprising to find a correlation between binge years and performance in Experiment 2 and not Experiment 1 given the larger sample size and greater range of bingeing scores in Experiment 2 with which to draw a correlation. Similarly, the lack of correlation between typical consumption and SSRT performance in Experiment 2 could be due to the age differences between samples. In Experiment 1, 68% of participants were aged 17 or 18 whereas only 58% of participants in Experiment 2 were

aged 17 or 18. There was a greater percentage of participants in Experiment 2 in late adolescence or early adulthood compared to Experiment 1. Given that our typical alcohol consumption variable provides a measure of current alcohol consumption behaviour and that the young-adult brain is less susceptible to alcoholic insult than that of an adolescent brain, it is not unreasonable that we failed to find a correlation between typical alcohol consumption and performance in Experiment 2.

In order to determine the consistency of each spatial pattern separation task, split-half reliability estimates were calculated to differentiate performance on the task due to random measurement error (a combination of subject-specific factors and test-specific factors). A higher coefficient of reliability indicates greater consistency and lower proportion of performance variance due to random measurement error. The closer a correlation coefficient is to the estimate of reliability, the greater the amount of variance in true performance is explained by the variable in question. Given the split-half reliability estimate of $P^*xx' = 0.581$ for the CMT, the correlations between CMT and depression as well as CMT and alcohol bingeing (absolute correlations of $r = 0.308$ and $r = 0.280$ respectively) indicate that these factors account for a substantial portion of the variance on the CMT, suggesting that the CMT provides a valid measure of pattern separation. Similarly, we observed significant SSRT performance differences between low and high BDI groups as well as a correlation coefficient of r = -.248 between binge years and SSRT performance in combination with a very high split-half reliability ($P^*xx' = 0.853$) estimate for the SSRT. Taken together, these findings suggest that the SSRT is providing a reliable measure of spatial pattern separation. Further, analysis of the semi-partial

correlations in both experiments allowed us to ascertain, potentially, a truer relationship between our predictor variables and task performance. For example, the semi-partial correlation between BDI score and CMT performance was quite a bit stronger after accounting for the variance in BDI score accounted for by typical alcohol consumption. The same was true in every case in which a semi-partial correlation was analyzed. This type of analysis further elucidates the independent contributions of our predictor variables to this potentially neurogenesis sensitive memory function. Across our two experiments, using two different spatial pattern separation tasks, our findings are consistent with the hypothesized spatial pattern separation deficit, reflecting a reduction in neurogenesis, in those who binge on alcohol and in those with elevated depression scores.

To the best of our knowledge, this is the first study to examine the effects of binge-like alcohol consumption on pattern separation ability in humans. This research is particularly important when we consider the prevalence of this destructive behaviour in adolescents. It has previously been reported that as many as 44% of college students binge drink every two weeks, while as many as 19% binge more than 3 times per week (Wechsler, Lee, Kuo, & Lee, 2000). In fact, adolescents rats are less sensitive to the sedative effects of alcohol (Little, Kuhn, Wilson, & Swartzwelder, 1996; Silveri & Spear, 1998) and this resistance may facilitate the propensity of the human adolescent population to binge drink. When coupled with heightened vulnerability to alcohol related toxicity in adolescence, this type of behaviour can have severe damaging effects on the brain and related behaviour (Monti et al., 2005; Silveri & Spear, 1998). In addition to the memory deficits described here, long-term

effects of alcohol use during adolescence include increased risk of alcohol dependence (Grant & Dawson, 1997), learning deficits, and more widespread cognitive and memory impairments (Zeigler et al., 2005). Given the prevalence of bingeing and the cognitive impacts illustrated here, it is imperative that the risks associated with alcohol consumption be made more readily apparent to youths at an age when they are most impressionable. In this way, it may be possible to reduce the prevalence of this physically and cognitively destructive behaviour.

The finding in Experiment 2 that performance differences manifested only at the large separations may seem counter-intuitive, considering that the smallest separations should cause the greatest interference, posing the greatest challenge to pattern separation mechanisms. It is these trials that possess the greatest potential for memory interference and were hypothesized to require the greatest contribution of DG neurogenesis to overcome this interference. On the other hand, this pattern of performance closely parallels our previous findings with visual object similarity on the BPS-O task, where differences between high and low BDI individuals were driven mainly by performance on less similar visual pairs (Déry et al., 2013). In fact, both high and low BDI groups performed below chance level at the smallest separations, suggesting a failure of pattern separation mechanisms to differentiate these highly spatially similar items, regardless of one's level of neurogenesis. We suggest that DG sparse coding and AHN are not able to overcome the interference associated with such highly similar spatial representations.

While studies of the mechanisms and behaviour associated with pattern separation make up a substantial field of research, it is interesting to note that false

recognition, a seemingly separate field of study, bares a striking resemblance to what might be expected of a pattern separation failure. False recognition was first demonstrated 50 years ago and refers to a phenomenon whereby participants, when tested on retention of a memorized word list, falsely recognized new words as having been a part of the memorized list (Underwood, 1965). These new words were either semantically or physically similar to words from the original list and as such, would have likely contributed to a relatively high potential for memory interference.

It is possible that this interference, due to pattern overlap in the CA3, is responsible for false recognition. For example, say participants are presented with a list of words, many of which are associated with candy, though the word candy itself is not part of the list. When the word "gobstopper" is presented to the participant, all gobstopper-related information from the occipital, parietal, fontal cortices etc., is sent via the EC to the DG where this information is orthogonalized and projected onto the CA3. At this point, our CA3 cortical representation of the word "gobstopper" consists of all gobstopper-related information relayed to the hippocampal formation from the aforementioned cortical areas and will likely possess some contextual information describing a gobstopper (Treves & Rolls, 1992). Contained within this representation may be the notion of candy. When subsequently presented with the semantically-related new word "lollipop", a representation might then be formed in the CA3 that overlaps somewhat with that of the word "gobstopper" due to the context of candy being shared between the two items. Given extreme similarity between two words, it is possible that, similar to very small spatial separations, the amount of pattern similarity is too great for DG sparse coding and AHN to overcome. Failure

to orthogonalize these highly similar inputs results in interference which makes it difficult to recall aspects of the affected memories. In the absence of recollection, the participant must now rely on familiarity to make a recognition judgement. Given significant pattern overlap following insufficient separation, presentation of "lollipop" may elicit much the same activation as "gobstopper". Without being able to recall the original word "gobstopper" due to extensive interference, one might experience the reactivation of the overlapping "lollipop-gobstopper" representation as familiarity. This activation of overlapping representations could represent the processing fluency described by Jacoby and Whitehouse (1989), which contributes to the feeling of familiarity that participants misattribute to being due to previous exposure to the new word, "lollipop". If when presented with the new word "lollipop" the participant could recall having been presented with the word "gobstopper" during the initial encoding, it is less likely that the individual would falsely recognize the word "lollipop" as having been a part of the initial word list. This is because the individual can recollect having seen the word "gobstopper" and can potentially attribute any feeling of familiarity, following presentation of "lollipop", to having seen the word "gobstopper" previously.

Similar to the effects described by Underwood (1965), the effects of false fame can potentially be explained by a failure to successfully pattern separate. In a study of false fame by Jacoby, Woloshyn, and Kelley (1989), researchers had participants read a list of non-famous names out loud under the belief that name pronunciation was being tested. Later, participants were presented with a new list of names that contained famous, new non-famous, and non-famous names from the previously read

list. Participants were required to identify whether a name was famous or not and told to respond "non-famous" if the name was present on the previous list. Researchers found that when participants failed to recall having seen a non-famous name on the previous list, they were much more likely to falsely identify the name as famous. Jacoby and Whitehouse (1989) suggests that this is due to misattribution of the fluency of perceptual processing due to prior experience. That is, the feeling associated with the presentation of a word seen previously, in the absence of recollection, triggers a feeling of familiarity that gets misattributed to being due to fame rather than having seen the word previously. Again, it is possible that mechanisms of pattern separation and pattern completion underly false recognition in the false fame paradigm. If presented with a list of names that are read passively while performing a second task toward which attention is directed, the performance of the second task could create the underlying context associated with each passively read name. In this way, the context associated with each name is shared and highly overlapping. When judging fame, it is possible that when presented with a non-famous name from the original list, the interference associated with the encoding of a large number of items with overlapping context, may prevent the participant from recalling having seen the name previously. The presentation of the name, however, still elicits reactivation of its representation, albeit an overlapping one. This reactivation, in the absence of recollection, could elicit the feeling of familiarity that gets misattributed to fame.

If pattern separation and completion underly the type of false recognition described by Underwood (1965), a number of predictions could be made about performance on such a task. By determining the ratio of immature to mature neurons

present in the DG at any given time, and given the importance of immature neurons to pattern separation processes, AHN would likely be an important contributor to the avoidance of false recognition. Those with healthy AHN would be better equipped to lay down orthogonalized representations of highly similar inputs than would those with reduced AHN. As such, one might predict that individuals who score highly on measures of depression and alcohol bingeing may commit more false recognition errors on a word recognition task like that employed by Underwood (1965) and be more susceptible to the false fame effect. One might also suspect to find a correlation between spatial pattern separation performance and number of false recognition and false fame errors. Testing of these predictions in the future may better help elucidate the underlying processes responsible for false recognition and potentially implicate pattern separation as a major contributing mechanism.

An important experimental consideration that stems from the dissociation of familiarity from recollection and the potential association of these processes with pattern separation and completion, is the necessity of posing these processes against each other in an experimental paradigm. In Experiment 1, participants must judge where an image was presented most recently. If making this recognition judgement using recollection, presumably following successful pattern separation, participants could recall the location of either image and deduce which image was presented most recently. Participants can, however, come to the same conclusion using familiarity. Following unsuccessful separation, one may not be able to recall exactly where an image was presented most recently due to interference. Having encoded the most recent location last, this representation within the CA3 will have been degraded the

least compared to those encoded further in the past. Activation of this representation, while too degraded for recollection, may still trigger the strongest feeling of familiarity. In this way, participants can be reasonably successful even without successful pattern separation. A slight experimental manipulation would be able to solve this issue in the future, by requiring participants to identify in which location they had seen an image in a game specified as containing the first appearance of a particular image.

An obvious limitation of our study is the inability to obtain a direct measure of neurogenesis. As a result, it is possible that one or more additional variables were affected by depression or alcohol consumption and that these variables may have caused or influenced the pattern separation deficits observed here. For example, depressive episodes in humans are associated with decreased serum levels of brain derived neurotrophic factor (BDNF) (Shimizu et al., 2003), a neurotrophin important for plasticity and long-term potentiation (Cunha, Brambilla, & Thomas, 2010; Jovanovic, Czernik, Fienberg, Greengard, & Sihra, 2000). A reduction in BDNF might be expected to cause more widespread learning and memory deficits in domains like working memory and paired associates learning. However, the results of the current study do not support this, as there was no such depression- or alcohol-related deficits on either control task, suggesting that the pattern separation deficits described here are likely not the result of general plasticity changes via BDNF expression. In the future, direct assessment of neurogenesis would be required to dissociate neurogenesis-dependent and -independent effects on memory.

An issue with Experiment 2 is the difficulty in disentangling the effects of early

adolescent bingeing from number of years bingeing. While the animal literature suggests that adolescent bingeing can have detrimental and even long-lasting effects on neurogenesis, it is difficult to tell whether that is the case in the present study. In the undergraduate population studied here, those who have binged the longest have done so because they started the earliest. Future research could examine individuals who binged in early adolescence but then stopped later into their teen years. Alternatively, selectively recruiting early adolescent onset drinkers and testing them longitudinally at different ages could resolve this issue. Another limitation of the present study is that the two control tasks, PAL and digit span, were administered after SSRT and on occasion the research assistant would not have time to run all the tasks if a participant arrived late or took especially long to fill out the mood inventory and questionnaire. This resulted in less available data for both the PAL and digit span tasks.

We believe this is the first study to demonstrate spatial pattern separation deficits associated with pre-clinical depression and alcohol bingeing in a sample of young adults. Importantly, this sample is drawn from a population that is generally believed to be healthy and widely used in cognitive experimentation. Further research is required to determine whether these potentially neurogenesis-mediated memory deficits signal early signs of broader hippocampal dysfunction and more serious psychopathology such as Major Depressive Disorder (MDD) and Alchohol Use Disorders. It is well recognized that having prior episodes of MDD predisposes one to relapse (Belsher & Costello, 1988). Similarly, heavy drinking during the teen years predisposes one toward developing an Alcohol Use Disorder later in life (Grant & Dawson,

1997). An important open question is whether the memory deficits observed in the present study associated with pre-clinical mood and/or alcohol abuse issues would resolve with early intervention or cessation of drinking. Alarmingly, in the undergraduate population that we have studied rather extensively in this and many other experiments, among those who started their bingeing in their early teens, it is extremely rare to find individuals who stopped bingeing; the latter could allow us to address this crucial question concerning recovery of neurogenesis-dependent memory.

Appendix A

Appendix

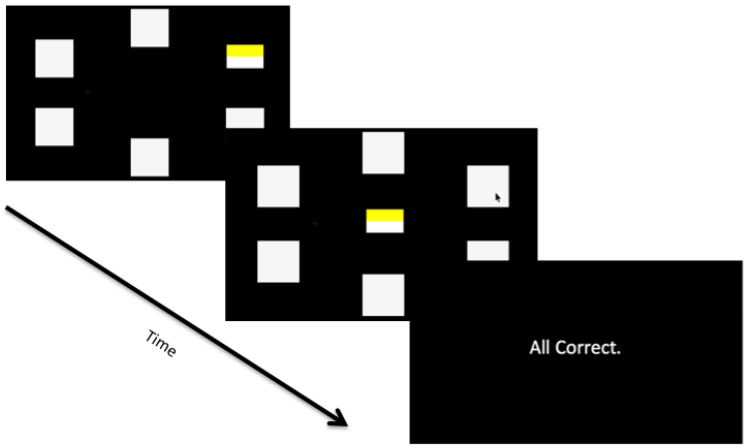


Figure A.1: PAL Control Task - Measure of Visuospatial Memory Using Paired Associates.

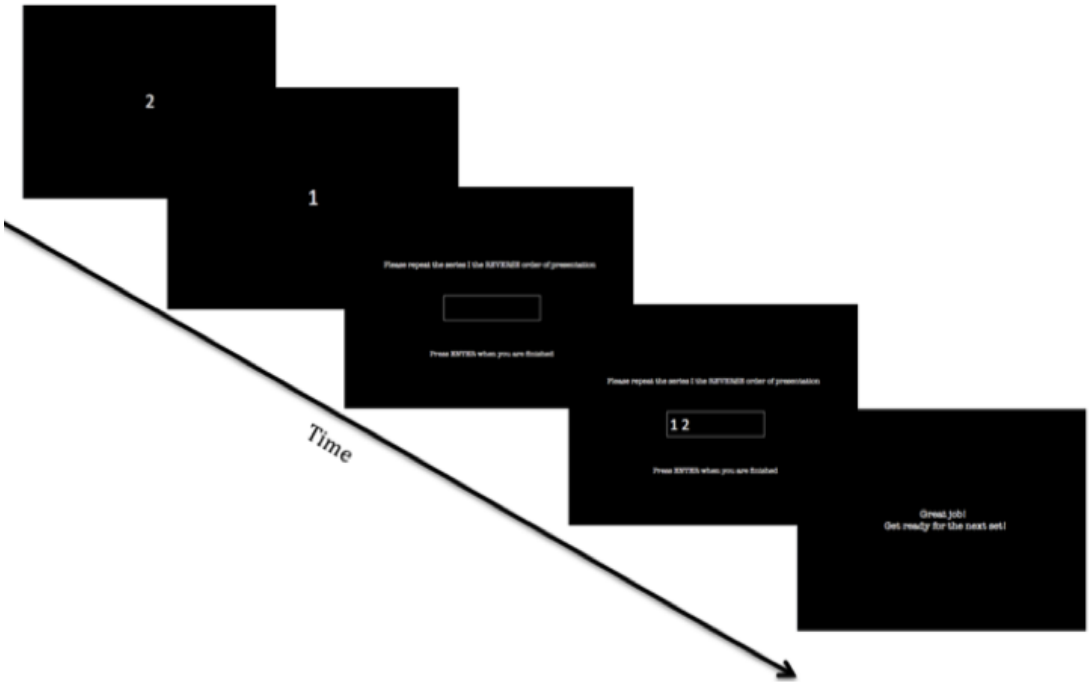


Figure A.2: Reverse Digitspan Control Task - Measure of Working Memory

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