Hippocampal Neurogenesis Leads to the Erasure of a Cocaine Conditioned Place Preference

by

Lyn Ann Vankampen

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Lyn Ann Vankampen

Masters of Arts

Department of Psychology, Program in Neuroscience

University of Toronto

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Abstract

Newborn neurons are generated in the hippocampus during adulthood. Little is known however about the functional role of these new neurons. Previous research proposed that ongoing neurogenesis clears established memories from the hippocampus (Frankland et al., 2013). Consistent with this, we recently found that high rates of neurogenesis induce forgetting of hippocampus-dependent spatial and fear memories (Akers et al., 2014). These tasks were all aversively-motivated. To test whether neurogenesis-mediated forgetting generalizes to appetitively-motivated, hippocampus-dependent memories, here we used a cocaine place preference paradigm. We found that increasing neurogenesis (by voluntary exercise) before training did not impact the acquisition of cocaine place preference. However, increasing hippocampal neurogenesis after training weakened an established cocaine place preference memory. This series of studies provides insight to the temporal impact of neurogenesis and suggests a possible link between exercise and memory functioning.

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Chapter 1

Introduction

1 Memory

Episodic memory can be described as the storage of our lived and conscious experiences. It often relies on the very rapid formation of new associations and requires the integration of information about spatial navigation, events, and time (Eichenbaum et al., 2010). The medial temporal lobe and in particular the hippocampus is the brain structure largely responsible for this task (Scoville and Milner, 1957; Eichenbaum, 2000). Through the use of humans and animals, a growing body of evidence from neuropsychology, lesion studies and electrophysiology have all linked the hippocampus and other medial temporal lobe structures with the ability to recount events and experiences (Scoville and Milner, 1957; Buzsaki 1986; Squire, 1984). In order to recapitulate our experiences, it requires the ability to link associations between information to form a coherent whole, for example the association between visual cues and the environment (Eichenbaum et al., 2010). This ability is required in order to recover our experiences at a later time point so that a given cue may assist in the retrieval of a particular memory. Experimental evidence has led to the observation that neurons in the hippocampus are responsible for the formation of associations (Komorowski et al., 2009; Tort et al., 2009). It is also generally believed that the hippocampus plays a time limited role in the storage of memories as they become more stable and long term, also referred to as systems consolidation (Squire, 1992; Nadel et al., 1997). Following the consolidation of a memory, memory traces are stored via distributed circuits within the cortex eventually becoming less hippocampal-dependent (Zola-Morgan and Squire, 1993).

2 Addiction and reward-related learning

Addiction is characterized as uncontrollable and compulsive behaviour which often leads to craving and seeking drugs of abuse (Neumann and Dong, 2013). The development and maintenance of addictions occurs due to a number of neuro-plastic changes that occur in the brain at the molecular and cellular level (Neumann and Dong, 2013). Addictive drugs such as cocaine, are able to take advantage of the molecular and cellular processes to create robust pathological memories (Neumann and Dong, 2013). Drugs of abuse rewire connections in the brain so that normal learning and memory mechanisms are strengthened which contributes to this pathological reward learning (Robinson and Berridge, 2003). Similar to normal declarative memories, emotional and motivational learning can occur subconsciously while having an impact on cognition and behaviour (Kalivas, 2007).

The major brain system implicated in the development and maintenance of addiction is the mesolimbic dopamine pathway (Hyman et al., 2014; Bardo, 2013). This reward pathway is comprised of several brain regions including the ventral tegmental are (VTA), the nucleus accumbens (NAc), the prefrontal cortex (PFC), amygdala and the hippocampus (HC). These regions are critical in the underlying pathology of addiction (Hyman et al., 2014; Bardo, 2013). The hippocampus is an important structure in the maintenance of reward based learning as it receives inputs from key structures in the reward pathway, such as the VTA. It then projects outputs to reward structures such as the NAc, PFC and BLA. Previous work has found that stimulating hippocampal outputs (ventral subiculum) alone can trigger drug seeking behaviour, which indicates the significant contribution of the hippocampus in addictions (Vorel et al., 2001).

Relapse to seeking drugs of abuse is common among addicts (McLellan et al., 2000; Ôô al., 1998; Weiss, 2010). The ability to tap into the neural circuitry involved in relapse is an important factor in treating addictions. It has previously been found that exposing cocaine addicts to previous cues associated with drug taking activates a circuit consisting of cortico-limbic structures, including the hippocampus (Kalivas, 2007).

3 Associative learning

Associative learning is an adaptive process that allows an organism to learn to anticipate events (Curzon et al., 2009). In order to study behaviour related to associative learning multiple paradigms have been utilized. A common paradigm which is used to study learning and memory is the fear conditioning paradigm. In a classical fear conditioning paradigm, an emotionally neutral conditioned stimulus (CS) is paired with an aversive unconditioned stimulus, usually a footshock (Phillips and LeDoux, 1992). The CS acquires aversive properties on its own and results in a response likened to threat i.e. freezing. This is also referred to as the learned response (Phillips and LeDoux, 1992; Blair et al., 2001). Fear conditioning uses either a cue or context in which the mice learn to associate. The major brain areas that are known to be involved in fear conditioning include the amygdala, hippocampus, frontal cortex, and cingulate cortex (Blair et al., 2001). It is generally believed that the hippocampus plays an important role in contextual processing. The hippocampus receives inputs from cortical areas that integrate information across sensory modalities (Phillips and LeDoux, 1992). Previous results suggest that the hippocampus supports contextual fear conditioning by storing a conjunctive representation of context (Rudy et al., 2002). Furthermore, damage to the hippocampal formation impairs contextual fear conditioning but not auditory cue conditioning (Kim and Fanselow, 1992).

Furthermore a growing body of evidence also suggests that the hippocampus encodes associations among stimuli (Eichenbaum, 2000). Episodic memory depends on the encoding of various contextual stimuli, including many external cues (Godden and Baddeley, 1975). The hippocampal system appears to be important for establishing episodic memory of both internal and external events within contexts as well as spatial memory (Eichenbaum, 2000). Therefore the hippocampus is critically important for the encoding of context discrimination and the conjunctive representation of associations and stimuli. It is of particular importance to target the hippocampus in order to eliminate reward related learning, as it plays a vital role in the establishment of drug and contextual associations (Mandyam, 2013).

4 Conditioned Place Preference

Unlike contextual fear conditioning, conditioned place preference can be employed with an appetitive stimulus rather than an aversive one but similarly uses context discrimination (Barr and Unterwald, 2015). The conditioned place preference paradigm has been most commonly utilized to examine motivation, reward and drug seeking behaviour (Tzschentke, 2007; Mueller & de Witt, 2011). The CPP procedure is based on the principles of Pavlovian conditioning. It assesses the association between two stimuli, an unconditioned stimulus (UCS) consisting of the drug effect and a conditioned stimulus (CS) consisting of the environment in which the drug is experienced (Mueller & de Witt, 2011). The rewarding property of the drug serves as a UCS, which is repeatedly paired with a previously neutral context. During the course of conditioning, the chamber acquires secondary motivational properties, becoming the CS.

The place preference procedure works on the assumption that an animal will approach stimuli that have been previously paired with the rewarding effects of a drug. Therefore when an animal approaches an environment where it has previously received a drug, then we infer that the animal

has a drug cue memory or a conditioned place preference (Mueller & de Witt, 2011). The conditioned place preference (CPP) paradigm has been widely used in addiction research and has been useful in aiding our understanding of relapse and drug seeking behaviour (Tzschentke, 2007). The dentate gyrus is a brain region that is particularly important for the acquisition of a cocaine CPP and has been implicated as a site that aids in the development of addiction (Barr and Unterwald, 2014). Inactivation of the hippocampus has been found to disrupt the acquisition of a cocaine CPP, which supports the role of the hippocampus in reward related learning particularly of a drug-context memory (Meyers et al., 2003; 2006; Hernandez-Rabaza et al., 2008).

The standard procedure employed with CPP includes differentially pairing distinct sets of environmental (contextual) cues with an appetitive stimuli, although an aversive stimuli may be used as well to assess a conditioned place aversion (Tzschentke, 2007). The environments can differ in multiple ways using olfactory, visual and texture as contextual cues. Learning takes place during the conditioning phase in which an appetitive stimulus becomes associated with a context. An increase in time spent in the paired context relative to the control context is taken as evidence that the unconditioned stimulus was rewarding and that learning has taken place (Tzschentke, 2007). A few of the advantages of using CPP include the ability to use a variety of appetitive stimuli, it requires no surgery and little training is needed (Bardo and Bevins, 2000). Although CPP has traditionally been used in the past to examine the motivational effects of drugs, we will take advantage of this procedure to study the role that neurogenesis plays in memory functioning. The use of drugs such as cocaine produce reliable and powerful long-lasting memory associations (Hsiang et al., 2014). Re-exposure to the environment associated with drug taking can evoke cravings capable of provoking drug seeking behaviours (Barr and

Unterwald, 2014). When environmental stimuli (cues) become associated with drug use, they become powerful motivators of continued drug use and relapse after abstinence (Torregrossa, et al., 2012). Previous studies have shown that a cocaine induced CPP can be found even after only two pairings (Tzschentke, 2007; Hsiang et al., 2014). By using the CPP paradigm we can investigate the effects of neurogenesis on a drug-cue memory.

The utilization of the conditioned place preference paradigm 1) allows for the formation of a contextually rich and long lasting memory, 2) provides multiple environmental cues that become associated with the drug memory, which is important for later recall as certain cues alone can trigger relapse (Shaham, 2003; Stefanik, 2013), and 3) will help address the question of whether neurogenesis induced forgetting can be generalized to an appetitive stimuli by using a highly rewarding stimulus.

5 Neurogenesis

The idea that new neurons are generated in the brain has only recently gained popularity in the scientific community as a bona fide phenomenon. The generation of newborn neurons in the adult brain was only discovered in the past 50 years (Altman and Das, 1965). Before this seminal work was completed, it was believed that following birth the total amount of neurons would remain constant across the lifespan with no new generation of cells in the brain (Gross, 2000; Gould et al., 2002).This long held belief was revoked when a plethora of published findings revealed convincing evidence for post-natal cell growth in the rodent, bird and human hippocampus (Spalding et al., 2013; Eriksson et al., 1998; Nottehbohm, 1985). Previous findings have revealed the discovery of two neurogenic niches; the subventricular zone of the lateral ventricles (SVZ), which gives rise to olfactory interneurons, and the subgranular zone which gives rise to granule cell neurons in the hippocampal dentate gyrus (Ming and Song,

2011; Alvarez-Buylla, 2002). Both neurogenic niches have been found to have high rates of cell turnover, the continuous restructuring of these circuits has raised debate as to what the functional significance of these newborn cells may be and what their contribution to behaviour is (Mongiat and Schinder, 2004; Cameron and McKay, 2001). The difference between neurogenesis that occurs during development and during adulthood is that the generation of new neurons are integrating into already established circuits, what the impact of new cells on the existing circuitry or the functional impact they have on existing memory processes is unknown (Esposito et al., 2005; Ming and Song, 2005).

Several behavioural paradigms have been utilized in order to study the functional significance of neurogenesis. These include hippocampal dependent tasks and hippocampal independent tasks such as the Barnes maze, incidental context learning, the Morris water maze, contextual fear conditioning, radial arm maze, trace eye-blink conditioning, and active shock avoidance (Saxe et al., 2007; Akers et al., 2014; Rola et al., 2004; Bruel et al., 2005; Song et al., 2012; Arruda-Carvalho et al., 2011). Accumulating evidence has shown that manipulating hippocampal neurogenesis has no effect on hippocampal independent tasks (i.e. delay eye blink conditioning and active shock avoidance) suggesting that newborn neurons in the DG have a select role in hippocampal dependent tasks.

Multiple functions have been hypothesized for the role of newborn neurons in the DG, such as pattern separation (Clelland et al., 2009; Leutgeb et al., 2007), the coding of temporal dissociations (Aimone, Wiles and Gage, 2006), the ability of providing new substrates for memory (Mongiat and Schinder, 2011; Ge et al., 2008) and the clearance of previously encoded memories (Akers et al., 2014; Frankland et al., 2013; Weisz and Arigbay, 2012; Meltzer et al., 2005). Theoretical modeling in computational studies predicts significant advantages of new

neurons over mature neurons for both temporary storage and the clearance of memories (Cechi, 2001). Replacement models of neurogenesis predict that although there seems to be learning improvement following an increase in new neurons it is also accompanied by the loss of information that has already been encoded (Deng, 2010). In particular recent experimental evidence has revealed a role of newborn neurons in the forgetting of established memories (Akers et al., 2014).

How this forgetting occurs however remains somewhat of a mystery. However a few hypotheses exist as to why forgetting occurs. One idea is that when newborn neurons integrate into the existing circuit this makes it more difficult in order to reactivate the previous memory trace (as these new neurons compete for new inputs and outputs with existing connections). New neurons may outcompete and replace existing synaptic connections in both the DG and CA3 (Frankland et al., 2013). In addition to this possible structural remodelling of the circuit, the integration of newborn neurons may also modify the strength of existing synaptic connections. It is known that new neurons contribute to an increase in the excitability of the circuit which is hypothesized to lead to a decrease in the ability to reactivate that same pattern of activity (Meltzer et al., 2005; Frankland et al., 2013). What is common to both of these hypotheses is that the pattern of connections changes in a time-dependent manner. Although both of these ideas have not currently been experimentally tested and further research is warranted.

5.1 Regulating neurogenesis

Recent technological advances have been used to manipulate neurogenesis by either increasing or decreasing rates in the DG (Vadodaria and Jessberger, 2014). Adult generated neurogenesis is bi-directionally regulated by a number of factors including stress, anti-depressants, exercise, seizures, learning, environment, and neurotrophic factors (van Praag et al., 1999; Gould et al.,

1999; Malberg et al., 2000; Zhao et al., 2008; Balu and Lucki, 2008;). Each of these can affect neurogenesis at different stages of neuronal development (i.e. proliferation, survival and differentiation) which makes it difficult to determine which factors are mitigating the behavioural effects. Additionally there are various side effects other than increasing or decreasing neurogenesis that need to be taken into consideration. For example stress has been shown to increase glucocorticoids as well as decrease neurogenesis (Schloesser, et al., 2009). Anti-depressants have been shown to increase neurogenesis while also regulating mood and stress (Malberg et al., 2000). Exercise substantially increases neurogenesis while also increasing the amount of neurotrophic factors as well as increasing the amount of non-neurogenic benefits on health (Gould et al., 1999; van Praag et al., 1999). Therefore these side effects need to be taken into consideration when interpreting findings.

5.2 Environmental manipulation

One of the most effective and naturalistic ways in which neurogenesis is regulated is through voluntary exercise (van Praag et al., 1999). Running in particular has been shown to induce neurogenesis which has been found to increase new neurons in the DG by approximately two-fold (van Praag et al., 1999). Running has also been found to increase the survival rate of newborn neurons in the DG (van Praag et al., 1999; Couillard-Despres et al., 2005).

Previous studies have examined an increase in newborn neurons in the DG following exposure to an enriched environment as well as leading to enhanced cognitive abilities (Nilsson et al., 1999; Kempermann et al., 1998). These studies have predominantly included a running wheel in the environment which confounds results with work showing an increase in neurogenesis following exercise (van Praag et al., 1999). However results from these studies have been varied with some showing that it is indeed exercise alone that increases neurogenesis

rather than exposure to an enriched environment that does not contain running wheels (Mustroph et al., 2012; Fabel et al., 2009; Grégoire et al., 2014). Therefore the ability of an enriched environment to increase neurogenesis must be carefully interpreted.

5.3 Genetic manipulation

In order to examine the impact of newborn neurons on the existing hippocampal circuit researchers have employed the use of transgenic mice to increase or suppress neurogenesis levels (Imayoshi et al., 2011; Yang et al., 2011; Couillard-Despres et al., 2006). Recent advancements in gene targeting strategies have allowed for the visualization of newborn neurons during development as well as the ability to manipulate neurogenesis to examine the impact it has on performance. One of the most popular strategies is to use mice targeting the nestin gene because nestin is selectively expressed in dividing cells (Dhaliwhal and Legace, 2011). In order to selectively decrease neurogenesis levels the nestin-TK mouse has been employed in which the targeted expression of herpes simplex virus thymidine kinase in neural stem cells which results in the inducible ablation of dividing cells when combined with the drug ganciclovir in those cells expressing nestin (Imayoshi et al., 2011). The use of site-specific recombinases have been utilized as tools in the analysis of gene function and the genetic manipulation of neurogenesis in the DG (Branda and Dymecki, 2004). The use of these transgenic mice allows for the ability to induce the deletion, insertion or inversion of DNA sequences by breaking and joining DNA molecules at specific sites. The use of the Cre-recombinase system allows for temporal control to restricted cell populations including newborn cells in the neurogenic niche in the DG (Imayoshi et al., 2011). Two important mouse strains have been used to examine the impact of increased neurogenesis these include the iBax and p53 mouse (Sahay et al., 2011; Akers et al., 2014). Both of these mouse strains take advantage of the Cre-recombinase system in order to increase

neurogenesis. The Cre-ER is activated by the administration of tamoxifen, a synthetic estrogen antagonist (Imayoshi et al., 2011).

5.4 Pharmacological manipulation

In addition to the genetic manipulation of neurogenesis, pharmacological means have also been used to alter neurogenesis levels (Deng et al., 2009). The use of mitosis dividing drugs such as memantine, an NMDA receptor antagonist, have been shown to substantially increase neurogenesis levels (Maekawa et al., 2009; Ishikawa et al., 2014). The use of anti-depressants, such as fluoxetine, have also been found to increase neurogenesis levels but the mechanism of how this occurs and how it affects depression is largely unknown (Cho and Kim, 2010; Duman et al., 2001; Malberg et al., 2000). Previous studies have also used the drug temozolomide in order to suppress neurogenesis levels (Niibori et al., 2012). Again although these methods have proven to be useful in order to manipulate neurogenesis, they also have additional side effects to take into consideration such as inflammation and can alter the health of the animal (Imayoshi et al., 2011).

6 Impact of manipulating neurogenesis

6.1 Anterograde

Previous results examining the impact of increased neurogenesis prior to learning have also been conflicting, with some studies showing an improvement in learning and memory performance on hippocampal-dependent tasks (Ishikawa et al., 2014; Sahay et al., 2011; van Praag et al., 2005; Trouche et al., 2009) and others finding no difference (Akers et al., 2014; Mustroph et al., 2015).

Previous studies that have examined the functional role of hippocampal neurogenesis have predominantly looked only at the anterograde effects of manipulating neurogenesis (Arruda-

Carvalho et al., 2011; Frankland et al., 2013). Most studies examining the impact of neurogenesis on memory functioning have reduced neurogenesis prior to the acquisition of a hippocampal-dependent memory task (Deng et al., 2010). Although these studies have not proven to be conclusive, with findings being mixed, the predominant view is that following the ablation or deletion of newborn neurons this leads to impaired learning and memory (Shors et al., 2001; Saxe et al., 2006; Dupret et al., 2008; Deng et al., 2009; Jessberger et al., 2009).

6.2 Retrograde

There has been very little research that has focused on the effects of manipulating neurogenesis levels following the acquisition of a task, also known as retrograde effects (Frankland et al., 2013). Computational models have predicted that the addition of new neurons into the DG degrades established memory traces as mature DGCs are replaced (Weisz and Arigbay, 2011). A theoretical model previously examined the retrograde effects on neurogenesis and suggested that increased neurogenesis following learning reduces the probability of successful memory retrieval (forgetting) of old memories (Weisz and Arigbay, 2011; Frankland et al., 2013). Whereas decreasing neurogenesis increases the probability of successful memory retrieval and protects existing memory (Weisz and Arigbay, 2011; Frankland, et al., 2013).

In a recent investigation, researchers determined through both genetic and pharmacological means, that if neurogenesis was increased following training on a fear contextual paradigm this led to a decrease in their freezing response at test (6 weeks later). This indicated that the strength of the fear memory was weakened or forgotten (Akers et al., 2014).

However researchers did not find this same effect when neurogenesis was increased prior to training which suggests that neurogenesis is disrupting previously encoded (retrograde) memories. This is likely due to the addition of new neurons to the existing circuit. This fits with

the time frame of new neuron integration and is supported by findings in the neuro-computational literature as was previously mentioned (Weisz and Arigbay, 2011).

Researchers also wanted to determine whether memory was differentially affected in infant mice since neurogenesis levels are relatively high during infancy, both infant and adult mice were trained on the contextual fear memory paradigm. Results revealed that infant mice were impaired following training and displayed little to no freezing following a 28 day delay whereas adult mice displayed freezing whether tested at a one day or a 28 day delay. Only if mice were tested one day following training did infant mice display freezing behaviour, indicating that infant mice rapidly forgot the fear memory which was in line with their previous results when neurogenesis levels are high (Akers et al., 2014). Conversely, researchers found that when neurogenesis levels were decreased, using the transgenic mouse model nestin-TK, this resulted in the ability to block the forgetting effect indicating that neurogenesis has a causal effect on memory.

This study provided support for the finding that neurogenesis leads to the erasure of a previously encoded aversive context dependent memory. This finding is further supported when neurogenesis was induced by pharmacological and genetic means. In contrast, decreasing neurogenesis can prevent and reverse the forgetting of an established fear memory. The contribution of these cells in information processing and the impact they have on existing circuitry remains an important question and warrants further inquiry.

7 Forgetting

Forgetting has long been associated with aging and disease and is often viewed as negative, however the ability to forget unused memories allows for an adaptable and flexible system which would be evolutionarily advantageous to an organism. Our brains have evolved in order to be flexible and adapt, forgetting is a beneficially adaptive process and is a way in which irrelevant

information is cleared from memory. It is as much an essential process in normal memory functioning as is the retention of information in memory processing. Learning to forget is also beneficial in such cases as trauma or addictions (Torregrossa, et al., 2012).

7.1 Theories of forgetting

It is generally believed that the retrieval of a memory requires the reactivation of those same cells that were activated at the time of encoding (Taylor, 2013; Rolls, 2000; McClelland et al., 1995). Forgetting occurs predominantly due to the inability to access or retrieve this target trace (Bouton and Moody, 2004). The encoding specificity principle argues that retrieval depends on a match between the conditions present during learning and conditions that are present at test and a mismatch between the two results in forgetting (Thomson and Tulving, 1973). Forgetting implies that a memory was once encoded but is then either lost due to an error at retrieval or is somehow unable to be reactivated possibly due to interference. In line with this it is believed that the forgetting of a memory is due to one of two theories; decay or interference.

The decay theory of forgetting states that forgetting occurs simply due to the passage of time and that the recall of memory serves to promote retention possibly due to the strengthening of connections (Hardt et al., 2013). Whereas forgetting due to interference occurs when previous or subsequent information interferes with the recall of a previously encoded memory. In support of this, new memories are more susceptible to interference as they have not had the chance to undergo consolidation (Hardt et al., 2013). During the consolidation period, memories are vulnerable to disruption and overtime become less labile (Wixted, 2004). This example is best seen in retrograde amnesic patients in which memories are temporally graded, these patients are able to recall memories that have already been consolidated whereas the ability to recall more recent memories is impaired (Wixted, 2004). This temporal gradient is found after insult of the

hippocampus where the encoding of new memories is disrupted as well as previously learned information (Scoville and Milner, 1957; Wixted, 2004). Although older memories have been shown to be relatively spared (Scoville and Milner, 1957). This is likely due to the consolidation of those memories, whereas newer memories have had less of a chance to consolidate making them more vulnerable.

A likely explanation for forgetting is due to the disruption of a memory trace before it has had a chance to consolidate. In support of interference theory, researchers found that memory for a previously learned list was better when subjects had slept (thereby avoiding interfering material) then when they were awake prior to the memory test (Jenkins and Dallenbach, 1924). However proponents of decay theory state that everyday forgetting is due to a decay like process that serves to degrade memories over time in order to remove unwanted information (Hardt et al., 2013).

7.2 Neurocomputational models

In order to have a better understanding of forgetting mechanisms, theoretical and neuro-computational studies have been employed (Weisz and Argibay, 2012; Hardt et al., 2013). It has previously been postulated in theoretical studies that the process of forming new memories and associated changes in synaptic weights may create retrograde interference by degrading previously encoded information (Wixted. 2004). This is in line with the predictions made by neuro-computational models of neurogenesis in the DG (Crick and Miranker, 2006; Weisz and Arigbay, 2012). These models predict while neurogenesis leads to an increase in the networks capacity to learn new information, it also increases the chances that forgetting of old information will occur. In the model created by Weisz and Arigbay (2012) neurogenesis significantly increased the retrieval of recent memories but interfered with the retrieval of remote memories.

The replacement of neurons in a feedforward network resulted in the loss of previously stored memories while facilitating the encoding of new memories. In this model, the addition of new neurons could modulate the capability of the adult hippocampal network to handle the storage of new memories and the clearance of old memories (Weisz and Arigbay, 2012; Deisseroth et al., 2004). This would provide support for the finding that the facilitation of new memories and the degradation of old memories can both co-occur. Although the relationship is more complicated than a simple one for one replacement, rather new neurons contribute to an ongoing process in the existing hippocampal circuitry (Deisseroth et al., 2004; Ge et al., 2008; Song et al, 2012).

Theoretical and neuro-computational accounts converge to support the finding that neurogenesis is regulated in such a way to increase the stability of newly acquired information and the clearance of older memories (Bruel-Jungerman et al., 2007; Deisseroth et al., 2004; Frankland et al., 2013). However the drawback to this process is that adding new neurons could result in a destabilization of the network (Aimone & Wiskott, 2008; Deisseroth et al, 2004). By adding noise and additional excitation into the existing hippocampal circuit through the addition of newborn neurons this may disrupt existing connections and therefore lead to forgetting of established memory traces (Deisseroth et al., 2004). This concept is not a new one as McGeoch (1932) previously argued that memory is lost because new learning interferes retroactively with existing memory and compromises its expression (Hardt et al., 2013). The neurobiological account of this process remains to be elucidated. Both theoretical accounts and neuro-computational models predict that neurogenesis presumably may be the underlying mechanism in place that regulates forgetting and memory processing.

8 Objectives

Previously researchers were able to provide convincing evidence that neurogenesis mediates forgetting of previously acquired memories using a contextual fear conditioning paradigm (Akers et al., 2014). In this study however, researchers exclusively looked at using an aversive stimuli and whether this finding can translate to other types of memories still needs to be determined. It will be important to determine whether this finding can be generalized to an appetitive stimuli and in uncovering the role of adult-generated neurogenesis.

The current study will examine both the retrograde and anterograde effects of increasing neurogenesis on a reward memory. Also to examine whether neurogenesis is mediating this effect, neurogenesis will be genetically ablated using a transgenic mouse model, nestin-TK and the suppression of neurogenesis. Finally the last study seeks to answer the question of whether neurogenesis-induced forgetting has an effect following the consolidation period. This will be the first series of studies, to our knowledge, examining the impact of neurogenesis on memory and forgetting using an appetitive stimulus. This study will have implications for addictions research, as well as contribute to our understanding of the role that neurogenesis has in memory functioning.

Previous work in the field has predominantly only examined the role of neurogenesis prior to the acquisition of a task after which memory is tested on a hippocampal-dependent task (Anderson et al., 2001; Deng et al; 2009, 2011).The predominant finding in these studies has been that when neurogenesis levels are reduced, memory is subsequently impaired on the task (Niibori et al., 2012; Deng et al., 2009; 2011; Drew et al., 2010), whereas increasing neurogenesis facilitates memory on select hippocampal dependent tasks (Sahay et al., 2011; Stone et al., 2013). Although previous studies have examined the impact of neurogenesis on learning and memory acquisition,

little work has been completed on the effect of neurogenesis on established memories. Here we will examine the anterograde effects of neurogenesis on learning an appetitively-motivated hippocampal-dependent task as well as the retrograde effects of neurogenesis. This will help us answer the question of what the effect of neurogenesis is on the acquisition of a memory as well as on previously stored memories. To further address these questions neurogenesis will be manipulated environmentally, genetically and pharmacologically by increasing as well as decreasing neurogenesis levels.

8.1 Goals

1) First it is important to ensure that voluntary running leads to increased levels of neurogenesis in the hippocampus as previous results have shown (van Praag, 1999). Mice provided with a running wheel should show higher levels of neurogenesis compared to sedentary controls.

2) To determine whether increasing neurogenesis (through voluntary exercise) before the acquisition of a cocaine cue memory has an effect on the formation of a cocaine conditioned place preference.

3) To determine whether increasing neurogenesis (through voluntary exercise) after the acquisition of a cocaine cue memory has an effect on memory retention.

4) To determine whether decreasing neurogenesis after the acquisition of a cocaine memory has an effect on memory retention. In order to find a causal link between neurogenesis and memory, manipulating neurogenesis in both ways is important to understanding its effects on memory functioning. This will help determine whether reducing neurogenesis levels has an inverse effect on memory thus providing a causal link between memory and neurogenesis.

5) To examine the temporal profile of neurogenesis and any changes in memory functioning, we wanted to examine the effects of neurogenesis following the consolidation of a cocaine memory. After memory consolidation, which requires an extended amount of time, the memory trace is said to become independent of the hippocampus (Squire, 1992). It will be determined what the effect of neurogenesis is following the consolidation period and to see whether the cocaine cue-memory was still hippocampal dependent.

8.2 Hypotheses

1) Access to a running wheel will increase the amount of newborn cells in the DG of the hippocampus.

2) All mice will learn to associate the drug cocaine with its paired context also known as a conditioned place preference (CPP). However mice provided with access to a running wheel prior to the acquisition of a cocaine cue memory will have a higher CPP score compared to sedentary controls, indicating that increased neurogenesis leads to the facilitation of a memory.

3) Mice provided with access to a running wheel following training of a cocaine conditioned place preference will have a lower CPP score compared to sedentary controls. Which will indicate that running induced neurogenesis leads to the forgetting of a previously encoded cocaine memory.

4) The nestin-tk mouse expresses herpes simplex virus thymidine kinase (TK) under the control of the nestin promoter, which drives expression selectively in neural progenitors. Administration of ganciclovir (GAN) kills actively dividing cells expressing this transgene. In mice that express this transgene it is predicted that they will exhibit decreased levels of newborn neurons and will have a higher CPP score compared to WT controls. Voluntary running will increase neurogenesis

in WT mice but neurogenesis will be blunted in TK+ mice. Exercise induced neurogenesis will lead to forgetting in WT mice but not in TK+ mice (in which neurogenesis is suppressed).

5) Providing access to a running wheel and increasing neurogenesis 4 weeks following cocaine conditioning will have no effect on memory due to the effects of consolidation. Running mice and sedentary mice will show similar CPP scores and therefore both will have retained the cocaine memory. Neurogenesis should not have an effect on memory following the consolidation of a cocaine cue-memory.

Chapter 2
Methods

1 Mice

Adult (8-12 weeks of age) wild-type (WT) female and male hybrid mice were used (C57BL/6N X 129S6/SvEv) were used in experiments 1A, 1B, 2A, 2B and 3. For experiment 4A and 4B a transgenic mouse line was used, nestin-tk, in which a nestin promoter drives the expression of a modified herpes simplex virus (HSV) gene encoding thymidine kinase (Akers et al., 2014). In TK+ mice, administration of the drug ganciclovir (GAN) leads to diminished levels of only dividing cells that express the TK transgene.

Mice were bred at the Hospital for Sick Children and group housed (3-5 mice per cage) on a 12 hr light/dark cycle with food and water available *ad libitum*. Behavioural experiments took place during the light phase of the cycle. All procedures were conducted in accordance with the policies of the Hospital for Sick Children Animal Care and Use Committee.

2 Drug

Cocaine

Cocaine HCl (Health Canada) was dissolved in sterile PBS and delivered intraperitoneally. The dose differed depending on the experiment (see experiment protocol).

Ganciclovir

GAN was mixed into powdered food and given to TK+ and WT mice *ad libitum* following conditioning and remained in home cage during the entire duration of the experiment.

3 Running wheels

Mice in running groups were provided with voluntary access to a single running wheel in home cage. Mice in the sedentary control group were similarly housed but not provided with running wheels.

4 Apparatus: Conditioned place preference

CPP was performed using an unbiased, counterbalanced protocol. The CPP apparatus contained two 15 X 20 cm Plexiglas chambers connected by a guillotine door. Each chamber had a distinct combination of visual, tactile, and olfactory characteristics. One side had white walls and a transparent textured floor, whereas the other side had black and white striped walls and a smooth white floor that was wiped with acetic acid 3% before each conditioning and test trial. The two chambers were separated by a guillotine door (which remained open during the habituation and test sessions but was closed during the conditioning sessions). The loco-motor activity and location of mice was measured using an overhead CCD camera which was connected to a computer which ran Limelight software (Colbourn Instruments).

5 Experiment protocol

The CPP procedure consists of 5 sequential phases: habituation, conditioning, post-test, extinction and reinstatement. During habituation, mice are allowed to free roam the two chambers of the CPP apparatus for a total of 10 minutes. The time spent in each chamber was measured for each mouse. To measure this, we used Limelight software to visually divide the CPP apparatus into three zones; chamber 1 zone, middle zone and chamber 2 zone (15 X 18 cm; 15 X 4 cm; 15 X 18 cm; see Figure 6). Time spent in the middle zone was excluded from the calculations. Conditioning sessions took place twice for 3 days consecutively with a minimum of

4 hours between each session. On each conditioning day, mice were confined to one side of the CPP chamber for 15 minutes immediately following either a saline injection (morning) or cocaine injection (afternoon) administration. The location of the cocaine paired chamber was randomized and counterbalanced across groups. Mice were tested either 18 hours, 28 days or 42 days following the final conditioning session.

5.1 Experiment 1A: The effect of exercise-induced neurogenesis on the acquisition of a reward memory.

Four weeks (28 d) prior to habituation, WT hybrid mice (n = 20) in the running group were provided with exercise wheels to induce neurogenesis prior to training. During this time sedentary mice (n = 20) remained conventionally housed. Cages of mice were chosen at random to be included in the running group. One day following habituation, all mice were trained for cocaine-cue CPP over 3 consecutive days. In the morning of each conditioning day (between 8:00 and 11:00 A.M), saline (SAL) was administered directly before mice were placed in one chamber (counterbalanced) and in the afternoon (between 2:00 and 6:00 P.M), mice received cocaine (COC; 15mg/kg, i.p) before directly being placed in the other chamber. Approximately 18 h following the final conditioning session, mice were assessed during a post-test session to measure CPP memory. Mice had free access to each chamber for 10 minutes. Approximately 12 h later, mice received extinction training of SAL-SAL training sessions for 2 days consecutively (in which each chamber was paired with saline administration). Extinction tests took place at the end of each extinction-training day in which mice had free access to each chamber for 10 minutes. Before the final reinstatement test, mice received an injection of 7.5mg/kg of cocaine immediately before being placed in the CPP apparatus (See Figure 6). During this time mice had free access to both chambers for 10 minutes, in which CPP memory was assessed.

5.2 Experiment 1B: The effect of exercise-induced neurogenesis on a previously encoded reward memory.

One day following habituation, WT hybrid mice were trained for cocaine-cue CPP over 3 consecutive days. In the morning of each conditioning day (between 8:00 and 11:00 A.M), saline (SAL) was administered directly before mice were placed in one chamber (counterbalanced) and in the afternoon (between 2:00 and 6:00 P.M), mice received cocaine (COC; 15mg/kg, i.p) before directly being placed in the other chamber. Following the final conditioning session, cages of mice in the running group (n = 20) were provided with an exercise wheel to induce neurogenesis. Cages of mice were chosen at random to be included in the running group. The exercise wheel remained in the cage for four weeks (28 d) following conditioning. During this time sedentary mice (n = 20) remained conventionally housed. All mice were assessed during a post-test session to measure CPP memory following the 4 weeks. Mice had free access to each chamber for 10 minutes. Approximately 12 h later, mice received extinction training of SAL-SAL training sessions for 2 days consecutively (in which each chamber was paired with saline administration). Extinction tests took place at the end of each extinction-training day in which mice had free access to each chamber for 10 minutes. Before the final reinstatement test, mice received an injection of 7.5mg/kg of cocaine immediately before being placed in the CPP apparatus. During this time mice had free access to both chambers for 10 minutes, in which CPP memory was assessed.

5.3 Experiment 2A: The effect of exercise-induced neurogenesis on the acquisition of a reward memory.

Four weeks (28 d) prior to habituation, WT hybrid mice in the running group (n = 16) were provided with exercise wheels to induce neurogenesis prior to training. Cages of mice were

chosen at random to be included in the running group. During this time sedentary mice (n = 16) remained conventionally housed. One day following habituation, mice were trained for cocaine-cue CPP over 3 consecutive days. In the morning of each conditioning day (between 8:00 and 11:00 A.M), saline (SAL) was administered directly before mice were placed in one chamber (counterbalanced) and in the afternoon (between 2:00 and 6:00 P.M), mice received cocaine (COC; 7.5mg/kg, i.p) before directly being placed in the other chamber. Approximately 18 h following the final conditioning session, mice were assessed during a post-test session to measure CPP memory. Mice had free access to each chamber for 10 minutes. Approximately 12 h later, mice received extinction training of SAL-SAL training sessions for 2 days consecutively (in which each chamber was paired with saline administration). Extinction tests took place at the end of each extinction-training day in which mice had free access to each chamber for 10 minutes. Before the final reinstatement test, mice received an injection of 7.5mg/kg of cocaine immediately before being placed in the CPP apparatus. During this time mice had free access to both chambers for 10 minutes, in which CPP memory was assessed.

5.4 Experiment 2B: The effect of exercise-induced neurogenesis on a previously encoded reward memory.

One day following habituation, WT hybrid mice were trained for cocaine-cue CPP over 3 consecutive days. In the morning of each conditioning day (between 8:00 and 11:00 A.M), saline (SAL) was administered directly before mice were placed in one chamber (counterbalanced) and in the afternoon (between 2:00 and 6:00 P.M), mice received cocaine (COC; 7.5mg/kg, i.p) before directly being placed in the other chamber. Following the final conditioning session, cages of mice in the running group (n = 20) were provided with an exercise wheel to induce neurogenesis. Cages of mice were chosen at random to be included in the running group. The

exercise wheel remained in the cage for four weeks (28 d) following conditioning. During this time sedentary mice (n = 16) remained conventionally housed. After which mice were assessed during a post-test session to measure CPP memory. Mice had free access to each chamber for 10 minutes. Approximately 12 h later, mice received extinction training of SAL-SAL training sessions for 2 days consecutively (in which each chamber was paired with saline administration). Extinction tests took place at the end of each extinction-training day in which mice had free access to each chamber for 10 minutes. Before the final reinstatement test, mice received an injection of 7.5mg/kg of cocaine immediately before being placed in the CPP apparatus. During this time mice had free access to both chambers for 10 minutes, in which CPP memory was assessed.

5.5 Experiment 3: The effect of neurogenesis following consolidation of a reward memory.

One day following habituation, WT hybrid mice were trained for cocaine-cue CPP over 3 consecutive days. In the morning of each conditioning day (between 8:00 and 11:00 A.M), saline (SAL) was administered directly before mice were placed in one chamber (counterbalanced) and in the afternoon (between 2:00 and 6:00 P.M), mice received cocaine (COC; 7.5mg/kg, i.p) before directly being placed in the other chamber. All mice were housed conventionally for 4 weeks (28 d) after which in some of the cages, chosen at random, an exercise wheel was introduced. After an additional 4 weeks (28 d), a total of 56 d, following the final conditioning session the post-test session took place and CPP memory in each group (running vs. sedentary) was assessed. Mice had free access to each chamber for 10 minutes. Approximately 12 h later, mice received extinction training of SAL-SAL training sessions for 2 days consecutively (in which each chamber was paired with saline administration). Extinction tests took place at the end of each extinction-training day in which mice had free access to each chamber for 10 minutes.

Before the final reinstatement test, mice received an injection of 7.5mg/kg of cocaine immediately before being placed in the CPP apparatus. During this time mice had free access to both chambers for 10 minutes, in which CPP memory was assessed.

5.6 Experiment 4A: The effect of suppressing neurogenesis on a reward memory.

One day following habituation, adult WT and TK+ mice were trained for cocaine-cue CPP over 3 consecutive days. In the morning of each conditioning day (between 8:00 and 11:00 A.M), saline (SAL) was administered directly before mice were placed in one chamber (counterbalanced) and in the afternoon (between 2:00 and 6:00 P.M), mice received cocaine (COC; 7.5mg/kg, i.p) before directly being placed in the other chamber. Following the final conditioning session, mice were provided with an exercise wheel simultaneously while being treated with GAN. The exercise wheel remained in the cage for six weeks (42 d) following the final conditioning session. Also during this time the regular food that mice previously had access to, was switched to powdered food. One gram of GAN was added and mixed with powdered food in a glass container in which mice had access *ad libitum* for the entire 6 weeks. After which mice were assessed during a post-test session to measure CPP memory. Mice had free access to each chamber for 10 minutes. Approximately 12 h later, mice received extinction training of SAL-SAL training sessions for 2 days consecutively (in which each chamber was paired with saline administration). Extinction tests took place at the end of each extinction-training day in which mice had free access to each chamber for 10 minutes. Before the final reinstatement test, mice received an injection of 7.5mg/kg of cocaine immediately before being placed in the CPP apparatus. During this time mice had free access to both chambers for 10 minutes, in which CPP memory was assessed.

5.6 Experiment 4B: The effect of suppressing neurogenesis on a reward memory.

One day following habituation, adult WT and TK+ mice were trained for cocaine-cue CPP over 3 consecutive days. In the morning of each conditioning day (between 8:00 and 11:00 A.M), saline (SAL) was administered directly before mice were placed in one chamber (counterbalanced) and in the afternoon (between 2:00 and 6:00 P.M), mice received cocaine (COC; 7.5mg/kg, i.p) before directly being placed in the other chamber. Following the final conditioning session, mice were treated with GAN. Also during this time the regular food that mice previously had access to, was switched to powdered food. One gram of GAN was added and mixed with powdered food in a glass container in which mice had access *ad libitum* for the entire 6 weeks (42 d). After which mice were assessed during a post-test session to measure CPP memory. Mice had free access to each chamber for 10 minutes. Approximately 12 h later, mice received extinction training of SAL-SAL training sessions for 2 days consecutively (in which each chamber was paired with saline administration). Extinction tests took place at the end of each extinction-training day in which mice had free access to each chamber for 10 minutes. Before the final reinstatement test, mice received an injection of 7.5mg/kg of cocaine immediately before being placed in the CPP apparatus. During this time mice had free access to both chambers for 10 minutes, in which CPP memory was assessed.

6 Statistical analyses

SPSS version 20 was used to perform one-way and two-way repeated measures ANOVAs; t-tests were used for post-hoc comparisons between means. CPP memory was assessed during the test sessions in which mice were given free access to both chambers of the apparatus. Only the first 5 minutes of data were used for analysis to exclude possible effects of within-test extinction. A

CPP score was measured for each mouse (time in seconds spent in the cocaine paired side minus the time spent in the saline paired side). CPP scores were analyzed using t-test, one-way (Condition x Day) ANOVA and 2-way ANOVA with condition (Sedentary, Runner) x genotype (positive, negative) as factors.

Chapter 3
Results

Exercise induced neurogenesis leads to the forgetting of a cocaine cue memory, depending on the strength of the initial memory

1 Experiment 1A: Anterograde 15mg/kg:

1.1 Pre-test

To determine whether the mice had any initial inherent bias to the chamber, a habituation test was conducted in which mice had free access to both chambers of the CPP apparatus prior to drug conditioning. Results determined that mice had no initial preference to either side of the chamber, $F_{(1, 26)} = 0.07$, $p = 0.80$ (Fig.1A).

1.2 Post-Test

Adult (8-11 weeks) WT mice were provided access to an exercise wheel for 4 weeks prior to the acquisition of a cocaine CPP in order to increase neurogenesis, or remained sedentary in home cage. Figure 1a shows that exercise induced neurogenesis had no significant effect on the acquisition of a cocaine cue memory, $F_{(1, 38)} = 0.52$, $p = 0.48$ (Fig.1A).

1.3 Extinction

In order to examine whether exercise induced neurogenesis had an effect on the rate of extinction for a cocaine cue memory, mice learned to associate both chambers with saline administration after which a place preference test was given. This leads to the degradation of the initial cocaine cue memory. We wanted to determine whether exercise induced neurogenesis before cocaine exposure would lead to a slower rate of extinction compared to sedentary controls. It was found

that the rate of extinction did not differ between groups (sedentary vs. runner) on either day 1, $F_{(1, 38)} = 0.04$, $p = 0.84$, or day 2 of extinction training, $F_{(1, 38)} = 0.01$, $p = 0.91$ (Fig.1A).

1.4 Reinstatement

Reinstatement is used in order to cue a memory once it has been extinguished and mimics a relapse situation (Mueller and Stewart, 2000). We wanted to ask the question of whether exercise induced neurogenesis before cocaine exposure played a role in reinstating a cocaine cue-memory. Exercise induced neurogenesis had no effect on reinstatement, $F_{(1, 38)} = 0.29$, $p = 0.59$ (Fig.1A).

2 Experiment 1B Retrograde 15mg/kg:

2.1 Pre-test

A habituation test was conducted in which mice had free access to both chambers of the CPP apparatus prior to drug conditioning. Results determined that mice had no initial preference to either side of the chamber $F_{(1, 38)} = 0.06$, $p = 0.70$ (Fig.1B).

2.2 Post-Test

Adult (8-11 weeks) WT mice were provided access to an exercise wheel for 4 weeks following conditioning of a cocaine CPP in order to increase neurogenesis, or remained sedentary in home cage. Figure 1B shows that exercise induced neurogenesis had no significant effect on a previously encoded cocaine cue memory, $F_{(1, 38)} = 0.15$, $p = 0.70$ (Fig.1B).

2.3 Extinction

We wanted to test the hypothesis of whether exercise induced neurogenesis after cocaine exposure would lead to a faster rate of extinction compared to sedentary controls. It was found

that the rate of extinction did not differ between groups (sedentary vs. runner) on either day 1, $F_{(1, 34)} = 0.09$, $p = 0.77$, or day 2 of extinction training, $F_{(1, 38)} = 0.19$, $p = 0.67$ (Fig.1B).

2.4 Reinstatement

We wanted to test the hypothesis of whether exercise induced neurogenesis after cocaine exposure played a role in reinstating a cocaine cue-memory. Exercise induced neurogenesis had no effect on reinstatement, $F_{(1, 38)} = 0.01$, $p = 0.92$ (Fig.1B).

3 Experiment 2A Anterograde 7.5mg/kg:

3.1 Pre-test

A habituation test was conducted in which mice had free access to both chambers of the CPP apparatus prior to drug conditioning. Results determined that mice had no initial preference to either side of the chamber $F_{(1, 30)} = 1.28$, $p = 0.27$ (Fig.2A).

3.2 Post-Test

Adult (8-11 weeks) WT mice were provided access to an exercise wheel for 4 weeks prior to drug conditioning in order to increase neurogenesis, or remained sedentary in home cage.. Figure 2A shows that exercise induced neurogenesis had no significant effect on the acquisition of a cocaine cue memory, $F_{(1, 30)} = 0.07$, $p = 0.80$ (Fig.2A).

3.3 Extinction

We wanted to test the hypothesis of whether exercise induced neurogenesis after cocaine exposure would lead to a faster rate of extinction compared to sedentary controls. It was found that the rate of extinction did not differ between groups (sedentary vs. runner) on either day 1, $F_{(1, 30)} = 0.22$, $p = 0.65$, or day 2 of extinction training, $F_{(1, 30)} = 0.34$, $p = 0.57$ (Fig.2A).

3.4 Reinstatement

We wanted to test the hypothesis of whether exercise induced neurogenesis before cocaine exposure played a role in reinstating a cocaine cue-memory. Exercise induced neurogenesis had no effect on reinstatement, $F_{(1, 22)} = 0.00$, $p = 0.99$ (Fig.2A).

4 Experiment 2B Retrograde 7.5mg/kg:

4.1 Pre-test

A habituation test was conducted in which mice had free access to both chambers of the CPP apparatus prior to drug conditioning. Results determined that mice had no initial preference to either side of the chamber $F_{(1, 34)} = 0.47$, $p = 0.50$ (Fig.2B).

4.2 Post-Test

Adult (8-11 weeks) WT mice were provided access to an exercise wheel for 4 weeks following conditioning of a cocaine CPP in order to increase neurogenesis, or remained sedentary in home cage. Figure 2B shows that exercise induced neurogenesis led to the forgetting of a previously encoded cocaine cue memory, $F_{(1, 34)} = 6.0$, $p = 0.02$ (Fig.2B).

4.3 Extinction

We wanted to test the hypothesis of whether exercise induced neurogenesis after cocaine exposure would lead to faster rate of extinction compared to sedentary controls. It was found that the rate of extinction did not differ between groups (sedentary vs. runner) on either day 1, $F_{(1, 34)} = 0.02$, $p = 0.97$, or day 2 of extinction training, $F_{(1, 34)} = 0.39$, $p = 0.54$ (Fig.2B).

4.4 Reinstatement

We wanted to test the hypothesis of whether exercise induced neurogenesis after cocaine exposure played a role in reinstating a cocaine cue-memory. Exercise induced neurogenesis had no effect on reinstatement, $F_{(1, 34)} = 0.93$, $p = 0.34$ (Fig.2B).

Suppressing neurogenesis

5 Experiment 3: Nestin-tk 7.5mg/kg

5.1 Pre-test

A habituation test was conducted in which mice had free access to both chambers of the CPP apparatus prior to drug conditioning. Results determined that mice had no initial preference to either side of the chamber as indicated by no significant main effect or interaction found (Fig.3).

5.2 Post-Test

Adult (8-11 weeks) nestin-TK+ and WT mice were provided access to an exercise wheel for 6 weeks following conditioning of a cocaine CPP or remained sedentary in home cage. No main effects were found for genotype ($F_{(3, 42)} = 0.82$, *ns*) or condition ($F_{(3, 42)} = 0.24$, *ns*); as well there was no significant interaction found between genotype and condition [genotype (positive, negative) x condition (sedentary, runner)], $F_{(3, 42)} = 6.95$, $p = 0.07$ (Fig.3).

5.3 Extinction

We wanted to test the hypothesis of whether levels of neurogenesis had an effect on the extinction of a cocaine memory. Results revealed no significant main effect of genotype ($F_{(3, 42)} = 0.72$, *ns*) or condition ($F_{(3, 42)} = 2.54$, *ns*) on extinction day 1 or on extinction day 2; genotype ($F_{(3, 42)} = 8.29$, *ns*) or condition ($F_{(3, 42)} = 8.2$, *ns*). Also a two-way interaction was not found which indicates that all groups extinguished at a similar rate, $F_{(3, 42)} = 6.02$, $p = 0.09$ (Fig.3).

5.4 Reinstatement

We wanted to test the hypothesis of whether exercise induced neurogenesis after cocaine exposure played a role in reinstating a cocaine cue-memory. No significant main effects were found for either genotype (positive, negative) or condition (sedentary, runner). As well no significant interaction was found however it did approach significance, $F_{(3, 19)} = 3.98$, $p = 0.06$ (Fig.3).

Neurogenesis-induced forgetting persists following consolidation

6 Experiment 4: Delay 7.5mg/kg

6.1 Pre-test

A habituation test was conducted in which mice had free access to both chambers of the CPP apparatus prior to drug conditioning. Results determined that mice had no initial preference to either side of the chamber, $F_{(1, 22)} = 0.24$, $p = 0.62$ (Fig.4).

6.2 Post-Test

Adult (8-11 weeks) WT mice remained sedentary for 4 weeks following cocaine conditioning and then were provided access to an exercise wheel for an additional 4 weeks to increase neurogenesis. Control mice remained sedentary in home cage for the entire 8 weeks. We wanted to examine the effects of neurogenesis following the consolidation period when a memory is no longer hippocampal dependent (Squire and Knowlton, 1995). Figure 4 shows that exercise induced neurogenesis led to the forgetting of a previously encoded cocaine cue memory even after the consolidation period, $F_{(1, 22)} = 13.8$, $p = 0.001$. Which indicates that the weakening of a cocaine cue memory occurs regardless of the timing in which exercise induced neurogenesis occurs (see Fig.4).

6.3 Extinction

We wanted to test the hypothesis of whether exercise induced neurogenesis had an impact on the rate of extinction of a cocaine memory. Results revealed no difference in the rate of extinction between groups on extinction day 1, $F_{(1, 22)} = 0.21$, $p = 0.65$ or extinction day 2, $F_{(1, 22)} = 0.07$, $p = 0.79$ (Fig.4).

6.4 Reinstatement

We wanted to test the hypothesis of whether exercise induced neurogenesis after cocaine exposure played a role in reinstating a cocaine cue-memory. Exercise induced neurogenesis had no effect on reinstatement, $F_{(1, 22)} = 0.21$, $p = 0.65$ (Fig.4).

Chapter 4
Discussion

This series of experiments has provided evidence for the role of hippocampal neurogenesis in memory functioning. This is the first study, to the best of our knowledge that examines the impact of neurogenesis on an appetitively- motivated, hippocampal dependent memory. By using the CPP paradigm, mice learned to associate, through olfactory, textile and visual cues, a distinct context with the drug cocaine. Mice had no initial preference for either context and after 3 consecutive days of drug-context pairings, mice exhibited a strong preference for the drug paired side also known as a conditioned place preference (CPP). This cocaine-CPP remained robust even after 4, 6 and 8 weeks following the last conditioning session, as indicated in the post-test session (see Fig. 1B, 2B, 3 & 4). Running wheels were introduced prior to or following cocaine conditioning. This was completed in order to see the temporal impact of neurogenesis on both the formation of a memory as well as on already established memories stored in the hippocampus circuit. We also wanted to see whether neurogenesis had an impact on the rate of extinction and the reinstatement of a cocaine-CPP.

Just as previous results have shown and as expected, running increased neurogenesis in the hippocampus (see Fig. 5). This was measured by calculating the amount of DCX+ cells (a marker of immature neurons) in the hippocampus (Knoth et al., 2010). There was a substantial number of DCX+ cells found in the hippocampus of running mice compared to mice that remained sedentary in home cage. This is line with previous results that have shown that running increases neurogenesis in the DG of the hippocampus (van Praag, 1999).

Results revealed that increasing neurogenesis (through voluntary exercise) led to the degradation of a previously encoded weak memory but not a strong memory. This suggests that stronger memories are resistant to neurogenesis induced forgetting. This finding is in line with results found in a contextual fear conditioning paradigm with the degree of forgetting dependent on initial memory strength (Akers et al., 2014). In contrast to this finding it was found that exercise induced neurogenesis had no effect on the acquisition of a cocaine-CPP with a high (15mg/kg) or a low dose (7.5mg/kg; see Fig. 1A & 1B). Traditionally an extinction protocol is utilized in CPP to examine the ability to reinstate a drug memory and in order to mimic relapse (Mueller et al 2011). The extinction protocol consists of a relearning process in which the mice learns to associate the previous cocaine paired side now with a saline injection instead so the mouse extinguishes the previous response until the mouse no longer has a preference for that chamber (Mueller et al 2011). Our results revealed that neurogenesis (through voluntary exercise) had no effect on the extinction rate of a cocaine cue-memory. This finding is most likely due to the effect of within-test extinction. Since mice are exposed to the chamber drug-free for 10 minutes during the post-test session, the cocaine cue-memory progressively extinguishes naturally over time. After only 1 day of extinction training, mice no longer exhibited a CPP. This indicates that mice effectively learned after only 1 SAL-SAL pairing that the original cocaine paired chamber is no longer associated with the drug.

We also wanted to examine whether neurogenesis had a bearing on the ability to reinstate the memory trace. Following the extinction of a cocaine memory when mice no longer prefer the cocaine paired chamber, we wanted to determine if neurogenesis had an effect on the ability to reinstate a cocaine-CPP. If the cocaine-CPP was already weakened, then it would be expected that it would be harder to reactivate the memory trace. This was in line with what we found,

running mice that showed a lower CPP score at post-test and therefore forgot the cocaine memory, were unable to reinstate the memory when given a cue (a single 7.5mg/kg cocaine injection). Although surprisingly there was also no difference found in the ability to reinstate the cocaine memory in sedentary controls either, both groups of mice showed no preference for the cocaine paired side following a cue (a single 7.5mg/kg cocaine injection).

2 The effects of suppressing neurogenesis on a reward memory

Next we wanted to determine whether suppressing neurogenesis could prevent the forgetting effect. We used a transgenic mouse line, the nestin-TK mouse in which when fed the drug ganciclovir leads to the ablation of newborn neurons (Imayoshi et al., 2011). Nestin-TK+ mice were housed with WT littermate controls. Mice were either provided access to a running wheel or remained sedentary for 6 weeks following cocaine conditioning. Preliminary results revealed that suppressing neurogenesis did not affect the recall of the cocaine CPP. Both nestin-TK+ mice and WT controls acquired a CPP however there was no significant difference found at post-test in any of the groups (See Figure 3). Since these results are preliminary findings it is difficult to determine any conclusive result at this stage. It also remains to be determined whether neurogenesis levels were actually decreased or not which would have an impact on the behavioural results. Histological quantification of DCX+ cells will need to be completed in order to deduce any conclusions.

We were able to show not just that an increase in neurogenesis leads to forgetting of a previously encoded memory but that forgetting persists even following the consolidation period when memories are said to be stable (Wixted, 2004). The first result is in line with previous findings in which an aversive stimulus was used, here we have provided evidence in order to generalize this finding using an appetitively motivated task. Therefore we can conclude that neurogenesis

mediates forgetting of previously stored memories. However it is of interest as to which mechanism is at play that is driving this effect.