Adult Hippocampal Neurogenesis, Cognitive Flexibility, and Depression

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Depression is a serious and complex mental health disorder that is becoming increasingly prevalent, and is among the leading causes of global disease burden. Although depression is thought to be primarily an affective disorder, there is growing evidence that it is associated with memory deficits. Adult hippocampal neurogenesis has been implicated in depression and may also contribute to cognitive flexibility. Therefore, the present study used an animal model of depression involving chronic corticosterone (CORT) injections to assess learning and memory, and changes in adult hippocampal neurogenesis. In Experiment I, rats were randomized to receive either CORT (40 mg/kg) or vehicle injections for 21 days. On the second day of treatment, all rats received injections of BrdU to label newborn neurons. Six days before the end of the CORT treatment, rats were subjected to behavioural testing in the Morris water maze (MWM). Neurogenesis was also assessed using immunofluorescence staining. Although CORT-treated rats performed on par with vehicle-treated rats during spatial learning of the MWM, cognitive flexibility of the CORT-treated rats was significantly impaired during the first day of reversal training. The probe test revealed enhanced memory retention of the platform location for the CORT-treated rats. Experiment II was similar to Experiment I in all respects, except that injections were stopped 3 days before behavioural assessment. In Experiment II, CORT-treated rats performed better in the spatial learning phase of the MWM, while reversal learning and memory retention in the probe test were no different than control animals. In both Experiment I and II, no difference in the number of newborn neurons between the two groups was observed. These findings suggest that cognitive flexibility is impaired in a CORT-induced animal model of depression, the effect is reversible and seems to be independent of suppressed hippocampal neurogenesis.

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List of Abbreviations

AUC	Area under the curve
BrdU	Bromodeoxyuridine
CA	Cornu Ammonis
CORT	Corticosterone
DCX	Doublecortin
DG	Dentate Gyrus
DG_{IP}	Infrapyramidal dentate gyrus
DG_{SP}	Suprapyramidal dentate gyrus
EC	Entorhinal cortex
fMRI	Functional magnetic resonance imaging
GR	Glucocorticoid receptor
HβC	Hydroxylpropyl-beta-cyclodextrin
HPA	Hypothalamic-pituitary-adrenal
LTP	Long-term potentiation
MDD	Major depressive disorder
M.O.M	Mouse-on-mouse
MR	Mineralocorticoid receptor
MWM	Morris water maze
NeuN	Neuronal Nuclei
NMDA	N-methyl-D-aspartate
NPHS	National Population Health Survey
NSC	Neural stem cell

OCT	Optimal cutting temperature
SD	Sprague Dawley
SSC	Saline sodium citrate
SUB	Subiculum
TBS	Tris-buffered saline
TSA	Tyramide single amplification
TMZ	Temozolomide
VEH	Vehicle
WHO	World Health Organization

Adult Hippocampal Neurogenesis, Cognitive Flexibility, and Depression

Major depressive disorder (MDD), commonly referred to as depression, is a serious and complex mental health disorder, with a lifetime prevalence of over 12% in Canada (Patten et al., 2006). Data from the Canadian National Population Health Survey (NPHS) indicate that the cumulative incidence of major depressive episodes was nearly 3% in 2003, rising to over 7% by 2007 (Wang et al., 2010). In 2004, the World Health Organization reported that depression was the third leading cause of disease burden, and the leading cause of disability in both males and females (WHO, 2008), although depression rates are consistently higher among women (Thorpe, Whitney, Kutcher, & Kennedy, 2001). Mental health illnesses are among the costliest conditions in Canada, with the estimated total burden costing $51 billion in 2003 (Lim, Jacobs, Ohinmaa, Schopflocher, & Dewa, 2008). Largely owing to the high prevalence, chronicity and age of onset, depression is among the highest burden of mental health illnesses in Ontario (Ratnasingham et al., 2013). Furthermore, 98% of suicide cases have a diagnosable mental health illness (Bertolote & Fleischmann, 2002), with the highest risk of suicide found in those with depression, bipolar disorder, borderline personality disorder, and anorexia nervosa (Chesney, Goodwin, & Fazel, 2014).

Depression is primarily characterized by pervasive and persistent low mood and anhedonia (loss of interest in pleasurable activities), often occurring with low self-esteem. A number of clinical symptoms are also seen with depression, including nervousness, irritability, psychomotor agitation and/or retardation, and changes in sleeping, appetite and weight (Nemeroff, 1998; Nestler et al., 2002). Individuals suffering from depression tend to experience decreased social functioning, and have a higher rate of physical illness and mortality (Nemeroff, 1998). Further adding to the complexity of the condition, depression often co-occurs with a number of other psychiatric illnesses, most notably anxiety (Mineka, Watson, & Clark, 1998). There is also growing evidence that cognitive disturbances are present in patients with depression, likely related to brain areas affected by the disorder (Marazziti, Consoli, Picchetti, Carlini, & Faravelli, 2010; Trivedi & Greer, 2014). Common cognitive symptoms of major depression typically manifest as negative self-evaluation and low self-worth, thoughts of death and suicidal ideation, rumination over minor past failings, delusions with a fixation on ideas of guilt, personal or situational shortcomings and feelings of persecution, and hallucinations (Marazziti et al., 2010).

Memory Deficits in Major Depression

A number of other cognitive deficits are often associated with depressive illness, such as difficulty making decisions, deficits in cognitive flexibility, and impaired memory and attention (Deveney & Deldin, 2006; Jaeger, Berns, Uzelac, & Davis-Conway, 2006; McCall & Dunn, 2003; Naismith, Longley, Scott, & Hickie, 2007; Rock, Roiser, Riedel, & Blackwell, 2014). Furthermore, deficits in the domains of executive function, processing speed, and episodic memory were found to correlate with the severity of depressive illness (McDermott & Ebmeier, 2009). With respect to memory-related dysfunction, patients with major depression were reported to have impairments in verbal (Bremner, Vythilingam, Vermetten, Vaccarino, & Charney, 2004) and spatial working memory (Gould et al., 2007), and episodic learning and memory (Austin, Mitchell, & Goodwin, 2001).

Memory deficits, in particular, may also represent a biomarker of depressive illness (Trivedi & Greer, 2014). In a group of high-risk subjects (who had a family history of depression, but did not themselves suffer from the disorder), brain regions associated with working memory have been shown to be over-active (Mannie, Harmer, Cowen, & Norbury, 2010). It was suggested that over-activity in brain regions associated with working memory may represent a marker for a vulnerability to develop depression. Additionally, as a pre-morbid marker of depression, poor free and cued recall performance (a measure of episodic memory) was associated with a subsequent diagnosis of depression (Airaksinen, Wahlin, Forsell, & Larsson, 2007).

Patients with depression have also been shown to have a global impairment in episodic autobiographical memory (Söderlund et al., 2014). With respect to autobiographical memory, people with depression tend to recall more negative than positive memories, have a high occurrence of intrusive memories of stressful life events, and have an increase in overgeneralized memory retrieval (Lemogne, Piolino, Jouvent, Allilaire, & Fossati, 2006). Cognitive flexibility also seems to be affected by emotional information, where individuals with depression showing less flexibility with negatively-valenced stimuli (Deveney & Deldin, 2006). Impaired executive function and impulsivity has also been shown in adolescents with acute and remitted depression (Maalouf et al., 2011). Moreover, attentional dysfunction and difficulty concentrating are thought to underlie impairments seen in memory (Marazziti et al., 2010). Similarly, poor concentration and indecisiveness may be factors that hinder functional recovery from cognitive disturbances in depressive illnesses (Trivedi & Greer, 2014). Overall, difficulty with memory and decision-making appear early on in the development of depression, and likely reflect changes to hippocampal and prefrontal cortex function (Trivedi & Greer, 2014).

Many of the cognitive deficits described above are related to dysregulation of structures implicated in learning and memory, including amygdala, hippocampus, and prefrontal cortex. The hippocampus is of particular interest in the study of cognitive impairment in depression because it plays a central role in declarative learning and memory, and seems especially important for integrating cognitive and emotional processes (Femenía, Gómez-Galán, Lindskog, & Magara, 2012; Millan et al., 2012; Small, Schobel, Buxton, Witter, & Barnes, 2011). Structural changes in limbic and forebrain regions, including the hippocampus, amygdala, and prefrontal cortex, have been seen with neuroimaging and post-mortem assessment of patients with depression (Drevets, 2000; Jaracz, 2008; Stockmeier & Rajkowska, 2004). Furthermore, hippocampal volume loss has been associated with prolonged major depressive illness (Sapolsky, 2000). More notably, structural abnormalities related to hippocampal shape, rather than a volumetric decrease has been shown to be important in characterizing neurological features of patients with depression (Posener et al., 2003). In general, there is strong support that dysregulation in the hippocampus is associated with the development of depression (Sheline, Mittler, & Mintun, 2002).

Structural and Functional Components of the Hippocampus

The hippocampus is a limbic structure crucially involved in long-term explicit memory consolidation and storage, learning, and spatial navigation. O’Keefe & Nadel (1978) originally characterized its function as providing an autobiographical context (situated in time and space) for which memories can associate. The hippocampus is a unique system of elaborate interconnected neural circuitry that is characteristically plastic and reactive, yet sensitive and vulnerable to a dynamic environment (Eichenbaum, 1997). This remarkable plasticity and sensitivity is what allows the structure to efficiently interact with the environment through its complex construction.

The hippocampus (Figure 1), located in the medial temporal region of the brain, is composed of the dentate gyrus (DG), and Ammon's horn consisting of the CA1 – CA3 pyramidal cell layers (O'Keefe & Nadel, 1978). Its distinctive cytoarchitecture is such that information can be assembled from various other neural networks; an important feature in the formation of memories. Integrated inputs from visual, auditory, and somatic associative cortices flow through the parahippocampal region to the perforant fibres of layer II of the entorhinal cortex (EC), ultimately relay information to the hippocampus (refer to Figure 1). Perforant path axons project to the dendritic branches of the DG granule cells. Information is then sent to the proximal apical pyramidal dendrites of the CA3 through DG axons (also known as mossy fibres). Finally, CA3 axons branch out, forming the Schaffer collateral pathway connecting to ipsilateral pyramidal cells of the CA1, completing the trisynaptic loop of the hippocampus. CA3 axons also project to the contralateral CA3 and CA1 pyramidal cells through commissural fibres projecting via the corpus callosum. Furthermore, CA3 cells interconnect on the same side, creating recurrent collaterals, which is a dense associative network, in addition to receiving direct input from layer II of the EC. CA1 axons ultimately project to the subiculum and deep layers of the EC, while also receiving direct input from layer III of the EC (Debuc, Robert, Paquet, & Daigen, 2002; Neves, Cooke, & Bliss, 2008).

The hippocampus at large is important for various types of learning behaviours; however its unique cytoarchitecture affords a further dimension of complexity that is finely tuned for cognitive flexibility. Although there is extensive cooperation among the subregions of the hippocampus, there is a diverse range of intrinsic information processing that is subregion-specific and behaviourally unique (Marr, 1971). For example, the DG and CA3 are thought to together support ‘spatial pattern separation’, which is the ability to discriminate similar, yet distinct memories (Deng, Aimone, & Gage, 2010). As previously outlined, the DG receives a collection of sensory inputs and environmental information from the entorhinal cortex (EC); however, the DG is much more densely packed with cells. As such, it orthogonalizes inputs coming from the EC to form a metric spatial representation of the environment (Kesner, Lee, & Gilbert, 2004). The densely packed cells of the DG allow for overlapping EC inputs to be encoded as distinct representations. Furthermore, the use of a sparse coding scheme of DG granule cells projecting to CA3 pyramidal cells allows for similar inputs to activate discrete granule cell populations, thereby driving a high fidelity of memory encoding (reviewed by Deng et al., 2010). Altogether, the DG and CA3 allow for experiences that are similar to be remembered as distinct (‘pattern separation’). The importance of this pattern separation property is thought to be indispensable in avoiding “catastrophic interference” of old memories when adapting and learning new information (Treves & Rolls, 1992; Wiskott, Rasch, & Kempermann, 2006).

Spatial navigational learning (akin to way finding in humans, a method of orienting one’s self in physical space that is independents of the starting location) is a hippocampal-dependent navigation strategy that specifically implements the use of landmark configurations to construct a cognitive map of the environment (Dupret, O'Neill, Pleydell-Bouverie, & Csicsvari, 2010; Eichenbaum, Stewart, & Morris, 1990; Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe, Nadel, Keightley, & Kill, 1975). In spatial learning, polymodal sensory information from the environment is incorporated together to create an understanding of position and how to navigate, in essence, forming a cognitive map (Tolman, 1948). Additionally, the hippocampus has been shown to participate in multiple stages of spatial memory processing, including encoding, consolidation and recall (O'Keefe & Nadel, 1978; Riedel et al., 1999).

The hippocampus in stress regulation and depression.

Limbic structures such as the hippocampus, amygdala, and prefrontal cortex, are responsible for regulating a system called the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is a system that responds to stress in conjunction with the sympatho-adreneromedullar system (the system responsible for the "fight-or-flight" response). The HPA axis is important for regulating the long-term neurological responses to stress to help face the imminent threat and prepare the organism to face similar threats in the future (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006). Through a cascade of intermediate steps involving hormone and transmitter secretion, the adrenal glands are stimulated to release corticosteroid hormones (corticosterone, or CORT, in most rodents, and cortisol in humans) in response to stress. Corticosteroid hormones bind to glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs), and are the main effectors of the HPA axis. Glucocorticoid hormones, a subclass of corticosteroid hormones, bind with high affinity to MRs, therefore, when circulating levels of glucocorticoids are low during periods of rest (and stress is not imminent), these receptors are extensively occupied. In contrast, glucocorticoid hormones bind to GRs with a low affinity and extensively occupy these receptors (in addition to MRs) during periods of elevated glucocorticoid secretion following stress, when the amount of circulating glucocorticoids are high (Reul & De Kloet, 1985). The hippocampus is a unique limbic structure in that it is one of the first structures to receive and process information from the environment. Furthermore, it contains an exceptionally high concentration of both GRs and MRs, particularly in the CA1 and CA2 regions and the dentate gyrus (Van Eekelen, Jiang, De Kloet, & Bohn, 1988). As a result, the hippocampus is critically involved in negative feedback regulation of the HPA axis through the action of glucocorticoid hormones. A delayed negative feedback system of glucocorticoids acts on the HPA axis to reduce and normalize its activity after a rise in stress hormone levels following a stressful event (Smith & Vale, 2006).

The hippocampus is therefore crucial for homeostatic control of stress. However, with prolonged stress, the hippocampus is also prone to damage. Therefore, because the hippocampus plays a critical role in episodic learning and memory, and is prone to the aversive effects of chronic stress, it is a key structure implicated in producing cognitive deficits associated with depression (Austin, Mitchell, & Goodwin, 2001; MacQueen et al., 2003). Furthermore, prolonged activation of the HPA axis can also lead to serious health risks that mirror many symptoms of depression, including a suppressed immune response, growth inhibition, sleep dysfunction, impaired memory, decreased sexual behaviour, and chronic dysphoria (D’Amato, Rizzi, & Moles, 2001; McEwen, 1998; Sandi & Pinelo-Nava, 2007). As a consequence of the abnormalities observed in the hippocampus and connected structures, depressed patients are often ill equipped to cope or adapt to the environment and stressful challenges. Furthermore, the clinical relevance of this may be that dysfunction in sensitive regions of the brain, such as the hippocampus, may be a vulnerability factor for poor treatment outcome (Frodl et al., 2008).

As mentioned, alterations to hippocampal volume and structure have been observed in patients with depression. In a study assessing hippocampal measures in depressed patients, both never-treated first-episode patients and patients with multiple episodes were found to have hippocampal dysfunction in various tests of recollection memory, although only multiple depressive episode patients had a reduction in hippocampal volume (MacQueen et al., 2003). Other studies have found that first-episode male patients show smaller left hippocampal volume, and significant left-right asymmetry in the hippocampus, suggesting that the left hippocampus might play an important role in the pathophysiology of early stages of major depression, particularly in male patients (Kronmüller et al., 2009). Overall, meta-analyses showed hippocampal volume reductions in both left and right hemispheres in patients with depression, and this is likely a consequence of repeated periods of depressive episodes (Campbell, Marriott, Nahmias, & MacQueen, 2004; Videbech & Ravnkilde, 2004). Diminished hippocampal volume seem to be most prominent in the main subfields (DG and CA1-3) of the hippocampus (Frodl et al., 2014; Huang et al., 2013). Moreover, while glia, pyramidal neurons, and granule cells are significantly more dense in postmortem hippocampi of patients with depression (Stockmeier et al., 2004), the overall hippocampal volume reduction seems to be related to volume loss in the neurpil, which subsequently under-represent large-scale losses in dendrites and synapses (Tata & Anderson, 2010). Furthermore, unmedicated depressed patients were found to have fewer mature granule cells in the anterior DG, as well as smaller DG and granule cell layer volumes, which suggests that these individuals have an impairment in maturation or survival of neuronal progenitor cells (Boldrini et al., 2013). This compliments findings in animal studies that show adult hippocampal neurogenesis is suppressed in animal models of chronic stress (Joëls, Karst, Krugers, & Lucassen, 2007; Schoenfeld & Gould, 2012) and depression (Sterner & Kalynchuk, 2010; Surget et al., 2008).

Adult Hippocampal Neurogenesis

Joseph Altman and colleagues were originally credited with the discovery of neurogenesis after publishing a series of papers outlining the generation of new neurons from stem cell with differentiation properties (Altman, 1962; Altman & Das, 1966). These newborn neurons were found to persist throughout adulthood in various structures within the mammalian brain, particularly in the neocortex (Altman, 1963; Altman, 1966), dentate gyrus (Altman & Das, 1965), and olfactory bulb (Altman, 1969), which is consistently conserved across phylogeny. Initially, Altman's proposition that neurons were born and integrated into the adult mammalian brain was not accepted within the scientific community. With improvements in molecular techniques, the scientific community had come to establish neurogenesis as a verified phenomenon nearly 30 years later. One such major technique was the in-vivo stereological use of the synthetic thymidine analogue 5-bromo-3'-deoxyuridine (BrdU) to label new cells (Kempermann, Kuhn, & Gage, 1997; Kuhn, Dickinson-Anson, & Gage, 1996). This thymidine analogue is taken up by cells during S-phase of mitotic division and therefore marks proliferating cells (Cooper-Kuhn & Kuhn, 2002; Nowakowski, Lewin, & Miller, 1989). In conjunction, newly generated cells also express specific cell-type markers that can be visualized using immunofluorescence staining (Figure 2). Therefore, although BrdU can be incorporated into any type of proliferating cell (neurons, astrocytes, oligodendrocytes), newly generated cells can also be co-labeled with neuron-specific markers. For example new neurons begin to robustly express neuronal nuclei (NeuN) at around 21 d after birth (when mature) and can be co-labeled with the antigen for NeuN (Mullen, Buck, & Smith, 1992). Therefore, together, mature neurons (that express NeuN) generated recently in adulthood (that incorporate BrdU) can be visualized (Figure 2). Techniques such as these have provided sufficient evidence for the production of adult-generated neurons, such that neurogenesis is now widely accepted, not only as a hippocampal- and olfactory-based event, but more recently as occurring within other structures, such as the hypothalamus (Kokoeva, Yin, & Flier, 2005; Markakis, Palmer, Randolph-Moore, Rakic, & Gage, 2004; Migaud et al., 2010; Rojczyk-Gołębiewska, Pałasz, & Wiaderkiewicz, 2014). Although hypothalamus neurogenesis has not yet been confirmed in humans, it is well established that adult neurogenesis occurs in the dentate gyrus (Eriksson et al., 1998; Bhardwaj et al., 2006) and olfactory bulbs of humans (Curtis et al., 2007; Curtis, Kam, & Faull, 2011).

The process of neurogenesis is comprised of three main physiologically and morphologically distinct stages: proliferation, differentiation, and survival. Adult neural stem cells (NSCs), cells with proliferating properties, give rise to neuronal tissue in the brain, including neurons and glial (astrocyte and oligodendrocyte) cells (Gage, 2000). Newly born neurons then undergo a phase of differentiation and fate determination into the various cell types, at which point surviving neurons can mature and integrate into the neural systems (Zhao, Deng, & Gage, 2008). The process of new neuron survival, maturation and integration into the hippocampal circuitry is believed to span between two to six weeks, during which time there is heavy apoptotic action. Kempermann and colleagues found that the number of new neurons decline heavily after division, where nearly 80% of neurons born in the adult brain do not survive. However after 4 weeks, the number of new neurons was shown to stabilize (Kempermann, Gast, Kronenberg, Yamaguchi, & Gage, 2003). Surviving neurons go on to functionally integrate into the neuronal network (Zhao et al., 2006). The period of interest to the present study is the 3-week time period leading up this maturation phase. During the 1-3 weeks after birth, the survival of new neurons is heavily mediated by the neurogenic niche (the microenvironment in which neurons are born). Here, the environment permits a close association with vasculature, and therefore vulnerability to extrinsic factors (Alvarez-Buylla & Lim, 2004; Palmer, Willhoite, & Gage, 2000). The action of local and distal cells can directly and indirectly influence new neurons (Zhao et al., 2008), as is the case of learning (Kee, Teixeira, Wang, & Frankland, 2007). Lastly, survival is heavily influenced by both positive experience – such as environmental enrichment (Kempermann et al., 1997; Tashiro, Makino, & Gage, 2007) or learning (Gould, Beylin, Tanapat, Reeves, & Shors, 1999) – and negative experiences – such as stress (Gould & Tanapat, 1999) and depression (Boldrini et al., 2013). Morphologically, three weeks after birth, new neurons form afferent and efferent dendritic connections with perforant path, hilar and CA3 targets, and are physiologically able to produce functional glutamatergic inputs and NMDA receptor signalling (Deng, Aimone, & Gage, 2010; Zhao et al., 2008). These morphological and physiological changes are also vulnerable to extrinsic factors. For example, Tashiro and colleagues (2007) found that 2-3 weeks after neuronal birth is a critical period whereby immature neurons are affected by experience. Importantly, experience during this critical period can influence the survival and population response of those new neurons, which may affect future neural representation of the experience in the DG. However, much of this evidence has originated from studies of mice. Notably, recent findings show differences between rats and mice in the maturation rate of adult-born hippocampal neurons. These findings suggest that adult-born hippocampal neurons in rats mature faster, showing a mature neuronal marker profile and activity-induced immediate early gene expression 1-2 weeks earlier than in mice, where newborn neurons are 2-3 weeks of age (Snyder et al., 2009). Furthermore, new neurons were more behaviourally involved in rats than mice, with spatial learning activating more new granule cells in rats (Snyder et al., 2009).

Functional theories of adult hippocampal neurogenesis.

The biological and functional significance of adult hippocampal neurogenesis has been a curious topic of interest to researchers within the field. Given the incredibly limited areas in the adult brain where neurogenesis occurs, and the fact that these neurogenic areas are highly conserved across phylogeny, this may suggest that neurogenesis is evolutionary advantageous in order to increase functionality in these areas. Many theories based on computational modeling and experimental studies have been proposed to suggest the functional role of adult born hippocampal neurons. This section outlines a few of the major theories proposed to explain the specific contribution of adult-born neurons to memory formation.

A few groups have focused efforts on explaining the significance of the continuous nature of neurogenesis. Neurogenesis is a constant process where the population of newborn neurons is perpetually changing through maturation or death. The cognitive impact of this turnover phenomenon is thought to allow long-term episodic memories to be temporally distinguished (Aimone, Wiles, & Gage, 2006). Another group experimentally tested hypotheses relating to the difference in excitability of mature vs. immature neurons, showing that upon maturing, new granule cells are no longer used for retrieval of previous experiences, and instead reach "early retirement" by becoming quiescent and unexcitable when previous memories are reinstated (Alme et al., 2010).

Others have looked at the behavioural consequence of neurogenesis. Aimone and colleagues (Aimone, Deng, & Gage, 2011) suggested that the formation of new neurons in the adult brain contribute to different functions depending on the stage of maturation. In their 2011 work, Aimone and colleagues explain that although many studies implicate the DG in behavioural 'pattern separation' of memories (discriminating similar yet distinct memories), a more precise description of DG function would be that it controls 'memory resolution'. Memory resolution is described as the extent of information that is encoded by the DG, which also affect downstream hippocampal regions during memory formation. Therefore, memories with more information encoded would be robust enough to support finer discrimination. Immature newborn granule cells add to information encoding by providing a complete but low-information representation of experienced events or features of the environment. Maturing neurons activate in an experience-dependent manner to salient environmental stimuli, gaining specificity and specialization over time. Mature neurons provide representations that are incomplete but high in information. Altogether, this maximizes the information encoded into hippocampal memories. At the behavioural level, this increases memory discrimination (i.e. memory resolution). At the hippocampal network level, this means pattern separation is increased by forming memories that are distinct and minimizing downstream interference.

Another model suggested by Wiskott and colleagues (2006) similarly focuses on the differences in function of immature and mature adult-generated neurons. Similar to other theories, Wiskott et al. follow the premise that immature adult-born neurons are broadly-tuned and highly excitable, and mature neurons are tightly-tuned to previous memories. They suggest that adult hippocampal neurogenesis is required in order to avoid catastrophic interference of old memories with new ones. Catastrophic interference is the notion that when new memories are learned, neural tuning would shift to favour the newly learned pattern. However, as more memories are learned and neural networks continue to shift to incorporate more patterns, the oldest patterns will lose valence and eventually be forgotten. This hypothesis uses the framework that for memories to form, inputs are relayed from the EC to the DG, where a sparse coding scheme and compression of inputs are used to efficiently encode patterns that are stored in the CA3. Since the role of the DG is to encode, catastrophic interference that occurs here would result in problems at any other point in retrieval of memories. Therefore, Wiskott and colleagues proposed that neurogenesis is necessary in the DG because the DG is the narrowest location along the trisynatpic loop and is also constantly flooded with new information from other networks that need to be encoded. Therefore, catastrophic interference endangers the proper consolidation of previously learned content. The addition of new neurons is hypothesized to solve this problem by allowing older surviving neurons to remain tuned to old memories, which is adaptive to earlier environments, while new neurons are sensitive to newer information from the environment.

Others have proposed that the role of neurogenesis is involved in a more global function – that is, accounting for its significance to health and disease across the lifespan. Kempermann and colleagues (Kempermann, 2008; Klempin & Kempermann, 2007) proposed neurogenesis as representing a 'neurogenic reserve'. Their neuogenic reserve hypothesis attempts to explain how the activity-dependent incorporation of relatively few adult-generated neurons in the hippocampal network can lead to lifelong functionality. They suggest that on a long timescale, neurogenesis allows a 'reserve' to be built that increases the potential for recruiting the necessary new neurons in time for computational need. Experience and activity sustain this reserve for plasticity over the lifetime with broadly-activated nonspecific mechanisms (such as activity in general) that regulate precursor cell proliferation, and specific learning stimuli that recruit the survival and incorporation of newborn cells.

Taken together, these theories provide a number of explanations for adult neurogenesis that outline function from a cellular and systematic to a global level. However, one key factor that is common among these theories is that neurogenesis operates toward serving learning and memory, and seems to be integrally involved in creating neural environment adept for plasticity and flexibility.

Recent work by Garthe and colleagues (2009) has provided behavioural and cellular evidence that neurogenesis also promotes cognitive flexibility to optimize the DG neuronal network. Working on the premise of Kempermann's (2002) theory that neurogenesis functions to increased complexity of the hippocampus such that it can accommodate for novel complexity of the environment, Garthe and colleagues provided evidence that new neurons added to the DG network are involved in increasing flexibility to some parameters of hippocampal-dependent learning in the water maze. In this study, mice were given temoxolomide (TMZ) to chemically supress adult neurogenesis. The hidden-platform version of the MWM for three days with two days of reversal was then used to assess the effects of TMZ-induced suppressed neurogenesis on learning and memory. Through qualitative analysis of the search strategies used in the MWM, they found that mice with supressed hippocampal neurogenesis did not advance to spatially precise search strategies, which was especially impaired in the reversal task. They concluded that adult hippocampal neurogenesis seems to be required to flexibly use a metric representation of the environment. Given that chemically supressed neurogenesis can cause such a specific behavioural deficit, it posits that suppressed hippocampal neurogenesis under other conditions such as depression, aging and chronic stress should also produce similar behavioural deficits. As cognitive flexibility in rodents may be examined using reversal training following the conventional Morris water maze training, the two tasks have been described in more detail in the following sections.

The Morris Water Maze (MWM)

In contemporary neuroscience research, the MWM is widely used for its simplicity yet significant implications to the neuroanatomical and neurochemical understanding of learning and memory. In the hidden-platform version of the MWM, a circular pool is filled with opaque water such that a platform is hidden just below the water surface. The objective during acquisition training is for the animal to use distal cues in the room to find the location of the hidden platform. A probe task is also often given 24-h after training to assess long-term memory retention (i.e. reference memory). In this task, the platform is removed from the pool and the amount of time spent in the target vs. other quadrants is assessed as a measure of memory retention (Vorhees & Williams, 2006). The MWM assesses spatial learning in a wide variety of species, most commonly rodents, but also in humans using a virtual maze (Kallai, Makany, Karadi, & Jacobs, 2005). In addition, it is an ideal assessment tool for learning and memory because it does not require pre-training, and results are reliable across a wide range of tank configurations and testing procedures (Vorhees & Williams, 2006). Furthermore, the MWM capitalizes on aversive motivation to complete the task, rather than appetatively motivated tasks like the radial arm maze, T- maze or Y-maze, which rely on food deprivation for motivation. This can be a problem because in experiments using chronic stress, motivation and appetite can be decreased, which can confound the ability for animals to complete an appetatively motivated task. Therefore, because the MWM does not rely on food reward to complete the task, this problem does not affect the ability to examine behaviour under chronic stress (Conrad, 2010). However, a disadvantage of the MWM task is that it uses a rodents' innate aversion to water to motivate escape, and is therefore stressful in some way to all animals subjected to the task.

Use of the MWM to assess hippocampal-dependent spatial navigation and reference memory has been validated extensively (Morris, 1993). However, the cues for which the animal depends on to find its way through the environment can affect how the animal learns and what brain regions are activated during learning (Vorhees & Williams, 2014). Allocentric navigation depends on distal cues in the environment – in the case of the MWM, placing distinct cues outside the pool, on the walls of the maze room but within the animals' visual range. Conversely, egocentric navigation depends on internal (ex. motor movement) and proximal cues to navigate through the environment, as in the case of the visible-platform version of the MWM. Egocentric and allocentric navigation are not per se controlled by distinct brain regions, and activate overlapping neural systems (Sherrill et al., 2013). However, the hippocampus seems more attune to allocentric navigation. For example, rats with hippocampal lesions show an impaired ability to use allocentric navigation, showing a preference to use egocentric navigational strategy, as well as impaired spatial mapping abilities (McDonald & White, 1994).

In order to encourage a hippocampal-dependent, allocentric learning strategy in the MWM, one can randomize the start location of the animal for every trial (Devan, Goad, & Petri, 1996). Randomization of the start location promotes the use of distal cues in animals navigating the MWM, which promotes allocentric navigation. Without a consistent start location, animals cannot rely on a set route to follow using internal and proximal cues to find the hidden platform. This randomization is done during acquisition training, when the memory for the task is being encoded. This type of spatial task is useful because it constitutes as an animal model of human explicit/declarative learning and memory (Eichenbaum, 2001), or at least a distinct subcomponent of such memory, namely episodic memory (Morris, 2013). Furthermore, studying this type of memory offers several other advantages: comparisons can be made across studies that use different species, since navigation is a natural behaviour for most species, including humans (Nekovarova, Nedvidek, Klement, Rokyta, & Bures, 2013); and behavioural outcomes can be supplemented with molecular, cellular and electrophysiological techniques to study how the brain processes information and gives rise to these behaviours. In addition to the acquisition training in the MWM, another increasingly common assessment in the MWM is reversal training. Reversal tasks are used to assess spatial flexibility, which is also sensitive to hippocampal function (Dong et al., 2013; Ramos, 2010) and in particular sensitive to hippocampal neurogenesis (Garthe et al., 2009).

Reversal learning as an assessment of cognitive flexibility.

In the broadest sense, cognitive flexibility is described as readiness with which concepts can be selectively altered in response to appropriate environmental stimuli (Scott, 1962). Within the realm of spatial learning and memory, cognitive flexibility is assessed based on an animals' ability to appropriately make shifts to a new destination based on a previously learned cognitive map. In paradigms like the radial arm maze, Barnes maze, and MWM, variations to the general procedure can add valuable information for understanding where deficits in learning may arise that may not be detected during initial acquisition training. One such variation is the reversal training, which assesses spatial flexibility, a form of cognitive flexibility. For instance in the MWM, after acquisition training, a reversal task can be given such that the hidden platform is relocated to the opposite quadrant of the pool. For learning to be successful in the reversal task, the animal must be able to inhibit irrelevant information and reconsolidate the initially learned position of the platform from the acquisition training, and learn the new goal position (Rossato, Bevilaqua, Medina, Izquierdo, & Cammarota, 2006). Similarly to the acquisition phase of the MWM, a probe task can be given 24 h later to assess if the new goal location was sufficiently learned. Reversal learning and flexible memory expression with the use of allocentric cues has been shown to be fundamentally dependent on proper hippocampal function (Ramos, 2010). At the cellular level, spatial cognitive flexibility has been associated with adult hippocampal neurogenesis, which may provide molecular support for the flexible abilities of the hippocampus at the behavioural level (Burghardt, Park, Hen, & Fenton, 2012; Garthe et al., 2009; Garthe, Huang, Kaczmarek, Filipkowski, & Kempermann, 2014; Kleinknecht et al., 2012).

Depression and Adult Hippocampal Neurogenesis

Accumulating evidence suggests that depression is related to hippocampal neurogenesis. Boldrini et al. (2013) found that untreated depression was associated with fewer mature granule neurons in the DG, which was the first of its kinds to present findings of a potential association between depression and neurogenesis in humans. Intriguingly, the primary catalyst that suggested a link between adult hippocampal neurogenesis and depression comes from the observation that most antidepressants, including environmental interventions that confer antidepressant-like effects (for instance, exercise), also stimulate adult hippocampal neurogenesis (Duman, 2004; Madsen et al., 2000; Malberg, Eisch, Nestler, & Duman, 2000; Yau et al., 2011). Likewise, in studies using human post-mortem brain tissue (Boldrini et al., 2009) and human cell line cultures (Anacker et al., 2011), antidepressants were shown to increase DG neural progenitor number and cell proliferation. Additionally, there is substantial evidence from multiple laboratories that shows neurogenesis is requires for antidepressants to properly exert their effect (Airan et al., 2007; Jiang et al., 2005; Santarelli et al., 2003; Snyder, Soumier, Brewer, Pickel, & Cameron, 2011). Even more compelling is the findings that when neurogenesis is ablated in mice, subsequent chronic stress does not allow for the efficient shut-off of the HPA axis, and consequently increase depressive-like behaviours in these mice (Snyder et al., 2011). These findings suggest that neurogenesis is important for proper regulation of the hormonal stress response as well as preventing the emergence of stress-related mood disorders like depression. In addition to its influence on mood, neurogenesis may also underlie some of the cognitive disturbances associated with depression. Specifically, cognitive inflexibility may be the behavioural outcome of suppressed hippocampal neurogenesis related to depression. Indeed, cognitive flexibility is impaired in patients with depression (Fossati, Ergis, & Allilaire, 2002), although investigating if this effect is a result of supressed neurogenesis is much more difficult to study in humans. Importantly, in mice, experimentally ablating hippocampal neurogenesis severely impairs flexible learning (Burghardt et al., 2012; Garthe et al., 2009). In order to address whether suppressed adult hippocampal neurogenesis underlies cognitive inflexibility in depression, a suitable animal model of depression is required.

Animal model of depression.

Animal models of depression have been developed not only to study the etiology and pathophysiology of the disease but also to identify and test new treatments. There are a number of animal models that can mimic some aspects of the pathophysiology of depression, although no single model fully meets all the criteria for validity (reviewed by Czéh, Fuchs, Wiborg, & Simon, 2016). There are four general criteria for validity of an animal model, namely face (similar behavioural and symptom phenotypes), predictive (similar effectiveness of antidepressant treatments), construct (similar neurobiological basis), and etiological (triggered by events important in the development of depression) validity (Czéh et al., 2016; Sterner & Kalynchuk, 2010). A number of animal models use genetic manipulation, for example, to overexpress or silence candidate genes, use optogenetic tools to block or stimulate neuronal activity, or selectively breed traits related to anxiety- or depressive-like characteristics. However, many models of depression are based in stress exposure because they often have good etiological, face, and construct validity (Czéh et al., 2016). These models involve paradigms such as social stress that involves conflict or defeat, chronic unpredictable stress (also known as chronic mild stress) that use multiple heterotypic stressors, chronic repeated stress that use a homotypic stressor such as restraint, chronic administration of exogenous CORT, uncontrollable stress that can induce learned helplessness, or early-life stress (Czéh et al., 2016; Sterner & Kalynchuk, 2010).

The value of these stress models come about because a likely culprit to provoke the development of depression is stress. Stress has been implicated as an important contributing factor to the onset of the initial depressive episode (De Marco, 2000). Furthermore, there is strong evidence that depressed patients shown dysfunction and hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Checkley, 1996), an important system for the regulation of stress response, and a system that involves participation of the hippocampus, prefrontal cortex, and amygdala (Joëls et al., 2006). Disrupted cortisol (the main stress hormone in humans) rhythmicity and hypercortisolemia have been observed in about half of depressed patients (Sachar & Baron, 1979). Interestingly, patients with Cushing’s disease, an illness where excess cortisol is characteristic, show disproportionately high rates of depression (Sonino, Fava, Raffi, Boscaro, & Fallo, 1998) – a finding that supports the notion that the development of depression may be influenced by chronic dysfunction in stress systems.

Overall, the chronic unpredictable (or chronic mild) stress model in rodents seems to be most analogous model of depression because it shows the most face, etiological, and construct validity of any other chronic stress model. Importantly, it best models anhedonic-like behaviours (Wiborg, 2013) and induces long-lasting changes in behaviour, neurochemical, neuroimmune, and neuroendocrinological dysfunction observed in depressed patients (Willner, 1997, 2005). However, one of the major problems with this model (and similarly with other models that use psychological/physical stressors) is that there can be considerable individual variability in stress susceptibility which can lead to substantial differences in the endogenous CORT response (Willner, Muscat, & Papp, 1992; Willner, 1997). Therefore, in order to limit variability, exogenous CORT administration can be used to provide more stringent control over stress hormone levels, thereby producing more robust and reliable behavioural symptomology (Gregus, Wintink, Davis, & Kalynchuk, 2005; Sterner & Kalynchuk, 2010). The amount of CORT delivered can be controlled through subcutaneous injections, pellet implantation, osmotic pump infusion, or passive administration through food or drinking water (Sterner & Kalynchuk, 2010). Injections of CORT is especially appealing because it offers better control over stress hormone levels in the body than passive administration of CORT. In fact, there is considerable evidence to support exogenous administration of CORT (particularly through injection) as a robust and reliable model – with high face, construct and predictive validity – to study the role of chronic stress in developing depressive symptoms (Gregus et al., 2005; Johnson, Fournier, & Kalynchuk, 2006; Marks, Fournier, & Kalynchuk, 2009; Sterner & Kalynchuk, 2010; Wu et al., 2013; Zhao et al., 2008). However, dosage (Johnson et al., 2006) and duration (Zhao et al., 2008) of CORT treatment are critical determinants for the development and expression of a depressive phenotype. For example, high chronic CORT injections administered at a dose of 40 mg/kg for 21 days have been shown to down-regulate mineralocorticoid receptor (MR) expression and trigger a number of depressive-like behaviours in mice (Wu et al., 2013). Other changes to hippocampal dendritic and cellular morphology also correlated with depressive-like behaviour, which was most prominent in rats given CORT injections of 40mg/kg for 21 days (Lussier et al., 2013). Furthermore, with 40 mg/kg for 21 days of CORT injections in rats, depressive-like behaviour has been shown in the forced-swim test (Gregus et al., 2005; Marks et al., 2009); an effect that was independent of changes in locomotor activity or muscle strength (Marks et al., 2009). Johnson and colleagues (2006) also showed a graded increase in depressive-like behaviours with increasing doses (10, 20, or 40 mg/kg) of CORT injections. They concluded that a dose of 40 mg/kg of CORT injections for 21 d seems to be the critical dose and duration to effectively produce a depressive phenotype (Johnson et al., 2006). Moreover, chronic administration of exogenous CORT has been shown to affect other depressive-like behaviours that are indicative of anhedonia, such as increased immobility in the forced swim test, and decreased sucrose preference, grooming behaviour and affinity to respond to food reward (David et al., 2009; Gourley, Kiraly, Howell, Olausson, & Taylor, 2008; Gregus et al., 2005; Johnson et al., 2006; Sterner & Kalynchuk, 2010). Importantly, behavioural symptoms of the chronic CORT animal model of depression has been shown to be reversed with anti-depressant treatment (David et al., 2009; Iijima, Ito, Kurosu, & Chaki, 2010).

Furthermore, stress hormone has been found to modulate adult hippocampal neurogenesis. Proliferation of progenitor cells in the DG is suppressed by both acute and chronic stress in numerous species (reviewed by Mirescu & Gould, 2006). A few studies have also reported chronic high dose of CORT injections is associated with suppressed survival of new neurons (Brummelte & Galea, 2010; Wong & Herbert, 2006; Wong & Herbert, 2004; Yau et al., 2011). As previously discussed, suppressed adult hippocampal neurogenesis has been implicated in depression (Boldrini et al., 2009, 2013), which provide further evidence to support the validity of an exogenous CORT injections as a model of depression. However, it is not currently clear whether suppressed adult hippocampal neurogenesis underlies memory deficits in individual suffering from depression. Previous findings from Garthe et al. (2009) provide evidence for the importance of functional neurogenesis in cognitive flexibility. As well, clinically depressed individuals do indeed seem to show difficulties with updating, shifting and inhibiting executive function processes, which are important for mental flexibility (Airaksinen, Larsson, Lundberg, & Forsell, 2004; Austin et al., 2001; Harvey et al., 2004). Therefore, it is hypothesized that rats chronically treated with CORT, with presumably reduced level of hippocampal neurogenesis, should have cognitive flexibility deficits in comparison to control group.

Effects of Recovery from Chronic Stress

Although short-term stress is adaptive, long term stress is often damaging. It is clear that prolonged stress can alter brain structure and function, influencing the development of depression in some individuals. The damaging effects of prolonged stress produce dysregulation of systems such as the HPA axis, which can lead to impaired cognitive function. Nonetheless, animal studies have provided evidence that many effects of chronic stress on brain and cognitive functions are reversible (McEwen, 2001; McEwen, 2004). Indeed, in the human example of Cushing's disease, hippocampal atrophy has been shown to be reversed following treatment of the disorder, once cortisol levels decrease (Starkman et al., 1999).

In animal models, providing a recovery period after chronic stress (by stopping the stressful stimuli) has shown to improve a number of detriments. Structurally, for example, 21 days of chronic restraint stress followed by a 21 day recovery resulted in an overextension of proximal dendritic arbors and spine growth as well as recovery of stress-induced impairments in synaptic plasticity (Goldwater et al., 2009). Furthermore, chronic restraint-induced CA3 dendritic retractions have been shown to recover to pre-stress conditions as soon as 10 days after the cessation of stress (Conrad, LeDoux, Magariños, & McEwen, 1999). With respect to neurogenesis, recovery for 21 days following chronic unpredictable stress was shown to normalize stress-induced suppression of proliferation and apoptosis (Heine, Maslam, Zareno, Joëls, & Lucassen, 2004). Twenty-one days of recovery following chronic restraint stress has also been shown to improve short- and long-term plasticity in the DG, as indicated by improved paired-pulse response and LTP (Radahmadi, Hosseini, & Nasimi, 2014). Sousa and colleagues (2000, 1998) also showed improvement following recovery after stress-induced damage to hippocampal neurites and synapses. Specifically, CORT injections (40mg/kg) for 1 month given during the neonatal period with 5 weeks of recovery increased DG volume (Sousa et al., 1998). Additionally, 1 month of chronic unpredictable stress or CORT injections (40mg/kg) have both been shown to produce CA3 dendritic atrophy, atrophy of granule and CA1 pyramidal cells, and changes in mossy fibre terminals and significant loss of synapses (Sousa et al., 2000). A 1 month recovery period in both cases allowed for significant structural reorganization throughout the entire hippocampus and improved dendritic retractions (Sousa et al., 2000).

Behaviourally, recovery from stress can also improve cognitive impairments. Spatial memory impairments in the radial arm maze induced by 21 days of restraint stress can be reversed with pharmacological treatment with anti-seizure medication that blocks excitatory amino acid action, or antidepressants that lower extracellular serotonin (Luine, Villegas, Martinez, & McEwen, 1994). With chronic unpredictable stress or CORT injections for 1 month followed by a 1 month recovery period, Sousa et al. (2000) showed comparable water maze learning and memory performance to that of control conditions. More recently, Hoffman et al. (2011) showed working and reference memory to be impaired following 21 days of restraint stress, and given a 21 day recovery period, even significantly enhanced reference memory in comparison to controls. Similarly, previous unpublished findings of our own also suggest that even within 8 days after the end of chronic CORT injection, spatial learning in the water maze could slightly improve (Satvat, Gheidi, Lui, Dhillon, & Marrone, 2012). However, not all brain regions are associated with improvement following recovery from chronic stress. In depressive disorders, chronic stress produced hyperactivity in the amygdala (Sterner & Kalynchuk, 2010). Additionally, chronic stress produces anxiety-like behaviours and increase amygdaloid neuronal hypertrophy, which were not shown to reverse even after 21 d of recovery (Vyas, Pillai, & Chattarji, 2004).

Taken together, most studies related to recovery from chronic stress uses a rest period of at least 21 days, for which, behavioural improvements have correlated with dendritic re-branching and complexity. Interestingly, hippocampal dendritic atrophy can possibly be reversed as early as 5-10 days after the cessation of stress (Conrad et al., 1999). In light of these findings however, a short recovery period after chronic CORT injection should not reverse the expected cognitive inflexibility, if such behavioural deficit is due to hippocampal neurogenesis. This is because, in rats, it takes at least 2 weeks for newborn neurons to functionally integrate into hippocampal circuitry (Snyder, Kee, & Wojtowicz, 2001). A very short recovery period following chronic CORT injection is not long enough to allow newborn neurons that are generated following the cessation of CORT to be mature enough to be recruited during the reversal training. Therefore, it is hypothesized that rats chronically treated with CORT, with presumably reduced level of hippocampal neurogenesis, should have cognitive flexibility deficits in comparison to control group, even when the CORT treatment is terminated a few days prior to behavioural training.

Rationale

The rationale behind the present thesis project was twofold: 1) to investigate whether cognitive flexibility is affected in chronic CORT animal model of depression, and 2) whether a deficit in cognitive flexibility is the result of suppressed adult hippocampal neurogenesis. To address these objectives, two experiments were designed. In Experiment I, rats were chronically injected with CORT and, in the final days of injection, underwent a spatial learning and memory task that requires cognitive flexibility. Multiple pulses of BrdU injections given one day after the start of CORT administration allowed identification of a pool of newborn neurons of the same age. Given cognitive flexibility has been shown to be mediated, at least in part, by functional adult hippocampal neurogenesis, and chronic CORT injection has been shown to suppress adult hippocampal neurogenesis, we expected to observe deficit in cognitive flexibility in CORT-treated rats. To assess whether cognitive inflexibility induced by chronic CORT injection in Experiment I was due to suppressed hippocampal neurogenesis, we carried out a second experiment that was similar to Experiment I in all respects, except that CORT and vehicle administration were terminated 3 days prior to behavioural testing. If the cognitive inflexibility seen in Experiment I was, in fact, due to suppressed adult hippocampal neurogenesis, a short rest period prior to the behavioural task in Experiment II should not be sufficient to ameliorate any cognitive flexibility deficits in CORT-treated rats. This is because a three-day rest period would not be long enough for new neurons generated after the CORT treatment to become functionally incorporated into the DG circuitry to be able to contribute to behaviour.

Experimental questions

1. Does this chronic exogenous CORT-injection model of depression affect spatial cognitive flexibility?
2. Are changes to cognitive flexibility the result of a suppression of adult hippocampal neurogenesis?

Hypotheses

Based on previous research, a pharmacological suppression of hippocampal neurogenesis can produce specific deficits in cognitive flexibility. Therefore, since chronic CORT potently suppresses neurogenesis, we hypothesize that, in Experiment I, the chronic stress model of depression used in the present study will produce a deficit in reversal training, a task that is used to assess cognitive flexibility. Furthermore, we hypothesized that any behavioural inflexibility seen in Experiment I should still persist in Experiment II if the suppression of adult hippocampal neurogenesis was the underlying factor to produce such cognitive inflexibility.

Method

Animals

Three months old, male Sprague-Dawley (SD) rats (Harlan, USA) were single-housed in standard shoe-box cages on a 12:12 h light/dark cycle (lights on at 07:00 h). Standard rat chow and water were provided *ad libitum*. All rats were left to acclimate to the laboratory conditions for 1 week after being received, and were gently handled daily for 1 week thereafter prior to beginning experimental testing. Body weights were measured daily in the morning at approximately the same time each day. All procedure followed the Canadian Council on Animal Care guidelines, and was approved by the University of Waterloo's Animal Care Committee.

Drug Administration

All rats received 50 mg/kg bromodeoryuridine (BrdU; Sigma-Aldrich) dissolved in 37°C 0.9% physiological saline. BrdU injections were delivered intraperitoneally in a volume of 2 ml/kg body weight, four times in one day, each injection being separated by a 2.5 h interval. In each experiment, animals were randomly assigned to receive either subcutaneous injections of 40 mg/kg corticosterone (CORT, Sigma-Aldrich) or vehicle (VEH). All drug administration was done at approximately the same time each day, starting at 10:00 h. CORT was dissolved in 37°C, 35% w/v 2-hydroxylpropyl-beta-cyclodextrin (2-HβC) and delivered in a volume of 1.25 ml/kg body weight. A similar volume of 2-HβC was used for control groups. Cyclodextrins are cyclic oligosaccharide compounds with a rigid doughnut-shaped structure, well suited as a complexing agent; the β form of which is particularly useful for solubilizing and stabilizing hormones and other molecules of similar size (Stella & He, 2008).

Morris Water Maze (MWM) Swim Task

All behavioural testing was done at approximately the same time of day for each day of testing. A circular pool (153 cm in diameter) was filled with 23 ± 1°C water, made opaque with dark non-toxic tempera paint. Tracking software (EthoVision XT Video Tracking System, Noldus Information Technology) was used to monitor each rats' movement in the water maze using a ceiling-mounted camera. A hidden platform (20 cm in diameter) was located in the centre of the North-East quadrant of the pool, 2 cm under the surface of the water for the first three days of training. Spatial training consisted of 1 block of 4 trials per day (separated by a 30 sec inter-trial interval), over three consecutive days. Prior to the first trial of the first day of training, rats were placed on the hidden platform for 20 sec to acclimate the rats to the pool environment and to reduce their anxiety. Each trial began by placing the rats in the pool facing the wall in a pseudo-random starting location (North, East, South, or West). If rats were unable to find the hidden platform within 60 sec, the experimenter manually guided the rat through the water to the platform where the rat remained for 20 sec.

To assess cognitive flexibility, reversal training was utilized by placing the hidden platform in the opposite (Southwest) quadrant of the pool on fourth and fifth day. Rats were once again given a pseudo-random location (North, East, South, or West) to start each trial. The rats were given two consecutive days of training of the reversal protocol and the probe test was administered on day 6. The probe test involved the removal of the platform from the pool 24 h after the final reversal training, to assess memory retention. The probe was a single 60 sec trial test to assess if the rats spent significantly more time swimming in the quadrant that previously contained the platform from the reversal training (Southwest).

Procedures

Experiment I: chronic administration of corticosterone.

Following a week of handling, eleven rats were injected subcutaneously once per day for 22 days with either CORT (n=6) or VEH (n=5) with the dosages described above. All rats received injections of BrdU, one day following the first administration of CORT or VEH. CORT and VEH injections continued during the subsequent water maze testing, for which injections were administered each day, post-training, until the last day of reversal training. Figure 3 depicts a visual representation of the timeline of Experiment I.

Experiment II: chronic corticosterone followed by a short rest period.

Following a week of handling, twelve rats were randomized to receive subcutaneous injection of either 40 mg/kg CORT (n=6; CORT-rest) or VEH (n=6; VEH-rest) once per day for 14 days. Similar to Experiment I, all rats received injections of BrdU, one day following the first administration of CORT or VEH. Drug treatment terminated three days preceding behavioural testing in the Morris water maze. Figure 4 depicts a visual representation of the timeline of Experiment II.

Histology

In both experiments, thirty min following the probe test, rats were anesthetised by isoflurane and were sacrificed by decapitation. Brains were rapidly removed, and hemispheres carefully separated and flash-frozen in a small container of isopentane bathed in a mixture of dry ice and 90% ethanol. Seven to ten right hemisections were mounted with optimal cutting temperature (OCT) compound (Fisher Scientific, Whitby, ON) such that at least one treatment group from each experiment (CORT, VEH, CORT-rest or VEH-rest) was present on each slide. Coronal sections (20 µm) were obtained with a cryostat (Leica) at -20 °C, through the entire dorsal hippocampus, thaw-mounted on Superfrost Plus slides (VWR), dried, and stored at –80°C.

Immunofluorescence staining

For each rat, 12 sections from the dorsal hippocampus [bregma -3.30 to -4.52 mm (Paxinos & Watson, 1986)] were randomly chosen. Sections were labeled for neuronal nuclear antigen (NeuN) and BrdU by method previously described (Ramirez-Amaya, Marrone, Gage, Worley, & Barnes, 2006). Briefly, tissue sections were fixed in a 2% formaldehyde solution for 8 min. The tissue sections were then rinsed three times with 0.1M tris-buffered saline (TBS) and quenched in TBS with 20% menthanol and 0.6% H_2O_2 for 20 min. Tissue was again rinsed with TBS before being blocked for 30 min with tyramide single amplification (TSA) blocking buffer (PerkinElmer, Boston, MA), after which it was incubated overnight at 4 °C with biotinylated mouse anti-NeuN antibody (1:2000; Millipore, Temecula, CA). The following day, tissue were washed once with TBS with 0.05% Tween 20 (TBS-T), then with TBS, and treated with VECTASTAIN ABC kit (Vector Labs, Burlington, ON) for 30 min. Following three washes with TBS, Coumarin fluorophore (PerkinElmer) was applied for 40 min for visualization of NeuN-labeled cells. Sections were then washed with TBS-T and TBS, quenched with 1% H_2O_2 in saline sodium citrate (SSC) buffer, washed again with TBS, and blocked with mouse-on mouse (M.O.M.) blocking reagent (Vector Labs) for 1 h. After being washed with TBS and SSC buffer, tissue sections were incubated in 50% formamide in SSC buffer at 65°C for 2 h, and treated with 2N HCl for 30 min at 37°C to denature DNA. Tissues were then neutralized with 0.1M boric acid, pH 8.5 for 5 min. After several washes in TBS, tissue sections were incubated with monoclonal mouse anti-BrdU (1:100, Roche Applied Sciences, Montreal, PQ) overnight at 4°C. Following three TBS washes, tissue sections were blocked with avidin/biotin blocking kit (Vector Labs), prior to incubation with biotinylated anti-mouse (1:200, Vector Labs) for 2 h at room temperature. After 3 more washes with TBS, fluorescein (TSA kit labelling, PerkinElmer) was used for 10 min to visualize BrdU-labeled cells. Lastly, the slides were washed with TBS, coverslipped with Vectashield mounting medium (Vector Labs), and sealed with nail polish the following day.

Image Acquisition and Cellular Data Collection

Microscopy and cell counts were done blind. Z-stacked images were collected using the 20x objective lens of an inverted fluorescent microscope (Carl Zeiss Microscopy, Canada). Image parameters (ex. exposure time, light intensity and slice distance) were kept constant for each slide. The mosaic feature of the microscope allowed reconstruction of the entire DG. Using ZEN 2012 SP2 software, the area of the suprapyramidal (DG_{SP}) and infrapyramidal (DG_{IP}) blades were measured for each section and the new granule cells co-labelled with both BdrU and NeuN were counted on each blade. To estimate the volume of each blade, the area was multiplied by the tissue thickness (20 µm). To obtain the density of newborn neurons for each blade, the number of new neurons on each blade was divided by the estimated volume (Cai et al., 2012). Finally, the newborn neurons' density of the DG_{SP} and DG_{IP} were added to calculate the newborn neuron density for both blades (total density).

Statistical Analysis

Data analyses were done separately for each experiment, and all analyses were performed using IBM SPSS statistical software. Body weight data was analyzed using an independent-samples t-test to compare the area under the curve (AUC) of the body weight over the entire experiment, plus three days of baseline body weights, for the CORT and vehicle-treated rats. Separate independent-samples t-tests were used to compare the mean velocity of the two treatment groups on each day of the water maze task. Mixed measures ANOVAs were used to analyze learning during the acquisition and reversal training of the MWM as measured by distance traveled and latency to reach the platform. For all mixed measure ANOVAs performed, the within-subjects factor was days (with 3 levels for acquisition training and 2 levels for reversal training), and the between-subject factor was the treatment conditions (2 levels: CORT vs. VEH in Experiment I, and CORT-rest vs. VEH-rest in Experiment II). If a significant interaction effect was found, independent-samples t-test was run for each day to assess where the differences may lie. The effect size correlation was then calculated using group means and pooled standard deviations. The probe trial data for each experiment was analyzed using a one-sample t-test. This test compared the percent of time spent in each quadrant for each treatment group against the test level of 25% (the percent of time to swim in any given quadrant by chance). An independent-samples t-test was used thereafter to assess any differences there may be between groups in each quadrant.

The cell density data for each experiment was separately analyzed. An independent-samples t-test was first used to compare total cell densities between the two groups. In addition, to compare newborn neurons' density of the two blades, mixed measures ANOVAs were performed, in which the within-subjects factor was the blade (2 levels: infra- vs. supra-pyramidal blades), and the between-subjects factor was the treatment (2 levels: CORT vs. VEH in Experiment I and CORT-rest vs. VEH-rest, in Experiment II). The level of statistical significance was set at $p \leq 0.05$.

Results

Experiment I: Chronic Administration of Corticosterone

Body weight.

Body weight data was represented by the area under the curve (AUC) for both CORT and vehicle-treated rats (Figure 5, Table 1). The independent-samples t-test comparing the AUC revealed a significant difference between groups, $t(9)=2.768$, $p=0.022$. As shown in Figure 5, body weights in the CORT rats were found to significantly decline over time. These data were taken to suggest that the chronic administration of corticosterone were effective (Jeong, Lee, & Kang, 2013).

Morris Water Maze task.

Velocity.

Independent-samples t-test for the mean velocity (cm/s) for each of the five training days found no significant differences between CORT and VEH rats, as shown in Figure 6 and Table 2. Therefore, motor impairment was not considered to be a factor that contributed to any differences found in distance traveled and latency between the two groups.

Acquisition Training.

The 2 x 3 mixed measures ANOVA on distance traveled over the 3 days of spatial training resulted in a significant main effect of day, $F(2, 18)=27.883$, $p\leq0.0001$. However, there was no significant main effect of treatment. Across all days of training, the distance traveled to reach the hidden platform decreased regardless of treatment, suggesting that all rats learned the location of the platform equally well (Figure 7A, Figure 8A, Table 3).

Similarly, a significant day effect for latency to reach the platform was found over the three days of spatial training, $F(2, 18)=25.907$, $p\leq0.0001$. There was no significant main effect of treatment on latency (Figure 7B, Figure 8B, Table 3). Figure 7B shows that, across all three days of acquisition training, both groups of rats took a similar amount of time to learn the location of the hidden platform.

Reversal Training.

To assess cognitive flexibility, a reversal task was performed on the fourth and fifth days of spatial training. A 2 x 2 mixed measures ANOVA of the distance traveled over the two days of reversal training found a significant day effect, F(1, 9)=13.693, p=0.005, and a significant day by treatment interaction, F(1, 9)=5.532, p=0.043 (Figure 7A, Figure 8A). No significant main effect of treatment was observed over the two days. However, to better understand the nature of this interaction, independent-samples t-tests were done to compare distance traveled between the two groups on each day of the reversal training. Although, no significant difference in distance traveled was found between the two groups for either day 4 or 5, a fairly substantive effect size was found on the first day of reversal training r=-0.470 (Table 3).

A 2 x 2 mixed ANOVA was also performed on the latency data and found a significant main effect of day, F(1,9)=15.220, p=0.004, and interaction between days and treatment, F(1, 9)=5.943, p=0.037. No significant main effect of treatment was observed. Independent-samples t-tests of latency between the two groups on each day of reversal training revealed a significant difference between the two groups on day 4 only, t(9)= 2.440, p=0.037, with a considerably large calculated effect size r=-0.588 (Figure 7B, Figure 8B, Table 3). Taken together these results indicate that CORT treated rats had no difficulty learning the initial location of a platform, although showed a deficit when the location of the platform was changed. However, by the second day of reversal training, performance of the CORT-treated rats was comparable to that of the vehicle-treated rats.

Probe Test.

To assess memory retention from the reversal task, a probe trial was given 24 h after the final reversal training day. A one-sample t-test was used to assess the percent of time spent in each quadrant compared with the chance level of randomly searching any given quadrant (i.e. 25%). This analysis revealed that the CORT group showed a preference for the target quadrant, as seen by a significantly higher percentage of time spent in that quadrant compared to chance levels, t(5)=3.961, p=0.011 (Figure 9, Table 4). The control group showed no significant preference above chance for the target quadrant (i.e. SW; the quadrant that held the hidden platform during the reversal trial). The CORT group spent a significant percentage of time below the level of chance in the opposite (NE) quadrant, where the platform was located during the initial acquisition, t(5)=-6.370, p=0.001, and NW quadrant, t(5)=-3.798, p=0.013, although not the SE quadrant. The vehicle group did not spend a significantly different percentage of time from chance in any quadrant except in the NW quadrant t(4)=-2.864, p=0.046. Furthermore, an independent-samples t-test comparing the CORT and VEH groups in each quadrant revealed that there was a significant difference in the percent of time spent in the target quadrant between the two groups t(9)=-2.442, p=0.037.

Quantification of adult hippocampal neurogenesis.

An independent-samples t-test was used to compare total density (both DG_{SP} and DG_{IP} blades) of new neurons in the DG and found no significant differences between the two groups (Figure 10). A 2 x 2 mixed ANOVA in which blades was the within-subjects factor, and treatment was the between-subjects factor, showed a significant main effect of blade only, F(1, 9)=10.274, p=0.011. Previous studies have also shown higher density of newborn neurons in the infra- than in the suprapyramidal blade of the dentate gyrus (Ramirez-Amaya et al., 2006; Satvat, Gheidi, Voll, Odintsova, & Marrone, 2012). These data are summarized in Table 5.

Experiment II: Chronic Corticosterone Followed by a Short Rest Period

Body weight.

Body weight data was represented by the area under the curve (AUC) for both CORT-rest and VEH-rest rats (Figure 11, Table 1). The independent-samples t-test comparing the AUC revealed a significant difference between groups, t(10)=5.904, p≤0.001. As shown in Figure 11, body weights of CORT-rest group were found to be significantly less than control (VEH-rest) group, taken to suggest that the chronic administration of corticosterone were effective (ex. Jeong, Lee, & Kang, 2013).

Morris Water Maze task.

Velocity.

Independent-samples t-tests for the mean velocity (cm/s) for each of the five training days found no significant differences between CORT-rest and VEH-rest groups, as shown in Figure 12 and Table 2. Thus motor deficits did not significantly contribute to any differences found in distance traveled and latency between the two groups.

Spatial training.

The 2 x 3 mixed measures ANOVA of the distance traveled found a significant main effect of day, F(2, 20)=10.465, p=0.001, and treatment, F(1, 10)=11.108, p=0.008 (Figure 13A, Figure 14A, Table 3). Similarly, the mixed measures ANOVA on latency to reach the platform (Figure 13B, Figure 14B, Table 3) showed a significant effect of day, F(2, 20)=11.069, p=0.001, and treatment, F(1, 10)=12.762, p=0.005. Taken together, these results suggest that both groups learned the location of the hidden platform over the 3 days of spatial training. However, the CORT-rest group learned the location of the hidden platform significantly faster and traveled significantly less distance to reach the platform than the VEH-rest group as shown in Figures 13A and 13B.

Reversal training.

Similar to Experiment I, to assess cognitive flexibility, a reversal task was performed on day 4 and 5 of water maze task. A 2 x 2 mixed ANOVA for distance traveled revealed no significant effects of day or treatment (Figure 13A, Figure 14A, Table 3). The planned independent-samples t-test comparison for day 4 revealed no significant difference between groups in distance traveled. Similarly the 2 x 2 ANOVA performed on latency data did not find significant effects of day or treatment (Figure 13B, Figure 14B, Table 3). The planned independent-samples t-test for day 4 also revealed no significant difference between groups in latency to reach the platform.

Probe test.

The percent of time spent in each quadrant compared against the level of chance to randomly search any given quadrant (25%) for the probe trial was analyzed using a one-sample t-test for each group. This analysis revealed that the CORT-rest group spent a significantly lower percentage of time swimming in the NW quadrant t(5)=-3.438, p=0.018. No preference above the level of chance was seen in any other quadrant for either group (Figure 15, Table 4). An independent-samples t-test comparing the CORT-rest and VEH-rest groups in each quadrant revealed no significant differences between groups in any quadrant.

Quantification of adult hippocampal neurogenesis.

An independent-samples t-test was used to compare total density (both DG_{SP} and DG_{IP} blades) of new neurons in the DG and found no significant differences between the two groups (Figure 16, Table 6). A 2 x 2 mixed measures ANOVA, in which blades was the within-subjects factor, and treatment was the between-subjects factor, showed a significant main effect of blade only, F(1, 10)=75.202, p=0.001. This effect of blade was not surprising since previous studies have also shown higher density of newborn neurons in the infra- than in the suprapyramidal blade of the dentate gyrus (Ramirez-Amaya et al., 2006; Satvat et al., 2012).

Discussion

High doses of prolonged CORT exposure has been shown to induce depressive-like symptomology, and shows good face, construct, and predictive validity as a rodent model of stress-induced depression, as it offers better control over the internal stress hormone state (Sterner & Kalynchuk, 2010). Thus, a chronic CORT injection animal model was used to address two main objectives: 1) whether this animal model of depression affects cognitive flexibility, and 2) whether changes to cognitive flexibility are the result of suppressed adult hippocampal neurogenesis. To address these objectives, two comparable experiments were designed. In both experiments, rats were randomized to receive either CORT or vehicle injections. Newborn neurons were labelled with BrdU, and 17 days following the first treatment, rats were subjected to water maze training. In the first experiment, treatment continued throughout behavioural assessment, where injections were administered post-water maze training. While initial acquisition learning in the water maze was not affected in CORT-treated animals, reversal learning, used as a measure of cognitive flexibility, was significantly impaired in the CORT-treated rats. In a similar second experiment, CORT and vehicle injections were terminated 3 days prior to beginning of water maze testing. We hypothesized that if suppressed adult hippocampal neurogenesis underlies cognitive inflexibility as suggested by Burghardt et al. (2012) and Garthe et al. (2009), then rats given CORT injections which is terminated 3 days before behavioural testing should still have persistent cognitive inflexibility. The behavioural results of this second experiment revealed enhanced performance of the CORT-treated rats during initial spatial acquisition in the water maze, as well as comparable performance to the control group in cognitive flexibility measured by reversal training.

Immunofluorescence analyses of newborn DG neurons revealed chronic CORT administration did not influence the survival of newly born neurons in either experiment, irrespective of a rest period. Taken together, these observations suggest that cognitive inflexibility is evident in an animal model of depression using chronic CORT injections. However, such cognitive deficit is unlikely to be the results of suppressed hippocampal neurogenesis. Other forms of neuroplasticity, unrelated to hippocampal neurogenesis, may underlie cognitive inflexibility in Experiment I and superior performance of the CORT-treated rats in spatial learning in Experiment II. These results will be discussed in light of previous related research.

Spatial Learning

The dose and duration of CORT administration used in the present study has been repeatedly shown to produce a depressive-like phenotype in rodents, and as such, this protocol is considered an animal model of stress-induced depression (Gregus et al., 2005; Johnson et al., 2006; Lussier et al., 2013; Marks et al., 2009; Sterner & Kalynchuk, 2010). Although we did not specifically assess levels of circulating CORT in the animals in the present study, the attenuated weight gain of the CORT group can be a good proxy for indicating the effectiveness of long-term CORT treatment in male rats (Conrad et al., 2007), and many other investigators have used it as such (ex. Coburn-Litvak, Pothakos, Tata, McCloskey, & Anderson, 2003; Conrad et al., 2007; Jeong et al., 2013; McLay, Freeman, & Zadina, 1998). Consistent with the body weight data seen in the present study (refer to Table 1, Figure 5 and 11), rodents subjected to chronic stress have also been consistently shown to have attenuated weight gain (Dallman et al., 2003; Jeong et al., 2013; Strack, Sebastian, Schwartz, & Dallman, 1995).

The results of Experiment I showed that chronic CORT administration specifically impaired cognitive flexibility in the Morris water maze without affecting initial acquisition of spatial learning. As seen in Figure 7, both groups of rats learned the location of the hidden platform equally well during the first three days of training. Interestingly, however, CORT-treated rats in Experiment II, in which CORT injection ceased only 3 days before spatial training, outperformed the vehicle treated rats during initial learning, as seen in Figure 13.

In regards to the effects of chronic stress and exogenous CORT administration on spatial learning and memory, contradictory results have been reported. Using chronic CORT administration (Coburn-Litvak, Pothakos, Tata, McCloskey, & Anderson, 2003; Darcet et al., 2014; Lee, Sur, Shim, Lee, & Hahm, 2014; McLay, Freeman, & Zadina, 1998) and a variety of other stress protocols, a majority of the literature report that chronic stress can produce spatial learning and memory deficits, with concomitant remodelling in the hippocampus that support the notion that remodelling is involved in producing learning deficits (reviewed by Conrad, 2010; Sandi & Pinelo-Nava, 2007). However, not all studies have reported such a deficit from chronic stress (Bodnoff et al., 1995; Cerqueira et al., 2005; Conrad et al., 2007; Hebda-Bauer, Morano, & Therrien, 1999; Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000). One study even found a moderate improvement in water maze learning and memory in the CORT-treated rats (Workman, Chan, & Galea, 2015). Chronic stress produces changes in hippocampal morphology, such as retractions of apical dendrites in the CA3 (Magariños & McEwen, 1995; McLaughlin, Baran, & Conrad, 2009; McLaughlin, Gomez, Baran, & Conrad, 2007; Watanabe, Gould, & McEwen, 1992), and modifications to hippocampal dendritic spine density, shape, and synaptic terminal structure (Magariños, Verdugo, & McEwen, 1997; Sunanda, Rao, & Raju, 1995). Such structural changes are thought to contribute to the impairment in hippocampal-dependent learning and memory seen following chronic stress (McLaughlin et al., 2009). It has also been suggested that chronic stress-induced remodelling results in a vulnerability of the hippocampal structure, which may lead to inadequate spatial learning and memory, as opposed to a direct impairment (Conrad, 2006). This may explain some of the anomalous findings in the literature (Conrad, 2006). For example, even when stress conditions produce CA3 dendritic retractions, behavioural impairments in hippocampal-dependent learning are not always found (Conrad et al., 2007). Others have also suggested that exogenous CORT affects the hippocampus differently than other types of chronic stressors with a psychological/physical component (such as restraint or chronic unpredictable stress). For instance, Sousa et al. (2000) found differences in spatial learning of rats exposed to 1 month of chronic unpredictable stress compared to CORT injections (40 mg/kg). Interestingly, unlike rats exposed to chronic unpredictable stress, CORT-treated rats did not show a significant impairment in MWM learning, which was concomitant with milder degenerative changes in hippocampal morphology as compared to chronic unpredictable stress. Exogenous CORT has also been shown to significantly impair memory in the Barnes maze, although deficits in the MWM were far less profound (McLay et al., 1998). Furthermore, exogenous CORT has been suggested to affect HPA axis function differently than other psychological stressors (Conrad, 2006; Wright, Lightner, Harman, Meijer, & Conrad, 2006). Exogenous CORT asserts powerful negative feedback action on the HPA axis, thereby inhibiting HPA activity (Magariños, Orchinik, & McEwen, 1998), whereas psychological/physical stressors act by increasing adrenal weight (Watanabe et al., 1992), and induce hypersecretion of endogenous CORT (Wright et al., 2006). This over-reactive HPA response from psychological stressors, in conjunction with long-term changes to hippocampal morphology (that occurs in both exogenous CORT and psychological stressors), has been proposed as the mechanism by which chronic stress impairs spatial learning and memory (Wright et al., 2006). The notion that exogenous CORT inhibits HPA activity (rather than producing an over-reaction of HPA activity) may explain why CORT-treated rats in Experiment I performed on par with control animals.

Another possible mechanism to explain the comparable water maze acquisition of the two groups of rats in the first experiment may relate to the involvement of glucocorticoid (GR) and mineralocorticoid (MR) receptors in the hippocampus. There is evidence to suggest that dysregulation of GRs and/or MRs in the hippocampus and other limbic structures can disrupt the negative feedback regulation of the HPA axis; this may be involved in the pathogenesis of depression (de Kloet, Joëls, & Holsboer, 2005; Gregus et al., 2005; López, Chalmers, Little, & Watson, 1998; Wu et al., 2013). Previous work has shown that chronic high doses of CORT can downregulate the expression of MRs in the hippocampus, while leaving GR levels relatively intact (Wu et al., 2013; Xu et al., 2011). This pattern has similarly been seen in non-medicated suicide victims with a history of depression (López et al., 1998). A number of works by Oitzl and de Kloet (de Kloet, Oitzl, & Joëls, 1999; Oitzl, de Kloet, Joëls, Schmid, & Cole, 1997; Oitzl & de Kloet, 1992; Oitzl, Fluttert, & de Kloet, 1994) have elucidated the role that these corticosteroid receptors play in learning and memory. MRs have been implicated in modulating behavioural reactivity (i.e. interpreting environmental stimuli and choosing appropriate behavioural responses), while GRs are more involved in consolidating learned information. In the MWM, when MRs are downregulated, behavioural reactivity is disrupted, as seen through altered exploratory behaviour and search-escape strategies. Downregulation of GRs correlates with increased escape latencies in the MWM; however, a downregulation of MRs change behavioural reactivity, which does not necessarily increase escape latencies. Altered behavioural reactivity does not substantiate a reduced ability to consolidate information. Although the present research did not explore this type of mechanism, given the present behavioural findings, it would be a topic of interest for future research. The lack of treatment differences in acquisition training in the first experiment may suggest that such a regime of chronic exogenous CORT can alter learning behaviour through the downregulation of MRs (rather than GRs) resulting in no learning deficit. Further investigation into this matter can better inform how cognition is altered in pathology related to chronic stress induced depression.

All of the chronic stress studies discussed above typically use a 21-day stress regimen or more. However, contradictory results have also been reported in regards to chronic stress exposure for less than the typical 21 days. For example, McLaughlin and colleagues (2007) assessed different intensities (2 or 6 h of restraint stress) and durations (10 or 21 days) of stress in relation to hippocampal morphology, and spatial learning and memory. They found that 21 days of high stress was the only stress regime to produced behavioural deficits in the Y-maze and CA3 dendritic retractions; but less intense stress or a shorter chronic stress protocol did not differ significantly from controls. Similarly, in another study, chronic restraint and unpredictable stress for 14 days did not show perturbed CA3c (the area of the CA3 closest to the DG hilus) dendritic arbors, although adrenocortical activation was altered (Magariños & McEwen, 1995). By contrast, other groups have found significant CA3 dendritic retractions (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002) and impaired learning and memory in behavioural tasks such as the water maze (Moosavi, Naghdi, Maghsoudi, & Zahedi Asl, 2007; Radecki, Brown, Martinez, & Teyler, 2005) with shorter periods (10 and 7 days of stress, respectively) and less intense (restraint stress for 2 h/day) chronic stress. An enhancement in learning in the radial arm maze following 13 days of stress (Luine, Martinez, Villegas, María Magariños, & McEwen, 1996) or in the MWM following 10 days of stress (Gouirand & Matuszewich, 2005) have also been reported. Clearly, there is no consensus on whether stress for less than the typical 21-days cause overt perturbations in learning and memory, and the differences in the literature may relate to differences in behavioural and structural assessment procedures, the type of stress given, and individual variability in animals' stress response.

Notably, in the aforementioned studies, no recovery period were incorporated between termination of the stressors and the learning tasks. A few studies have shown that a recovery period from stress can enhance learning (Sousa et al., 2000) or memory (Hoffman et al., 2011). However, in both these studies, the recovery period lasted for as long as the duration of chronic stress (1 month and 21 days, respectively). Another study used restraint stress for 21 days with a shorter (1 week) rest period and found impaired learning of the stress-rested animals in the MWM, although memory retention in the probe task was unaltered (Kasar, Mengi, Yildirim, & Yurdakos, 2009). Indeed, at the cellular level, CA3 dendritic retractions produced by chronic restraint stress for 21 days have been shown to recover to pre-stress conditions between 5 and 10 days after cessation of stress (Conrad, LeDoux, Magariños, & McEwen, 1999). Interestingly, yet another study found that an even shorter rest period (3 days) following chronic constant light stress slightly enhanced learning in the MWM during the first two days of acquisition training (Ma et al., 2007). Constant light stress may be consider to be a comparatively mild stressor, which may have a different influence on learning and memory than more intense stressors like. However, given the findings from Conrad et al. (1999) and Ma et al. (2007), there may be other ancillary factors aside from dendritic alterations, at least in the hippocampus, that may contribute to the impact of stress (enhancement or impairment) on learning and memory over short timescale (ex. 3 days) of rest. Furthermore, in a recent study that used similar animal model of depression as the present study, rats were assessed in the MWM one day following the last chronic CORT treatment, where a modest enhancement of spatial learning was also observed in the CORT-treated rats (Workman et al., 2015).

In the present study, the results of Experiment II in regards to MWM acquisition training showed that a short rest period before spatial training enhanced spatial acquisition. Both the latency and the distance traveled to the hidden platform were significantly better in the CORT-treated rats in comparison to the control group. A mechanism for this, initially proposed by Hoffman et al. (2011), could be that stress-enhanced learning involves the modulation of hippocampal-dependent spatial learning and memory by the amygdala (Akirav, Sandi, & Richter-Levin, 2001; Kim, Lee, Han, & Packard, 2001). Learning tasks such as the water maze are aversive to rodents, and the amygdala is engaged when animals perform well (Akirav & Richter-Levin, 1999), an effect that is partially mediated by CORT hormone (Akirav & Richter-Levin, 2002). Furthermore, alterations to dendritic structure in communicating brain areas during stress can influence the effect on learning. For example, chronic restraint stress for 10 days has shown to produce hypertrophy of dendrites in the basolateral region of the amygdala, which persist even after 21 days of recovery (Vyas et al., 2004). However, 21 days after stress, dendritic retractions in the CA3 region of the hippocampus can recover (Hoffman et al., 2011; Sousa et al., 2000; Vyas et al., 2004). As Hoffman and colleagues (2011) suggested, this hypertrophied amygdala may form strengthened connections with reformed dendrites of the hippocampus, thereby facilitating spatial learning and memory, and this effect may, in part, be mediated by CORT. Although the present experiment did not use a stress (21 days of stress) and rest (21 days of rest) regime like Hoffman and colleagues, a similar mechanism may explain the enhanced acquisition of learning seen in CORT-treated rats in Experiment II. As previously discussed, it is unclear if a 14-day stress regimen can produce CA3 dendritic retractions (compare, for example, Magariños

& McEwen, 1995; McLaughlin et al., 2007, who used 14 and 10 day stress respectively, with Vyas et al., 2002 who used 10 day stress). Even still, with a longer (21-day) stress regimen, CA3 dendritic atrophy can recover as soon as 5 – 10 days (Conrad et al., 1999). Although CA3 dendritic retractions was not specifically assessed in the present study, considering the previous literature, CA3 dendritic retractions may not have been present, and if they were, they most likely recovered, at least partially, within the 3 day rest period in Experiment II. This in combination with hypertrophy of the amygdala may have facilitated initial acquisition of learning seen in Experiment II.

Cognitive Flexibility and Probe Tests

Although, CORT-treated rats in the first experiment did not show spatial learning deficit, a deficit in cognitive flexibility, measured by their poor performance in the reversal task was observed (Figure 7). By the second day of reversal training, both groups of rats learned the new location of the hidden platform equally well. Vorhees & Williams (2006) have suggested that, under normal conditions, rats in the water maze can rapidly switch search strategies to find a new goal position during reversal learning, a task that requires cognitive flexibility. They suggest that this change can be so fast that an average of learning across a single day of training may not show preserved return visits to the original platform location above the level of chance. Reversal learning deficit has also been reported in a few studies that used chronic stress paradigms (Cerqueira et al., 2005; Darcet et al., 2014; Hill et al., 2005; Yu, Zhang, Chen, & Zhang, 2015). For example, Hill et al. (2005) found that, in rats, chronic unpredictable stress given for 21 days did not influence the acquisition of learning in the MWM, but impaired subsequent reversal learning. They found that the increase in escape latency in the reversal task in chronically stressed animals was due to perseveratory behaviour: stressed animals spent more time searching in the original training quadrant and were more reluctant to extinguish the initially learned behaviour. Interestingly, data from human studies also suggest impairment in cognitive flexibility in those who have highly stressful jobs (Ohira et al., 2011). Furthermore, a correlation between cognitive flexibility and mood has been suggested in both rats (Bessa et al., 2009) and humans (Fossati, Ergis, & Allilaire, 2002).

Although deficits in cognitive flexibility have often been linked with dysregulation in the prefrontal cortex (Cerqueira et al., 2005; Dalley, Cardinal, & Robbins, 2004; Ragozzino, Detrick, & Kesner, 1999), animals with hippocampal lesions have also been found to have impaired cognitive flexibility (Ramos, 2010), which suggest that multiple brain regions are involved. Interestingly, many of the regions required for appropriate flexible learning, such as the hippocampus, prefrontal cortex and striatum, are disrupted in stress-related mood disorders such as depression and anxiety (Anisman & Matheson, 2005; Beck, Brown, Steer, Eidelson, & Riskind, 1987; Philippe Fossati, Amar, Raoux, Ergis, & Allilaire, 1999). In light of recent experimental findings that suggest a role for hippocampal neurogenesis in cognitive flexibility (Burghardt et al., 2012; Garthe et al., 2009, 2014; Kleinknecht et al., 2012), we hypothesized that CORT-treated rats would perform poorly in the reversal training, given that chronic CORT treatment has shown to have detrimental effects on hippocampal neurogenesis (Brummelte & Galea, 2010; Wong & Herbert, 2006; Wong & Herbert, 2004; Yau et al., 2011). In fact, Sahay, Wilson, and Hen (2011) also suggested that the hippocampus, and in particular, the DG, plays an important role in flexible learning, and disruption in this area are associated with stress-related pathologies like anxiety and depression. They outlined that there is a normal adaptive response to changing environments that involves a balance between pattern separation (the ability to discriminate similar contexts) and pattern completion (the ability to reconstruct a context from a partial cue, or “completing the pattern”). Furthermore, this action seems to be regulated by neurogenesis, which is influenced by changes in the environment. Prolonged exposure to hostile environments (such as during stress, in poor aging, or with sensory deprivation) decrease neurogenesis, leading to diminished pattern separation, which, behaviourally, can manifest as an impairment in discriminating similar contexts, leading to overgeneralization. Generalization due to decreased pattern separation may be advantageous because it favours avoidance of potentially dangerous situations. However, when these adaptive responses become maladaptive, impaired pattern separation can lead to pathologies such as anxiety disorders and depression. Indeed, poor pattern separation and cognitive inflexibility have been correlated with people who have depression and anxiety disorders (Déry et al., 2013; Kheirbek, Klemenhagen, Sahay, & Hen, 2012; Leal, Tighe, & Yassa, 2014; Shelton & Kirwan, 2013). Cognitive flexibility in the context of reversal training in the water maze is a form of pattern separation (Garthe et al., 2009), where the animal is required to discriminate the new location of a platform from a previous location, within the same context (i.e. water maze with similar distal environmental cues). Impaired behavioural pattern separation performance in older adults is associated with higher activity of the CA3 (Yassa et al., 2011), and similar results have been reported in aged animals (Wilson, Ikonen, Gallagher, Eichenbaum, & Tanila, 2005). Thus, it has been postulated that disrupted pattern separation, increases activity of the CA3, leading to excessive pattern completion (Yassa et al., 2011). However, similar studies have yet to be done in individuals with clinical depression to investigating the ‘pattern separation’ and ‘pattern completion’ shift with respect to activity in hippocampal subregions like the CA3 and DG. Few studies have used fMRI analysis to assess hippocampal sub-field activity in depressed individuals, and disparate findings have been reported. For example, a study by Werner et al., (2009) did not find any differences in hippocampal activity during an associative learning task in patients with depression compared to matched controls. However, other studies have found depressed individuals to have dysregulation in hippocampal activation during encoding of an associative memory task (Fairhall, Sharma, Magnusson, & Murphy, 2010), as well as attenuated right hippocampal activity during memory recall (Milne, MacQueen, & Hall, 2012). However, with the evidence currently available in the literature, it is not yet known how the hippocampal sub-fields (in particular, the CA3 region and DG) respond in depressed individuals when encoding or consolidating pattern separation-related information, or when pattern completion-related information is retrieved.

Pattern completion is a function performed at the time of recall or retrieval of a memory (Hunsaker & Kesner, 2013), and as such, the probe test is a behavioural assessment that can reflect pattern completion. Accordingly, with respect to the probe test in Experiment I, which took place one day following the last reversal training, CORT-treated rats had a clear preference for the target quadrant (the quadrant where the platform was located during the reversal training). Their preference was significantly above the level of chance. The VEH control group, however, did not show a preference above the level of chance for any quadrant. Notably, the rats in the present study were trained in the reversal task for only two days. Under normal conditions, young male rats require at least 3-4 days of training to show a robust preference for the target location during the probe tests (Darcet et al., 2014; Dong et al., 2013; Touyarot, Venero, & Sandi, 2004; Wright & Conrad, 2008; Yu et al., 2015). Yet, in the present study, after being given only two days of training, the CORT-treated rats showed a greater preference for the target quadrant than the control rats. In animal models of stress, rodents tend not to prefer the target quadrant as much as controls (Darcet et al., 2014; Lee et al., 2014; Touyarot et al., 2004; Yu et al., 2015), but not always (Touyarot et al., 2004; Wright & Conrad, 2008). However, these studies vary in the stress regimen and water maze protocols. For example, among the studies that included reversal training followed by a probe test (Darcet et al., 2014; Yu et al., 2015), animals were trained in the reversal task for three or more days, where control animals showed higher preference for the target quadrant in comparison to the stressed animals. Thus, the fact that the CORT group in Experiment I showed such exceptional preference for the target quadrant in the probe test following only two days of training, yet had poor performance during the first day of the reversal task is in support of the idea that poor pattern separation increases pattern completion (Yassa et al., 2011).

Impaired pattern separation seems to be the results of suppressed adult hippocampal neurogenesis. Therefore, we hypothesized that chronic CORT induced suppression of adult hippocampal neurogenesis may underlie cognitive inflexibility. Cellular analysis for labelled new neurons, however, did not support this hypothesis. The proportion of new neurons double labeled with BrdU and NeuN in the DG were not significantly different between the two groups (Figure 10; Table 5). These findings may suggest that the survival of new, matured neurons in the DG is unaffected by the animals’ prolonged exposure to CORT. These results seem to be at odds with previous literature. Chronically elevated glucocorticoids potently inhibit neurogenesis in the DG (Mirescu & Gould, 2006), and has consistently shown to suppress proliferation and/or survival of new neurons (reviewed by Joëls, Karst, Krugers, & Lucassen, 2007; Schoenfeld & Gould, 2012). Furthermore, this phenomenon seems to be a general one, holding true for a wide range of species and stressors (Mirescu & Gould, 2006). An exogenous elevation of CORT has also been shown to have a dose-dependent impact on neurogenesis in the adult DG. Chronic high doses of CORT (40 mg/kg for 21 days) but not low doses of CORT (10 mg/kg) reduce cell proliferation and density of immature DG neurons in both male and female rats, and reduce immature neuron density in both dorsal and ventral DG areas in male rats (Brummelte & Galea, 2010). Furthermore, high doses of CORT (40 mg/kg) both before and after cell division reduce new neuron survival (Wong & Herbert, 2004) and discourage neuronal differentiation (Wong & Herbert, 2006). A possible explanation for this discrepancy between the findings of the present study and previous work is that the age of the newborn cells at the end of Experiment I was younger than those investigated in previous work (28 days in Wong & Herbert, 2004; 2006). The labelled population of new neurons at the end of the study were 22 days and may not have been old enough for robust expression of neuronal nuclei (NeuN). The postmitotic neuronal marker NeuN is a neuron-specific nuclear protein that marks mature neurons. Although this marker can be expressed in new cells within the DG as early as 1 day after cell birth (Kempermann et al., 2003), it is most robustly expressed in rats (with the greatest percentage of NeuN co-labelling with BrdU-positive cells, ~ ≥ 80%) 28 or more days after cell birth (Brown et al., 2003; Kempermann et al., 2003; Snyder et al., 2009; Zhao et al., 2008). New neurons at three weeks of age show much less expression of NeuN, with NeuN labelling ~ 50-60% of BrdU-labeled cells (Brown et al., 2003; Snyder et al., 2009). NeuN is considered a marker of cell maturity because NeuN-expressing cells are likely to have undergone fate determination, and become persistently integrated into the granule cell layer, thus depicting survival of a newly born cell (Kempermann et al., 2003). However, environmental manipulations known to affect net neurogenesis (absolute numbers of new neurons), such as exercise or stress, that can affect the developmental course of granule cells (Brandt et al., 2003; Wong & Herbert, 2006; Wong & Herbert, 2004). Given that no differences were found in either CORT or vehicle-treated rats in either Experiment I or II, it may be that cells at 22 days of age do not robustly express NeuN because they have not yet undergone fate determination. It would therefore be interesting to quantify the development of new neurons in the present study with other cell markers such as doublecortin (DCX), calretinin and calbindin. Doublecortin-expressing cells mark immature progenitor cells (Filippov et al., 2003). Calretinin has been suggested to mark maturing neurons that are in between the stages of being immature and fully mature (Brandt et al., 2003). Furthermore, calbindin is another marker for mature neurons (Sloviter, 1989), but seems to be expressed very shortly after calretin is no longer expressed (Brandt et al., 2003). This would allow closer examination as to whether the chronic stress model of depression used in the present study may specifically alter the developmental course of newborn neurons, as opposed to reducing the number of surviving neurons. Although such phenotyping of newborn cells is outside the scope of the present study, quantifying cells with the aforementioned cell markers may be of interest for further research.

Experiment II was designed to be identical to Experiment I in all respects except that the CORT and vehicle injections were terminated 3 days prior to initiation of water maze testing. We hypothesized that if suppressed adult hippocampal neurogenesis underlies cognitive inflexibility as seen in Experiment I, then rats given CORT injections followed by a short period of rest should still have persistent cognitive inflexibility. In other words, terminating CORT injection 3 days before initiation of the behavioural task should not be long enough to change the behavioural effects observed in Experiment I, if behavioural outcomes were due to suppressed hippocampal neurogenesis.

As previously discussed, there is considerable evidence showing that chronic stress-induced brain remodelling can be reversible (Hoffman et al., 2011; Sandi et al., 2003; Sousa et al., 2000). For example, chronic stress can produce retractions of CA3 apical dendrites, and change hippocampal dendritic spine density, shape, and synaptic terminal structure (Magariños et al., 1997; Magariños & McEwen, 1995; McLaughlin et al., 2007; Sunanda et al., 1995; Watanabe et al., 1992). Furthermore, it appears that CA3 dendritic retractions produced by chronic restraint stress for 21 days, recover to pre-stress conditions between 5 and 10 days (Conrad, LeDoux, Magariños, & McEwen, 1999). However, suppressed neurogenesis is not as quickly recover after termination of CORT treatment. In a recent study with a 10 day gap between CORT injection (40 mg/kg administered for 18 days) and tissue collection, fewer DCX-expressing new neurons were found in the ventral dentate gyrus only (Workman et al., 2015). Wong & Herbert (2006) also showed that with a similar dose of CORT (40 mg/kg) for 18 days, hippocampal neurogenesis was still suppressed, even after a 9-day delay between the final CORT injection and tissue collection. Thus, if the observed cognitive inflexibility in Experiment

I was due to suppressed adult hippocampal neurogenesis, ending the CORT injection only 3 days before starting the water maze training should not reverse the observed deficit in the cognitive flexibility task. Even if new neurons started to generate soon after termination of CORT treatment, they would not be mature enough to be incorporated into the hippocampal neural circuitry by the time rats underwent reversal training (and thus these new neurons would not be able to contribute to flexible behaviour). In addition, behavioural data from Experiment II, revealed no difference in performance of the rats in either the reversal training or the probe test. It can be argued that cognitive inflexibility induced by chronic CORT injection (as observed in Experiment I) is reversible when the CORT treatment terminates prior to behavioural training.

Immuonohistochemical analysis of the dentate gyrus in these rats revealed no difference in the number of labelled new neurons. Since the same BrdU protocol was used in both experiments, and thus the age of the populations of new neurons was also the same, it is reasonable to suggest that suppression of adult hippocampal neurogenesis may not be the underlying cause of deficits in cognitive flexibility, at least not in the animal model of depression used in the present study. Further research is needed to understand if and how neurogenesis may affect cognitive flexibility and how it may relate to depression.

Concluding Remarks

Behavioural outcome of the two experiments together with cellular data, suggest that chronic CORT injections impair cognitive flexibility and potentiate recall. These effects are not due to suppression of hippocampal neurogenesis and are only evident for as long as the CORT is present. Reversal learning is a task that requires cognitive flexibility, and likely recruits a number of different brain regions other than the hippocampus (Anisman & Matheson, 2005; Beck et al., 1987; Philippe Fossati et al., 1999). As mentioned, CA3 apical dendrites retract by prolonged stress (McEwen, 2007). However, interestingly, the recurrent collaterals of the CA3 that project to the basal dendrites (Li, Somogyi, Ylinen, & Buzsáki, 1994) are not affected by chronic stress (McEwen, 2007). In addition, recurrent collaterals play a key role in pattern completion (Hunsaker & Kesner, 2013). Thus, it can be speculated that recurrent collaterals become abnormally hyperactive following chronic CORT injections, perhaps to compensate the loss of DG outputs, given the mossy fibres projection of apical dendrites detract by chronic CORT treatment. Thus, hyperactivity of the recurrent collaterals may underline better performance of the CORT treated rats in the probe test in Experiment I. However, the comparable performance between groups observed in Experiment II may have been because of recovery, or at least partial recovery, of the mossy fibers projections. There is evidence that loss of mossy fibre synapses in the CA3 produced by chronic stress can be rapidly recover, even within 2-3 days, if animals are given an experience such as water maze training, that consist of both physical and intellectual stimulation (Sandi et al., 2003). It may be that 3 days of water maze acquisition learning prior to reversal and probe tasks, facilitated recovery of changes in hippocampal morphology. This may have normalized the potential effect on reversal learning and probe recall such that stressed animals could perform on par with their control counterparts. However, these possibilities were outside the scope of the current experiments, and should be tested at cellular level in the future studies. CA3 hyperactivity during chronic stress and perhaps in depression may lead to memory overgeneralization. Indeed, an overgeneralization of autobiographical memories are often evident with stress-related pathologies such as post-traumatic stress disorder (PTSD) and depression (Sumner, Griffith, & Mineka, 2010). Thus, these findings may have implications for depression because this may suggest that promoting recovery of dendritic structure, either by physical or intellectual stimulation, or through novel drugs that can promote regrowth of dendritic structures can have a beneficial impact on cognitive ability of those suffering from depression.

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Table 1: The body weight data presented as area under the curve (AUC) for CORT and vehicle-treated rats in Experiment I and II. Data are mean ± SEM. For Experiment I, n=6 for CORT and n=5 for VEH. For Experiment II, n=6 for both CORT and VEH.

Area under the curve for Experiment I and II				
Experiment	**CORT**	**VEH**	**P-value**	**Effect size Correlation (*r*)**
Experiment I	7999.750 ± 61.421	8369.500 ± 127.451	0.022	0.630
Experiment II	8190.000 ± 96.587	9081.250 ± 116.016	≤0.001	0.862

Table 2. Summary of the effect size correlations for the velocity between groups from Experiment I and II.

Effect size correlations velocity data from Experiment I and II		
Experiment	**Day of testing**	**Correlation (*r*)**
Experiment I	1	0.435
	2	0.283
	3	0.084
	4	0.118
	5	0.540
Experiment II	1	0.417
	2	0.359
	3	0.376
	4	0.457
	5	0.130

Table 3. Summary of the effect size correlations for distance travelled and latency between treatment groups across all days of behavioural training in Experiment I and II.

Effect size correlations for behavioural data from Experiment I and II			
Experiment	**Measure**	**Day of testing**	**Correlation (*r*)**
Experiment I	Distance	1 (Acquisition)	0.600
		2 (Acquisition)	0.179
		3 (Acquisition)	0.276
		4 (Reversal)	-0.470
		5 (Reversal)	0.229
	Latency	1 (Acquisition)	0.514
		2 (Acquisition)	0.117
		3 (Acquisition)	0.257
		4 (Reversal)	-0.588
		5 (Reversal)	0.135
Experiment II	Distance	1 (Acquisition)	0.561
		2 (Acquisition)	0.482
		3 (Acquisition)	0.490
		4 (Reversal)	0.425
		5 (Reversal)	0.277
	Latency	1 (Acquisition)	0.538
		2 (Acquisition)	0.475
		3 (Acquisition)	0.454
		4 (Reversal)	0.404
		5 (Reversal)	0.252

Table 4. Summary of the effect size correlations for the probe task for each quadrant between groups from Experiment I and II.

Effect size correlations for probe task from Experiment I and II		
Experiment	**Quadrant**	**Correlation (*r*)**
Experiment I	SW (Target)	0.599
	NE (Opposite)	0.493
	NW	0.125
	SE	0.369
Experiment II	SW (Target)	0.140
	NE (Opposite)	-0.063
	NW	0.384
	SE	-0.336

Table 5. Summary of the cell densities of the DG for CORT and VEH treated rats in Experiment I. Total density depicts the summed density for the supra and infrapyramidal blades together. The p-value and effect size correlations are also presented. Data are mean ± SEM. n=6 for CORT and n=5 for VEH.

Experiment I cellular densities				
Area	**CORT**	**VEH**	**p-value**	**Correlation (*r*)**
Total Density (Brdu+/area)	0.0000237344 ± 0.0000018192	0.0000265657 ± 0.0000031149	0.540	0.053
Supra. Density (Bdru+/area)	0.0000099117 ± 0.0000020176	0.0000103512 ± 0.0000013691	0.861	0.246
Infra. Density (Brdu+/area)	0.0000138227 ± 0.0000018192	0.0000162145 ± 0.0000022116	0.421	0.190

Table 6. Summary of the cell densities of the DG for CORT-rest and VEH-rest treated rats in Experiment II. Total density depicts the summed density for the supra and infrapyramidal blades together. The p-value and effect size correlations are also presented. Data are mean ± SEM. n=6 for both CORT and VEH.

Experiment II cellular densities				
Area	**CORT-rest**	**VEH-rest**	**p-value**	**Correlation (r)**
Total Density (Brdu+/area)	0.0000388882 ± 0.0000046472	0.0000427049 ± 0.0000039494	0.418	0.237
Supra. Density (Bdru+/area)	0.0000136530 ± 0.0000023505	0.0000159693 ± 0.0000014097	0.701	0.113
Infra. Density (Brdu+/area)	0.0000252352 ± 0.0000024197	0.0000267356 ± 0.0000029240	0.545	0.178

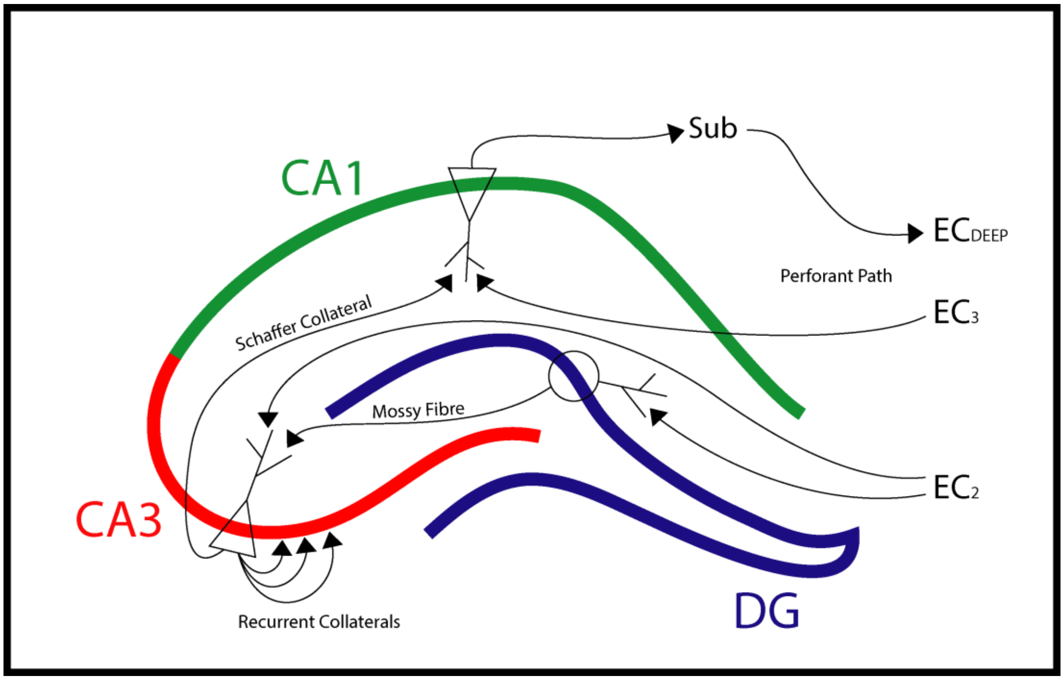

Figure 1. Schematic representation of the hippocampus. Information travels from the entorhinal cortex (EC) layers (sub-layers denoted by subscripted numbers) to the dentate gyrus (DG), CA regions, and subiculum (SUB), back to the EC. Various layers of the EC also communicate with the various components of the trisynaptic loop. The DG's granule cells are more densely packed than the other regions along the trisynaptic loop, a feature that allows the DG to orthogonalize information.

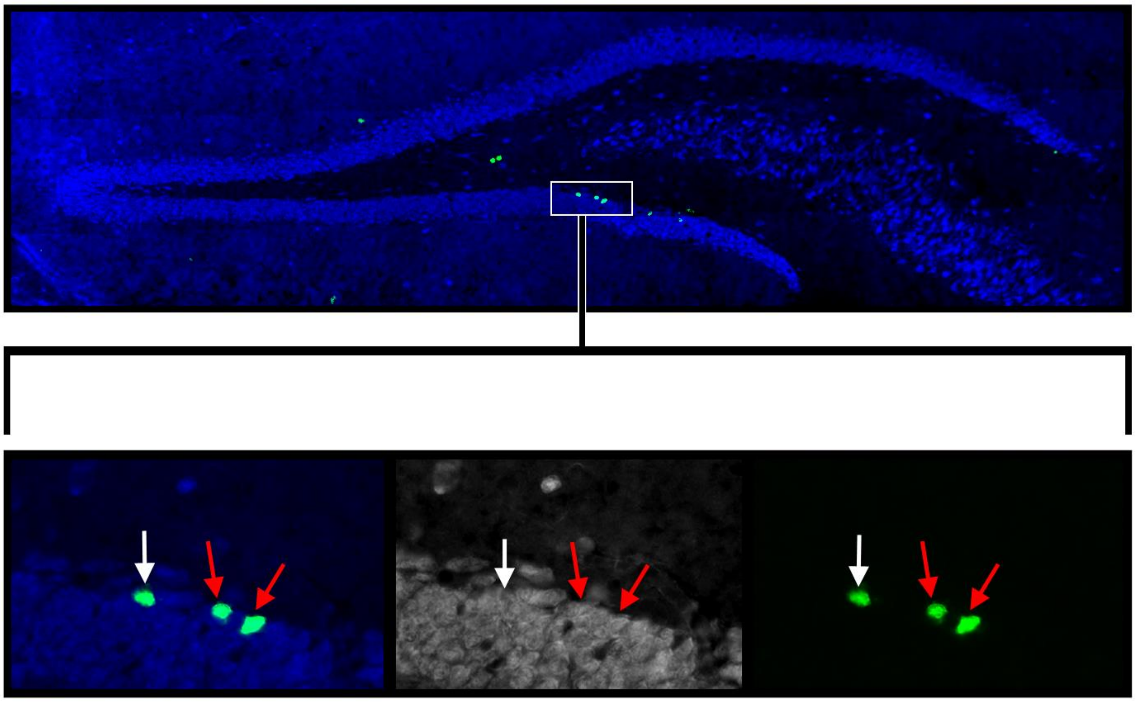

Figure 2. Representative inverted microscope image of the DG (upper) of the hippocampus with BrdU-labeled newborn neurons (green). The inset below depicts a representation of the quantification of new neurons. BrdU-labeled neurons (left inset; red arrows) are shown to overlap NeuN-labeled cells (middle inset) with BrdU (right inset). A BrdU-labeled cell that is not a neuron (left inset, white arrow) is not shown to have overlapping NeuN-labelling (middle inset) with BrdU (right inset).

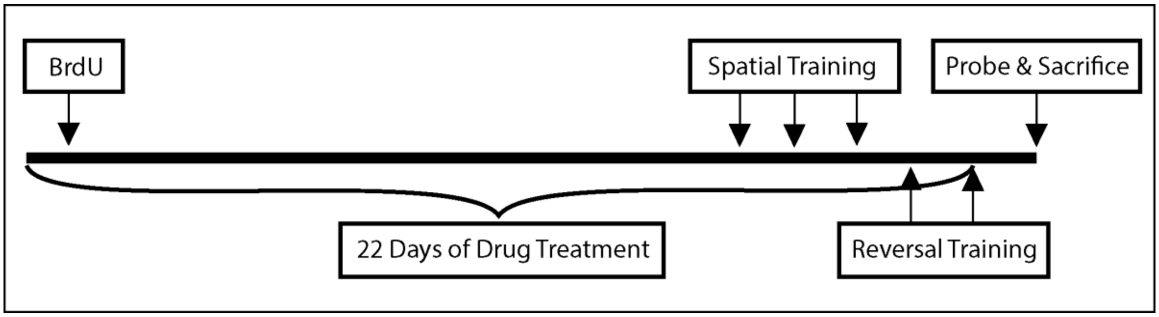

Figure 3. Timeline of procedures for Experiment I.

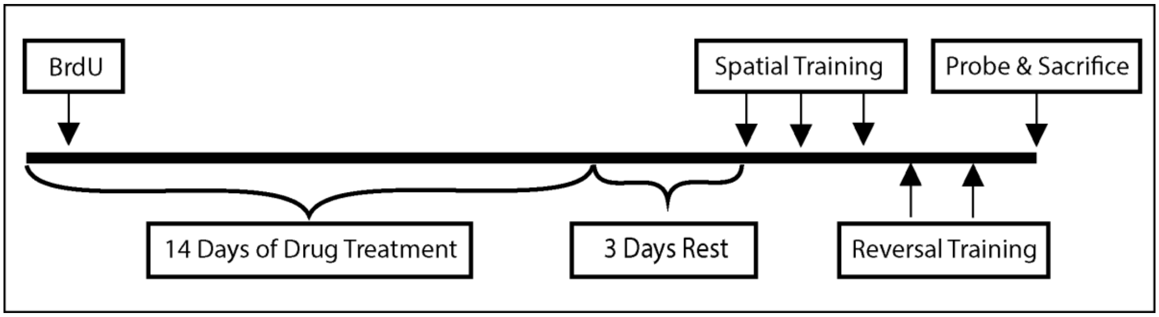

Figure 4. Timeline of procedures for Experiment II.

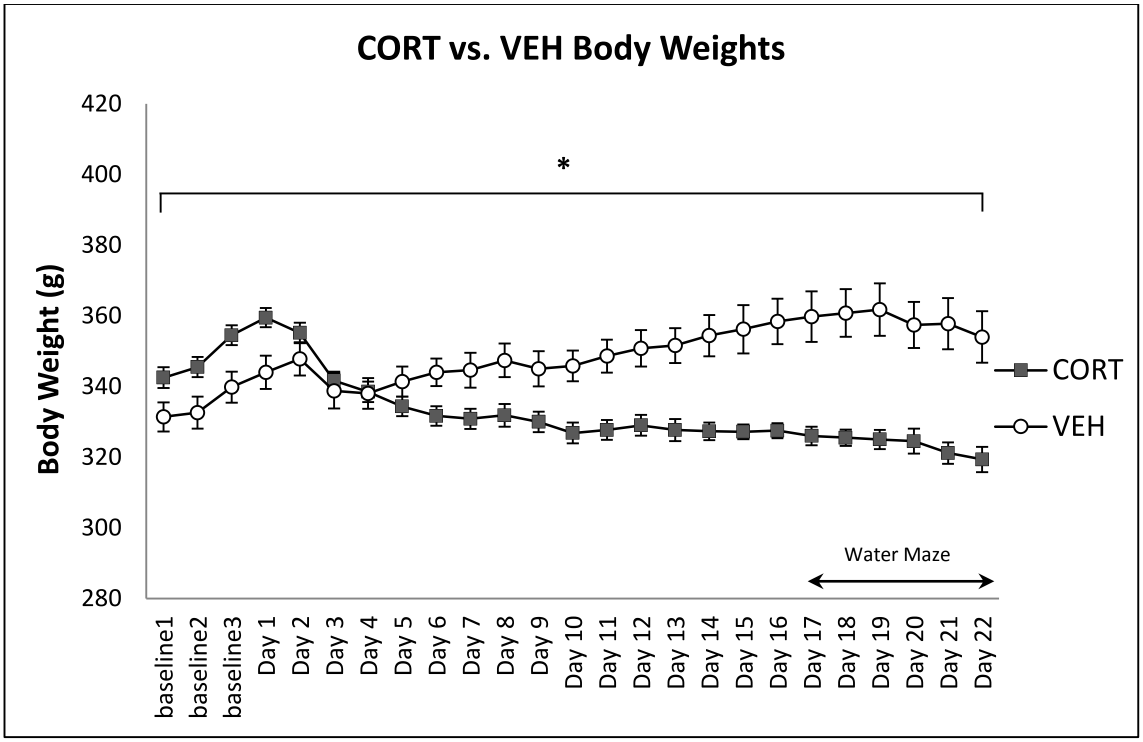

Figure 5. Body weights of CORT and VEH rats over the course of Experiment I. Comparisons were performed for the area under the curve (AUC). Body weights differed significantly between the two groups ($p=0.022$). Data are mean ± SEM for each group. $^*p\leq0.05$. $n=6$ for CORT and $n=5$ for VEH.

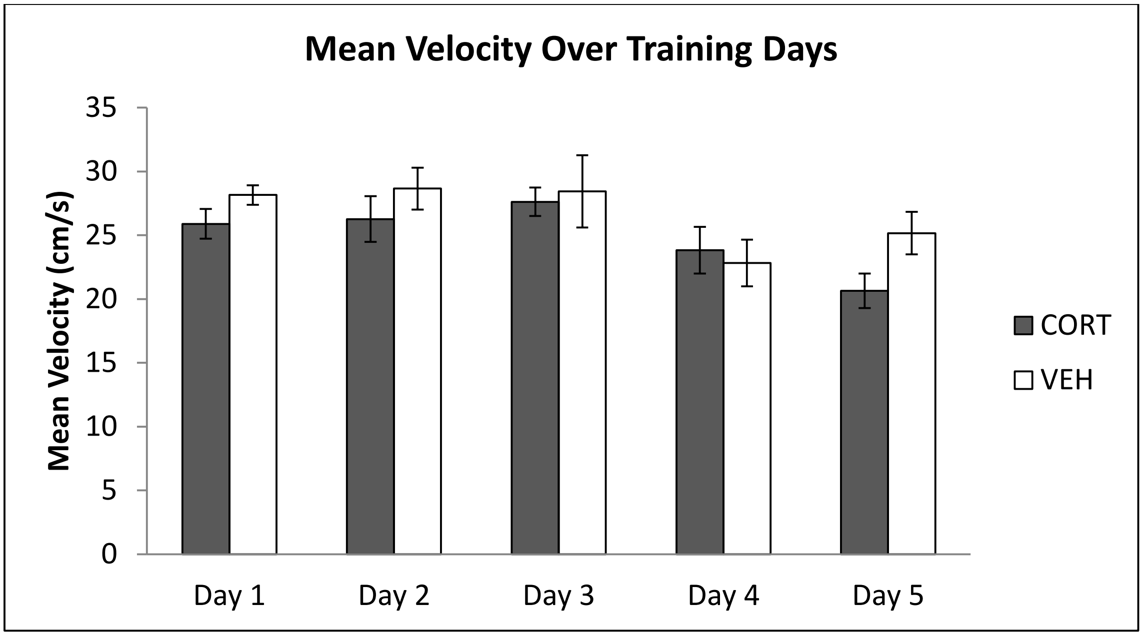

Figure 6. Mean velocity (cm/s) for CORT and VEH groups for Experiment I for spatial (day 1-3) and reversal (day 4-5) training. There were no statistically significant differences between groups on any of the training days. Data are mean ± SEM. n=6 for CORT and n=5 for VEH.

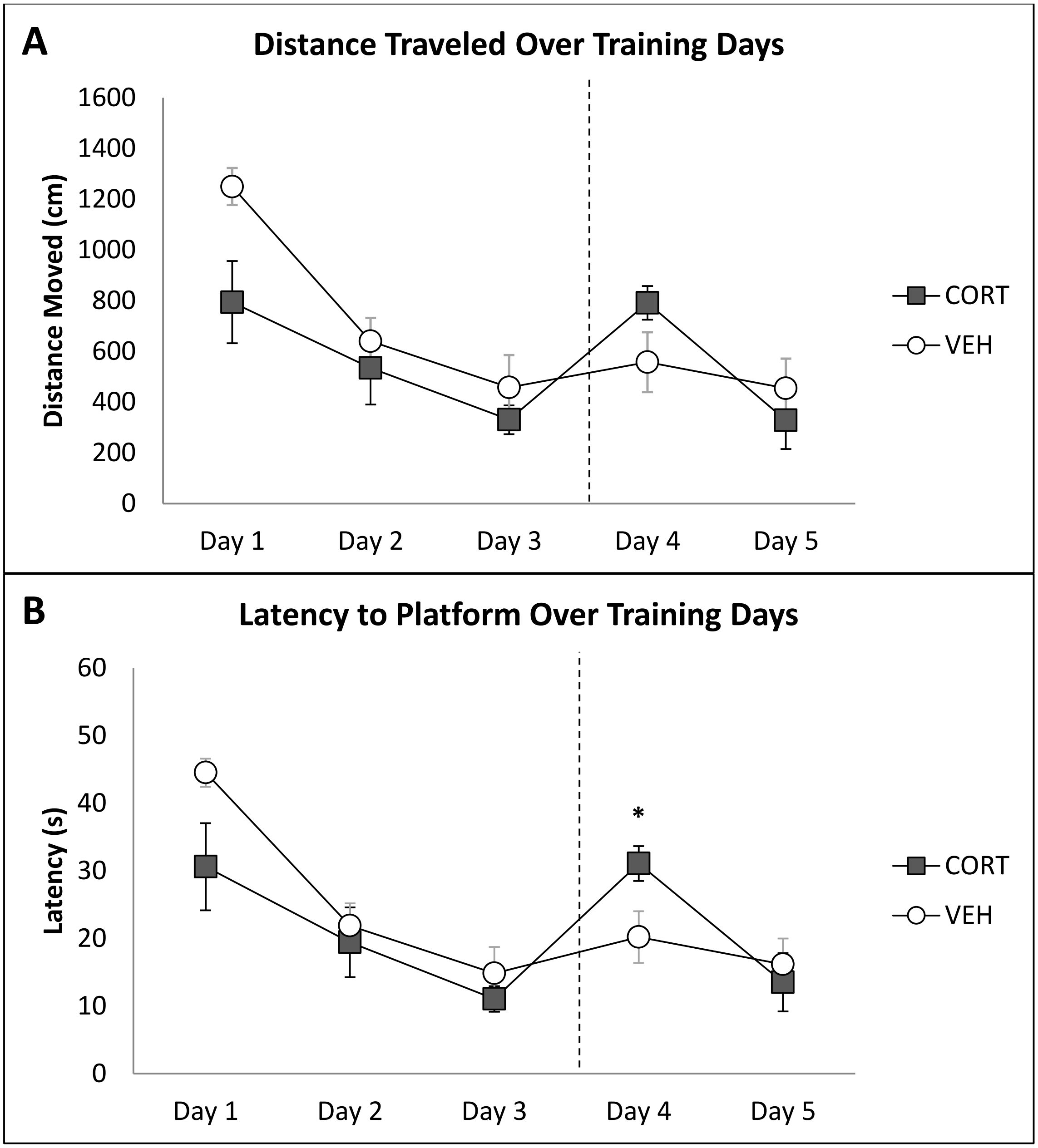

Figure 7. Average distance traveled (A) in the water maze arena and latency to reach the platform (B) in CORT and VEH rats for Experiment I over the acquisition and reversal training phases. Data are mean ± SEM. n=6 for CORT and n=5 for VEH. *p≤0.05.

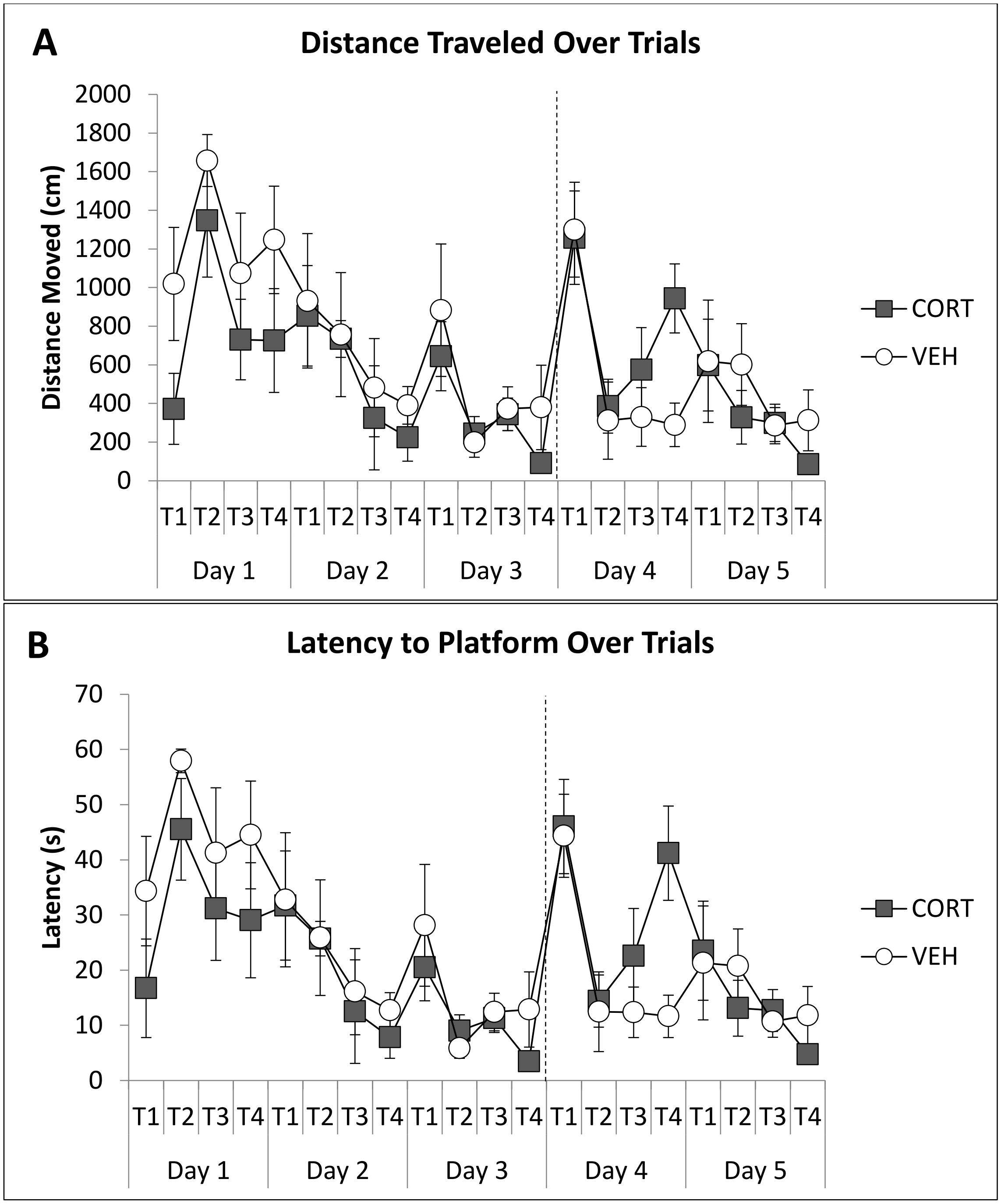

Figure 8. Average distance traveled (A) in the water maze arena and latency to reach the platform (B) across trials between groups for Experiment I. Data are mean ± SEM. n=6 for CORT and n=5 for VEH. *p≤0.05.

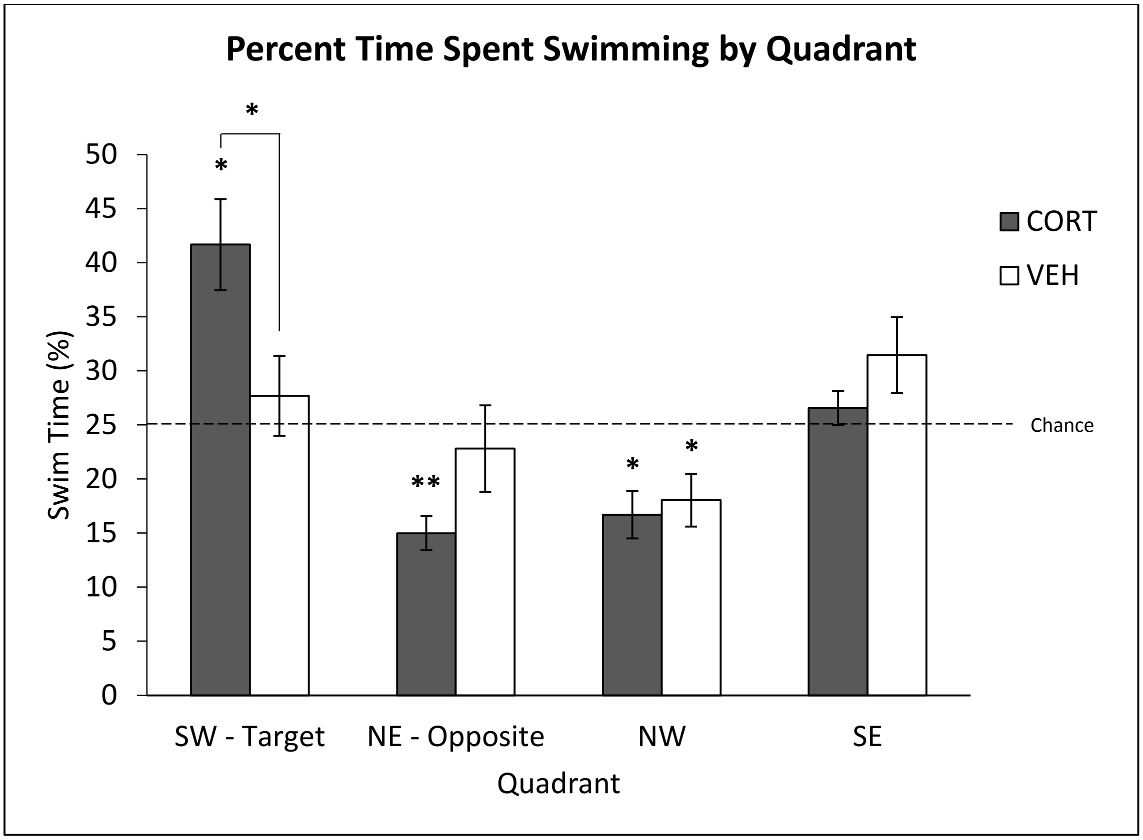

Figure 9. The percent of time spent in each quadrant during the probe trial for Experiment I. The dotted line represents the percent of time an animal would spend in each quadrant by chance alone during a random search (i.e. 25%). Data are mean ± SEM. n=6 for CORT and n=5 for VEH. $*p \leq 0.05$; $**p \leq 0.001$.

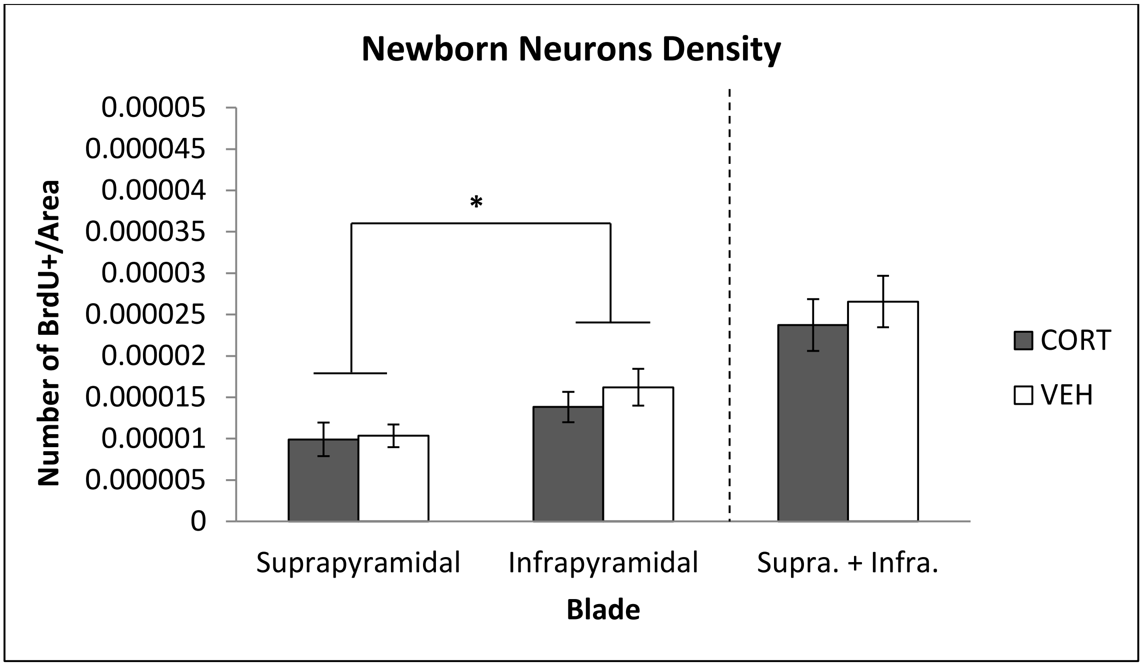

Figure 10. The density of newborn neurons in the supra and infrapyramidal blades of the DG, as well as the total density of newly generated neurons in each treatment group (CORT or VEH) for Experiment I. Data are mean ± SEM. n=6 for CORT and n=5 for VEH. *$p \leq 0.05$.

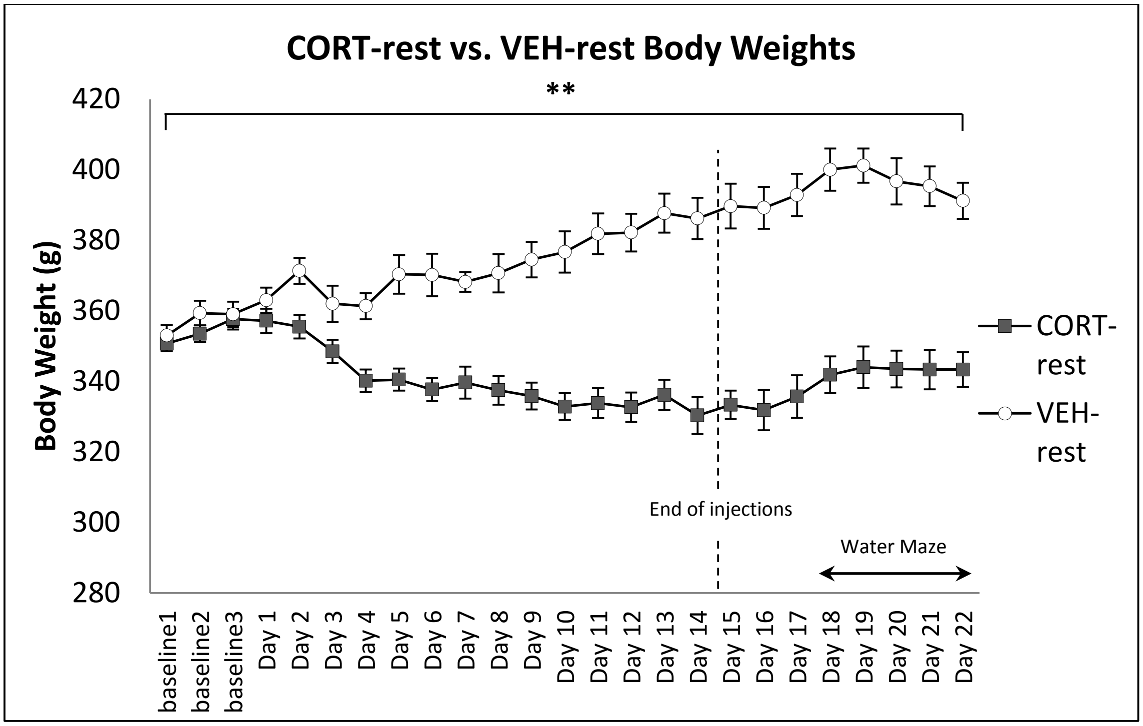

Figure 11. Body weights of CORT-rest and VEH-rest rats over the course of Experiment II. Comparisons were performed for the area under the curve (AUC). Body weights differed significantly between the two treatment groups (p<0.001). Data are mean ± SEM for each group. **p≤0.001. n=6 for both CORT-rest and VEH-rest.

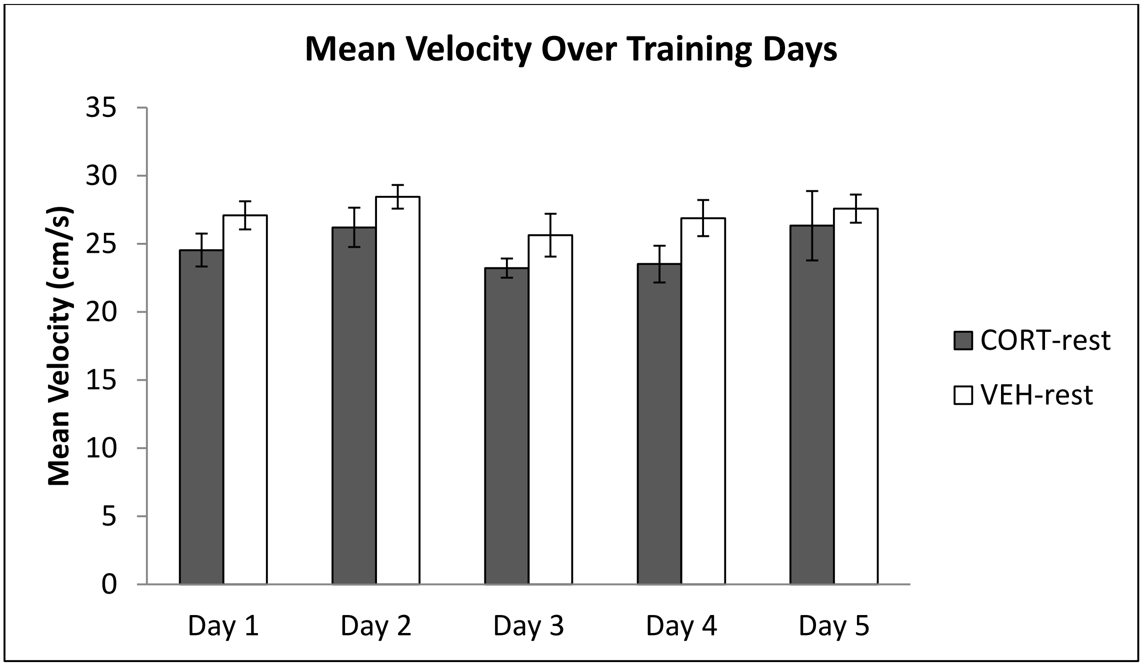

Figure 12. Mean velocity (cm/s) for CORT-rest and VEH-rest groups for Experiment II for the acquisition (day 1-3) and reversal (day 4-5) training. The differences between groups were not statistically significant on any of the training days. Data are mean ± SEM. n=6 for both CORT-rest and VEH-rest.

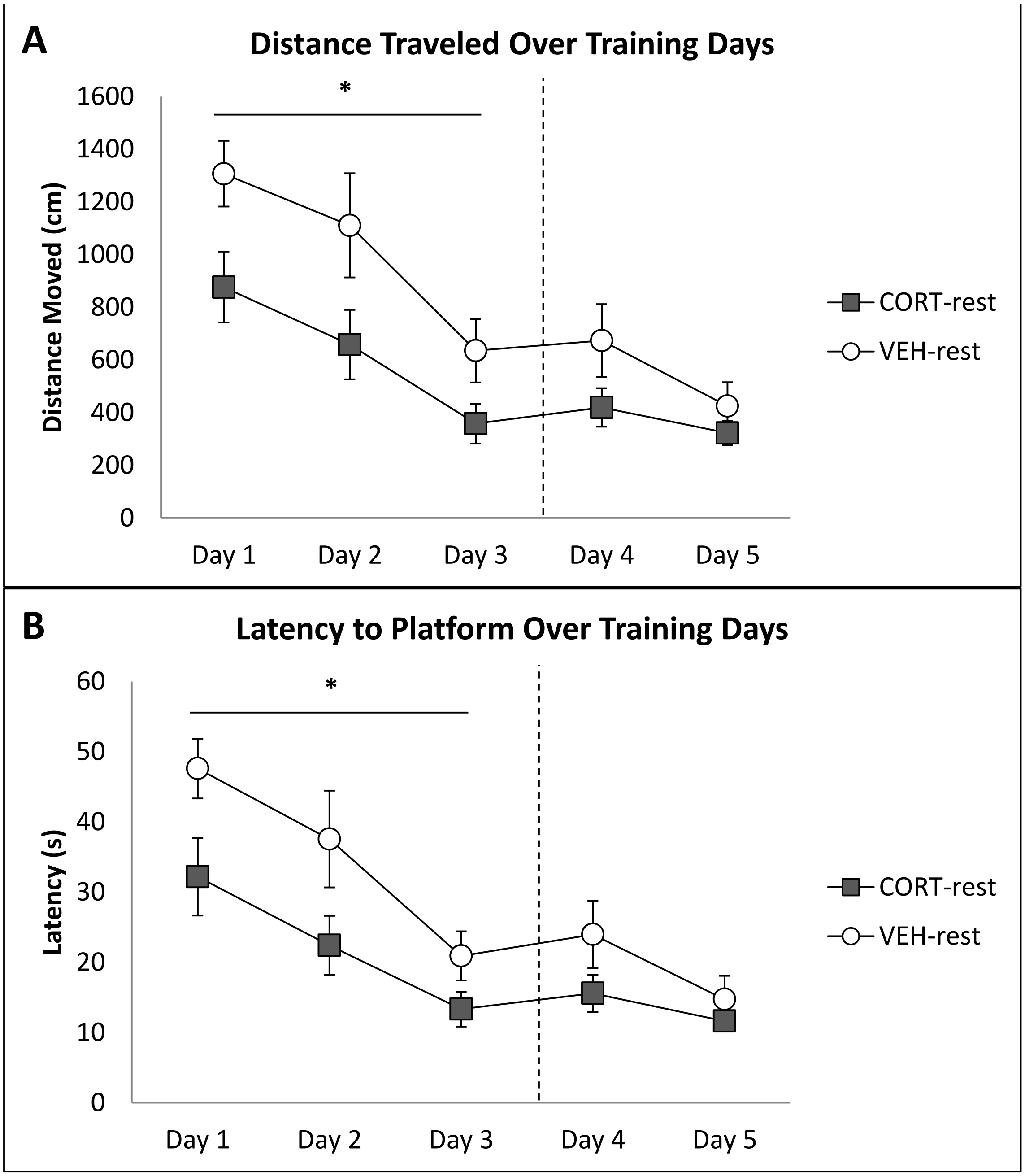

Figure 13. Average distance traveled (A) in the water maze arena and latency to reach the platform (B) in CORT-rest and VEH-rest rats for Experiment II over the acquisition and reversal training phases Data are mean ± SEM. n=6 for both CORT-rest and VEH-rest. *$p \leq 0.05$.

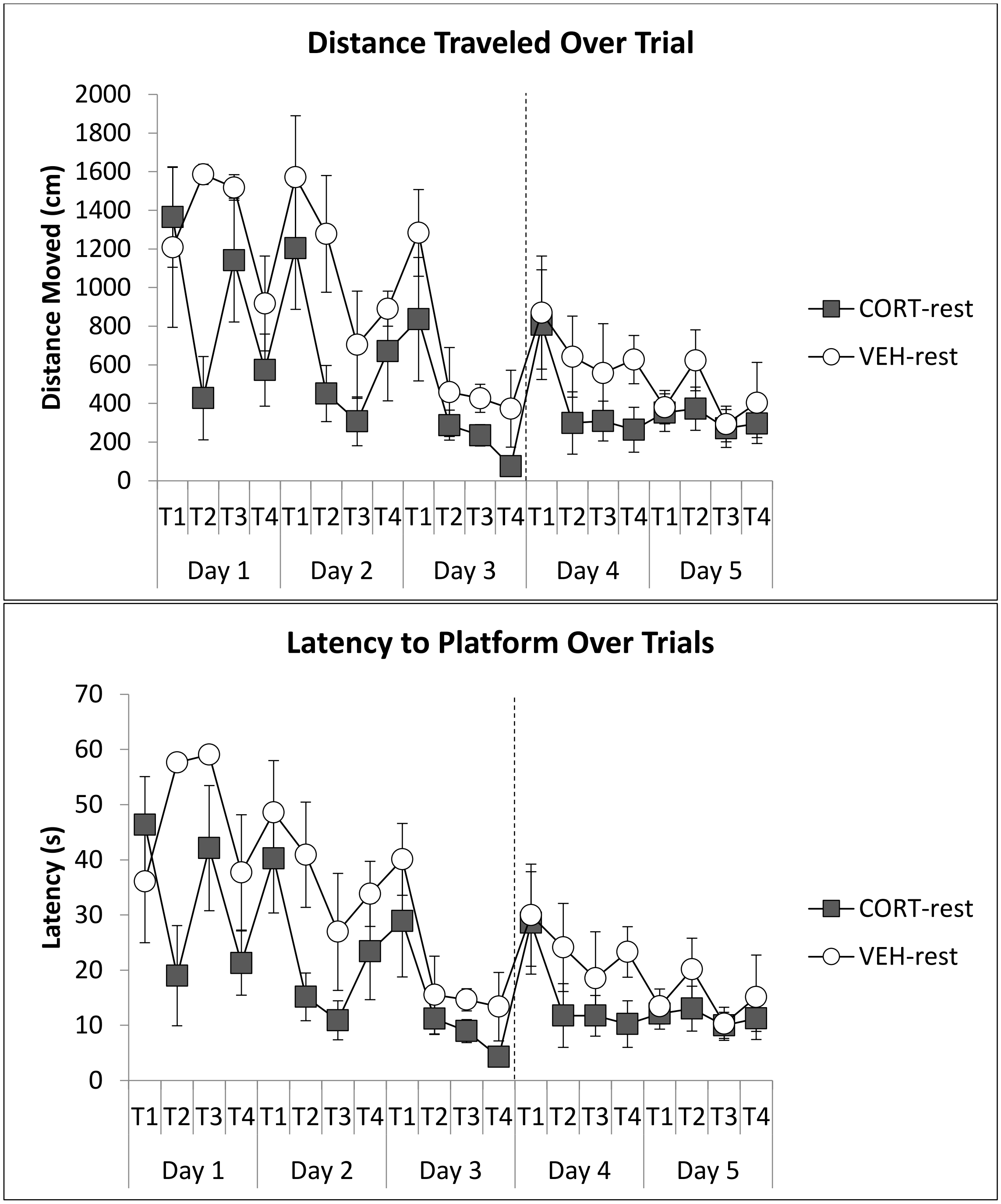

Figure 14. Average distance traveled (A) in the water maze arena and latency to reach the platform (B) across trials between groups for Experiment II. Data are mean ± SEM. n=6 for CORT and n=5 for VEH. *p≤0.05.

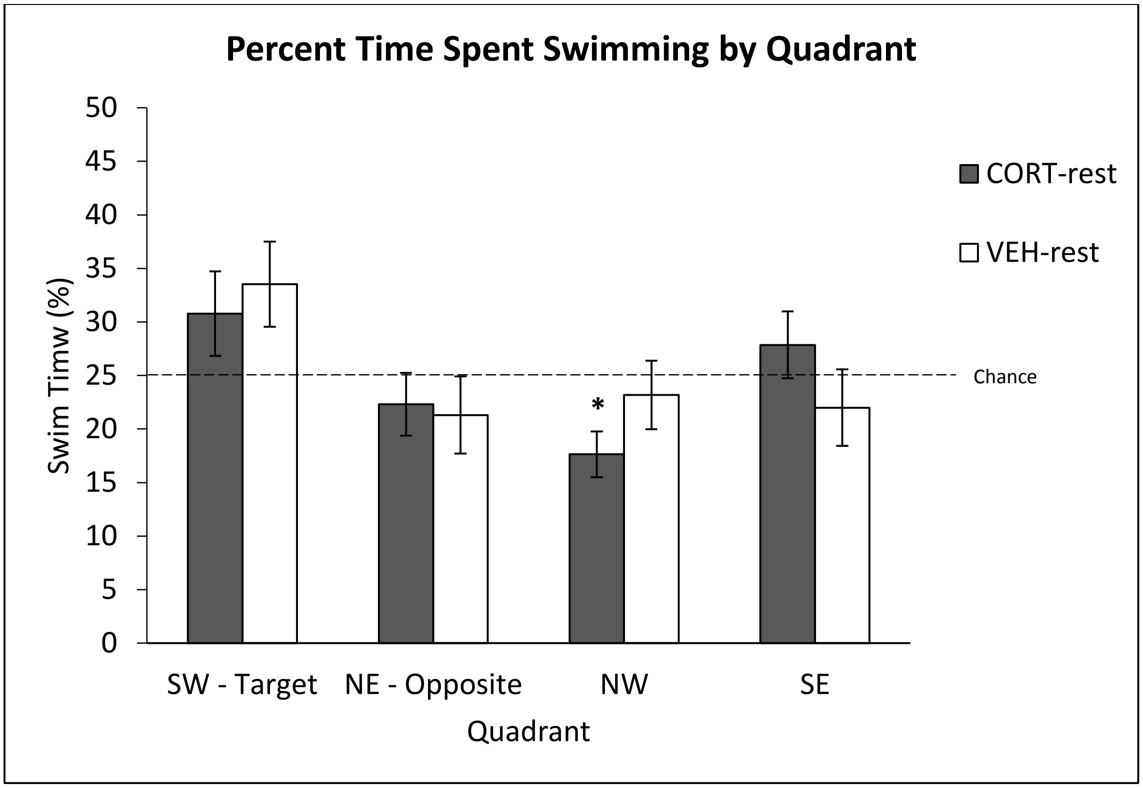

Figure 15. The percent of time spent in the target and opposite quadrants in the probe trial for Experiment II. The dotted line represents the percent of time an animal would spend in each quadrant by chance alone during a random search. Data are mean ± SEM. n=6 for both CORT-rest and VEH-rest. *p≤0.05.

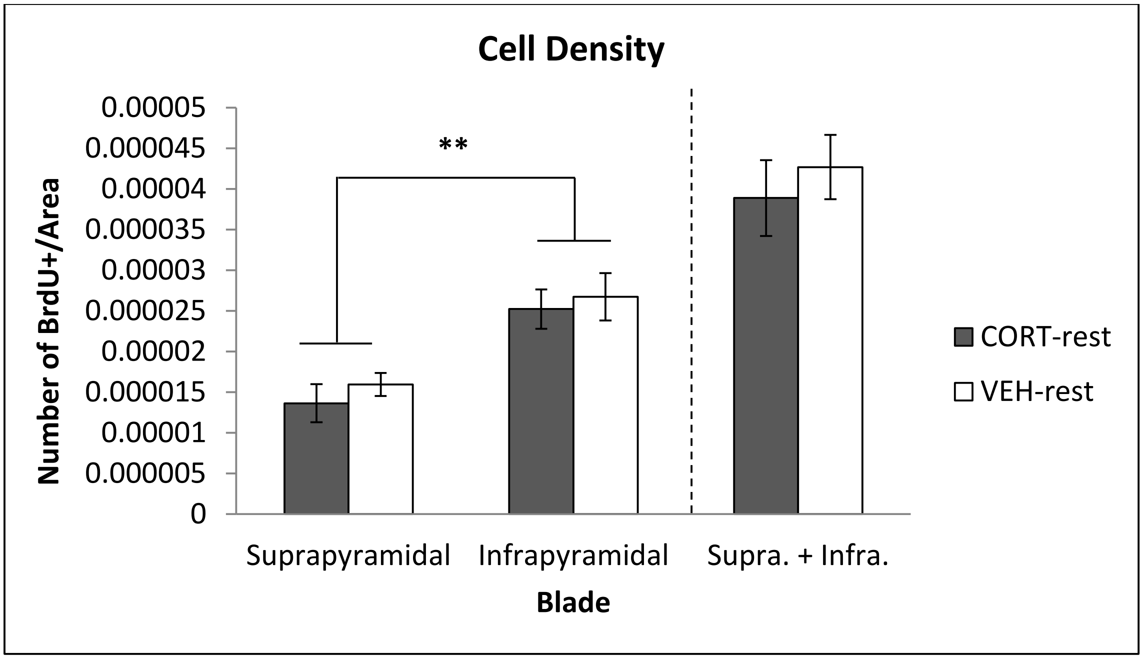

Figure 16. The density of newborn neurons generated in the supra and infrapyramidal blades of the DG, as well as the total density of newly generated neurons in each treatment group (CORT-rest or VEH-rest) for Experiment II. Data are mean ± SEM. n=6 for both CORT-rest and VEH-rest. **$p \leq 0.001$.