FURTHER DEFINING THE FUNCTIONAL PROPERTIES OF THE DENTATE GYRUS: CONTRIBUTIONS TO SPATIAL REPRESENTATIONS

by

Andrea M. Morris

A dissertation submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

Department of Psychology

The University of Utah

August 2011

The University of Utah Graduate School

STATEMENT OF DISSERTATION APPROVAL

The dissertation of **Andrea M. Morris**

has been approved by the following supervisory committee members:

Raymond Kesner	, Chair	**4/22/2011** Date Approved
Sarah Creem-Regehr	, Member	**4/22/2011** Date Approved
Kristen Keefe	, Member	**4/22/2011** Date Approved
Jeanine Stefanucci	, Member	**4/22/2011** Date Approved
Jason Watson	, Member	**4/22/2011** Date Approved

and by **Cynthia Berg**, Chair of the Department of **Psychology**

and by Charles A. Wight, Dean of The Graduate School.

ABSTRACT

The hippocampus (HPP) plays an important role in episodic memory, or memory for an event that occurs in a specific place and time, and there is evidence to suggest that the HPP is involved in processing spatiotemporal information in order to form contextual representations of memory events. The HPP is not a homogeneous structure, but instead is comprised of anatomically distinct subregions, including the dentate gyrus (DG), CA3, and CA1, associated with separate mnemonic processing functions that contribute to episodic memory formation. Specifically, the DG is thought to support spatial processing functions, whereas the CA1 subregion has been implicated in temporal processing. Despite considerable advances in our understanding of the unique contributions of HPP subregions to learning and memory processes, the role of the dorsal DG (dDG) in spatial processing as it relates to spatial representations is not entirely understood or agreed upon. Given the importance of spatial representations to spatial navigation and episodic memory function, the current investigation sought to further define the role of the dDG in spatial processing through a series of studies that explored the nature of spatial memory representations. The results suggest that the dDG plays a critical role in (1) the integration of multimodal information into unique representations of the spatial environment via conjunctive encoding, (2) the reduction of interference among similar spatial locations via spatial pattern separation, and (3) the formation of temporal associations among distinct spatial events via temporal integration. Taken together, the

present findings provide evidence for a dynamic role for the dDG in spatial processing by demonstrating the importance of an intact dDG across a variety of spatial tasks and under a variety of learning and memory demands.

To my loving family for their support and encouragement.

TABLE OF CONTENTS

ABSTRACT iii

LIST OF FIGURES viii

ACKNOWLEDGEMENTS x

Chapters

1 GENERAL INTRODUCTION 1

References 5

2 STUDY ONE: THE ROLE OF THE DENTATE GYRUS IN THE FORMATION OF CONTEXTUAL REPRESENTATIONS 9

Abstract 9
Introduction 10
Materials and Methods 12
Results 18
Discussion 22
Acknowledgements 27
References 27

3 STUDY TWO: SELECTIVE LESIONS OF THE DENTATE GYRUS PRODUCE DISRUPTIONS IN PLACE LEARNING FOR ADJACENT SPATIAL LOCATIONS 30

Abstract 30
Introduction 31
Materials and Methods 35
Results 40
Discussion 43

Acknowledgements 47
References 48

4 STUDY THREE: THE ROLE OF THE DENTATE GYRUS IN THE FORMATION OF TEMPORAL ASSOCIATIONS FOR SPATIAL LOCATIONS 53

Abstract 53
Introduction 54
Materials and Methods 57
Results 63
Discussion 66
Acknowledgements 71
References 71

5 GENERAL DISCUSSION 74

References 78

LIST OF FIGURES

Figure Page

1. Histological representation of a dDG lesioned rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997). 19

2. Histological representation of a vehicle-infused control rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997). 19

3. Mean (± SE) percent correct performance of DG lesioned rats and control rats on the contextual associative learning task as a function of days (1-10). 20

4. Mean (± SE) of dDG lesioned rats and control rats on the olfactory discrimination task. 21

5. Mean (± SE) of dDG lesioned rats and control rats on the contextual discrimination task. 22

6. Schematic of eight-arm radial maze configuration for the adjacent condition of the place-learning task. 39

7. Schematic of eight-arm radial maze configuration for the separate condition of the place-learning task. 40

8. Histological representation of a dDG lesioned rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997). 41

9. Histological representation of a vehicle-infused control rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997). 41

10. Mean (± SE) trials to criterion for dDG lesioned rats and control rats on the separate and adjacent conditions of the place-learning task. 42

11. Schematic representation of behavioral procedures for cued recall for spatial location task. 60

12. Schematic representation of behavioral procedures for novelty detection for spatial location task. 62

13. Histological representation of a dDG lesioned rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997). 64

14. Histological representation of a vehicle-infused control rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997). 64

15. Mean (± SE) preference ratio of dDG lesioned rats and control rats for cued recall of temporally based spatial location associations. 65

16. Mean (± SE) preference ratio of dDG lesioned rats and control rats on the novelty detection condition for cued recall of temporally based spatial location associations. 67

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge Raymond Kesner, Ph.D., and Paul Gilbert, Ph.D., for their mentorship and encouragement. In addition, I am very thankful to my committee members Jeanine Stefanucci, Ph.D., and Sarah Creem-Regehr, Ph.D., for their support and helpful advice. I also would like to thank my committee members Jason Watson, Ph.D., and Kristen Keefe, Ph.D. I would like to acknowledge the students who have helped with these projects. Additionally, I would like to thank all of my friends, family, and especially John C. Churchwell, Ph.D., for their support. These investigations were supported by NIH grant #MH065314 to Raymond P. Kesner.

CHAPTER 1

GENERAL INTRODUCTION

The hippocampus (HPP) plays an important role in learning and memory processes. Specifically, theories of hippocampal function suggest that the HPP supports episodic memory, or memory for unique personal events that include detailed information about where and when the event occurred (Squire, 1992; Tulving, 1983). In support of this claim, there is evidence to demonstrate that the HPP processes spatiotemporal information in order to form a contextual representation of the memory event (Clayton & Dickinson, 1998; Rolls, 2010). In addition, previous research shows that damage to the HPP produces profound anterograde amnesia, or the inability to form new episodic memories (Scoville & Milner, 1957).

The HPP is not a homogeneous structure, but instead is comprised of anatomically distinct subregions including the dentate gyrus (DG), CA3, and CA1 (Amaral & Witter, 1995). The DG receives its major cortical input from the entorhinal cortex (EC) via the perforant pathway (Amaral & Witter). The EC also has direct projections to CA3 and CA1; however, the DG is considered to be the primary termination site for EC projections (Amaral, Scharfman, & Lavenex, 2007). The EC inputs into the DG can be divided into a medial and lateral component (Hargreaves, Rao, Lee, & Knierem, 2005). The medial EC (MEC) input to the DG conveys spatial

information and the lateral EC (LEC) input conveys nonspatial information (e.g., auditory, olfactory, somatosensory, and visual; see Hafting, Fyhn, Molden, Moser, & Moser, 2005; Hargreaves et al., 2005). This multimodal information is fed forward from DG granule cells to CA3 pyramidal cells along the mossy fiber projection system. Information is then projected from CA3 neurons via the Schaffer collaterals to CA1 neurons. CA1 neurons, in turn, project to the subiculum, the primary output structure of the hippocampus (Amaral & Witter, 1995; Johnston & Amaral, 2004).

Computational models, electrophysiological recording data, and evidence from behavioral studies support the idea that distinct subregions of the HPP are associated with separate mnemonic processing functions that contribute to episodic memory formation (Jung & McNaughton, 1993; Kesner, Lee, & Gilbert, 2004; O'Reilly & McClelland, 1994; Rolls & Kesner, 2006; Treves & Rolls, 1994). Specifically, the DG hippocampal subregion is thought to support spatial processing functions, whereas CA1 has been implicated in temporal processing (Gilbert, Kesner, & Lee, 2001; Kesner et al., 2004; Rolls & Kesner, 2006).

Based on the intrinsic circuitry of the hippocampus, Amaral and colleagues (2007) suggest that, "it is reasonable to consider the dentate gyrus as the first step in the processing of information that ultimately leads to the production of episodic memories" (p. 3). In support of this claim, the dorsal DG (dDG) plays a prominent role during encoding of spatial information and the formation of distinct spatial representations (Gilbert et al., 2001; Jerman, Kesner, & Hunsaker, 2006; Lee & Kesner, 2004; Rolls & Kesner, 2006). Previous research suggests that the HPP plays a critical role in the construction of cognitive maps of the environment built upon the accumulation of spatial

representations (O'Keefe & Nadel, 1978). According to O'Keefe and Nadel, as we navigate through our environment we gain knowledge about external stimuli and the relationships among stimuli. These experiences provide the basis for the formation of internal spatial representations. Spatial representations serve a variety of functions because they allow us to understand the relationship among places and objects in the external world. Spatial representations also play a significant role in episodic memory by providing a spatial context for episodic events, of which time is an important component (Eichenbaum, Dudchenko, Wood, Shapiro, & Tanila, 1999).

More recently, it has been suggested that the dDG mediates the initial formation of spatial representations through a conjunctive encoding process whereby incoming multimodal information is integrated into a single higher-order contextual representation of the spatial environment (Kesner, 2007). Despite anatomical evidence in support of this claim (Amaral et al., 2007; Amaral & Witter, 1995), there is a paucity of behavioral evidence to demonstrate dDG involvement in the formation of conjunctive spatial representations. In order to provide further support for the role of the dDG in the formation of integrated contextual representations, the first study in this dissertation tested animals with DG lesions on a contextual associative learning task described by Luu, Pirogovsky, and Gilbert (2008; developed by Rajii, Chapman, Eichenbaum, & Greene, 2006) that required the formation of an association between a cue (odor) and a context.

There is considerable support for the role of the DG in spatial pattern separation, a process for separating highly overlapping spatial information into distinct representations (Bakker, Kirwan, Miller, & Stark, 2008; Gilbert et al., 2001; Goodrich-Hunsaker,

Hunsaker, & Kesner, 2008; Rolls & Kesner, 2006). Several studies have shown that lesions of the dDG in rodents result in inefficient spatial pattern separation on working memory tasks, or tasks that require use of information that is trial unique (Gilbert et al., 2001; Goodrich-Hunsaker et al., 2008; Talpos, McTighe, Dias, Saksida, & Bussey, 2010). However, it is unclear whether selective dDG lesions disrupt spatial pattern separation for reference memory, or memory for information that remains constant across time (Olton, Becker, & Handelman, 1979). Therefore, the second study in this dissertation investigated the role of the dDG in pattern separation during acquisition using a spatial reference memory task described by McDonald and White (1995) in order to demonstrate that spatial pattern separation is capable of operating across a variety of memory demands.

Although previous research suggests that the DG is not involved in temporal processing (Gilbert et al., 2001), a novel role for the DG in temporal processing for spatial information has begun to emerge due to the development of a computational model of neurogenesis (Aimone, Wiles, & Gage, 2006). Based on the maturation process of newly formed granule cells in the DG, Aimone, Deng, and Gage (2010) suggest that the DG may support a temporal integration process, or the formation of temporal associations for proximal spatial events. Time and space are intimately related and are critical components of an episodic event (Eichenbaum et al., 1999). In addition, there is evidence to suggest that events encoded close in time are more likely to be recalled together (Brown & Schopflocher, 1998). Currently, there is a lack of behavioral evidence to support the temporal integration theory proposed by Aimone and colleagues (2006). Therefore, for the third study of this dissertation, we developed a novel behavioral

paradigm in order to determine whether the dDG supports the formation of temporal associations for spatial events, where space is the critical factor.

Despite considerable advances in our understanding of the unique contributions of HPP subregions to learning and memory processes, the role of the dDG in spatial processing as it relates to spatial representations is not entirely understood or agreed upon. Given the importance of spatial representations to spatial navigation and episodic memory function, the current investigation sought to further define the role of the DG in spatial processing through a series of experiments that explored the formation of spatial representations and the nature of spatial memory representations. The aim of the present investigation was to provide insight into the dynamic nature of spatial representations and broaden our understanding of DG contributions to spatial learning and memory processes.

References

Aimone, J. B., Deng, W., & Gage, F. H. (2010). Adult neurogenesis: Integrating theories and separating functions. *Trends in Cognitive Sciences*, *14*(7), 325-337.

Aimone, J. B., Wiles, J., & Gage, F. H. (2006). Potential role for adult neurogenesis in the encoding of time in new memories. *Nature Neuroscience, 9*(6), 723-727.

Amaral, D. G., Scharfman, H. E., & Lavenex, P. (2007). The dentate gyrus: Fundamental neuroanatomical organization (dentate gyrus for dummies). *Progress in Brain Research, 163*, 3-22.

Amaral, D. G., & Witter, M. P. (1995). Hippocampal formation. In G. Paxinos (Ed.), *The rat nervous system* (pp. 443-493). San Diego, CA: Academic Press.

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*, *319*(5870), 1640-1642.

Brown, N. R., & Schopflocher, D. (1998). Event cueing, event clusters, and the temporal distribution of autobiographical memories. *Applied Cognitive Psychology, 12*, 305-319.

Clayton, N. S., & Dickinson, A. (1998). Episodic-like memory during cache recovery by scrub jays. *Nature, 395*, 272-274.

Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., & Tanila, H. (1999). The hippocampus, memory, and place cells: Is it spatial memory or a memory space? *Neuron, 23*, 209-226.

Gilbert, P. E., Kesner, R. P., & Lee, I. (2001). Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. *Hippocampus, 11*(6), 626-636.

Goodrich-Hunsaker, N. J., Hunsaker, M. R., & Kesner, R. P. (2008). The interactions and dissociations of the dorsal hippocampus subregions: How the dentate gyrus, CA3, and CA1 process spatial information. *Behavioral Neuroscience, 122*, 16-26.

Hafting, T., Fyhn, M., Molden, S., Moser, M. B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature, 436*, 801-806.

Hargreaves, E. L., Rao, G., Lee, I., & Knierim, J. J. (2005). Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. *Science, 308*, 1792-1794.

Jerman, J. T., Kesner, R. P., & Hunsaker, M. R. (2006). Disconnection analysis of CA3 and DG in mediating encoding but not retrieval in a spatial maze learning task. *Learning and Memory, 13*(4), 458-464.

Johnston, D., & Amaral, D. G. (2004). Hippocampus. In G. M. Shephard (Ed.), *The synaptic organization of the brain* (pp. 455-498). Oxford, UK: Oxford University Press.

Jung, M. W., & McNaughton, B. L. (1993). Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus, 3*(2), 165-182.

Kesner, R. P. (2007). A behavioral analysis of dentate gyrus function. *Progress in Brain Research, 163*, 567-576.

Kesner, R. P., Lee, I., & Gilbert, P. (2004). A behavioral assessment of hippocampal function based on a subregional analysis. *Reviews in the Neurosciences, 15,* 333-351.

Lee, I., & Kesner, R. P. (2004). Encoding versus retrieval of spatial memory: Double dissociation between the dentate gyrus and the perforant path inputs into CA3 in the dorsal hippocampus. *Hippocampus, 14,* 66-76.

Luu, T. T., Pirogovsky, E., & Gilbert, P. E. (2008). Age-related changes in contextual associative learning. *Neurobiology of Learning and Memory, 89,* 81-85.

McDonald, R. J., & White, N. M. (1995). Hippocampal and nonhippocampal contributions to place learning in rats. *Behavioral Neuroscience, 109*(4), 579-593.

O'Keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map.* Oxford, UK: Oxford University Press.

Olton, D. S., Becker, J. T., & Handelman, G. E. (1979). Hippocampus, space, and memory. *Behavioral and Brain Sciences, 2,* 313-322.

O'Reilly, R. C., & McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: Avoiding a trade-off. *Hippocampus, 4,* 661-682.

Rajii, T., Chapman, D., Eichenbaum, H., & Greene, R. (2006). The role of CA3 hippocampal NMDA receptors in paired associate learning. *Journal of Neuroscience, 26*(3), 908-915.

Rolls, E. T. (2010). A computational theory of episodic memory formation in the hippocampus. *Behavioural Brain Research, 215*(2), 1265-1274.

Rolls, E. T., & Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Progress in Neurobiology, 79,* 1-48.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery, and Psychiatry, 20,* 11-21.

Squire, L. R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychological Review, 99,* 195-231.

Talpos, J. C., McTighe, S. M., Dias, R., Saksida, L. M., & Bussey, T. J. (2010). Trial-unique, delayed nonmatching-to-location (TUNL): A novel, highly hippocampus-

dependent automated touchscreen test of location memory and pattern separation. *Neurobiology of Learning and Memory, 94*(3), 341-352.

Treves, A., & Rolls, E.T. (1994). Computational analysis of the role of the hippocampus in memory. *Hippocampus*, *4*, 374-391.

Tulving, E. (1983). *Elements of episodic memory*. London, UK: Oxford University Press.

CHAPTER 2

STUDY ONE: THE ROLE OF THE DENTATE GYRUS IN THE FORMATION OF CONTEXTUAL REPRESENTATIONS

Abstract

The hippocampus is involved in encoding and integrating contextual information. Recently, it has been suggested that the dorsal dentate gyrus (dDG) hippocampal subregion may mediate the formation of contextual representations of the spatial environment through a conjunctive encoding process whereby incoming multimodal information is integrated into a single higher-order representation. Despite anatomical evidence in support of this claim, behavioral evidence is limited. Therefore, a contextual associative learning paradigm was used to determine whether the dDG supports the formation of integrated contextual representations. Male Long-Evans rats were randomly assigned as controls or to receive bilateral intracranial infusions of colchicine into the dDG. Following recovery from surgery, each rat was tested on an appetitive task that required animals to form an association between a cue (odor) and a context in order to receive a food reward. Each rat received 10 trials per day and was tested for 10 consecutive days. Upon completion of testing, animals were tested on an additional

two-choice olfactory and contextual discrimination task. The testing order was counterbalanced across animals. Results showed that control animals successfully acquired the contextual associative learning task for olfactory stimuli as indicated by improved performance across the 10 testing days. In contrast, animals with dDG lesions were impaired in the ability to acquire the odor-context associations. Results from follow-up odor and context discrimination tests showed that both groups acquired the discriminations at similar rates. Therefore, it is unlikely that deficits in performance on the contextual associative learning task were due to an inability to discriminate between odors or contexts. Present findings provide further support for dDG involvement in the formation of conjunctive contextual representations.

Introduction

According to the conjunctive theory of hippocampal function, the HPP is involved in the formation of conjunctive associations, or the binding of multiple inputs into a single novel representation (O'Reilly & McClelland, 1994; O'Reilly & Rudy, 2001; Sutherland & Rudy, 1989). More recently, it has been suggested that this binding process may occur within the dDG hippocampal subregion (Kesner, 2007). For example, Kesner (2007) purported that the dDG may use a binding process, referred to as conjunctive encoding, to construct a contextual representation of the spatial environment. Contextual representations are an important feature of episodic memory and damage to the HPP produces deficits in tasks that require the integration of multimodal information (Langston & Wood, 2010; Rajii, Chapman, Eichenbaum, & Greene, 2006).

Support for the role of the DG in the formation of conjunctive contextual representations comes primarily from anatomical and electrophysiological recording studies (Amaral & Witter, 1995; Hafting, Fyhn, Molden, Moser, & Moser, 2005; Hargreaves, Rao, Lee, & Knierim, 2005). Based on anatomical descriptions of the hippocampal formation, the DG provides the main input zone for the HPP and receives its major cortical input from the EC via the perforant pathway (PP; Amaral & Witter, 1995). The EC inputs to the DG can be divided into a medial and lateral component (Hargreaves et al., 2005). The MEC input to the DG conveys spatial information and the LEC input conveys nonspatial information (e.g., auditory, olfactory, somatosensory, and visual; see Hafting et al., 2005; Hargreaves et al., 2005). It has been suggested that the DG may use a conjunctive encoding process to integrate multiple sensory inputs from medial and lateral portions of the EC into a single spatial representation (Kesner, 2007).

Currently, behavioral evidence in support of the dDG in conjunctive encoding is limited. However, two studies in particular have provided evidence for the formation of unitary contextual representations in the DG. For example, a study conducted by Hunsaker, Mooy, Swift, and Kesner (2007) was able to provide evidence for the integration of multimodal information in the DG based on a functional dissociation between EC inputs into the dDG. Direct infusions of either AP5 (an NMDA antagonist) or naloxone (an opioid antagonist) into dDG in rodents were used to disrupt medial and lateral perforant inputs, respectively. After receiving intracranial infusions into the dDG, animals were tested on an exploratory paradigm with a spatial and nonspatial component. Consistent with anatomical and electrophysiological recording data (Amaral & Witter, 1995; Hafting et al., 2005; Hargreaves et al., 2005), the results showed that rodents

infused with AP5 into the dDG were unable to detect a change in spatial location configurations, but displayed normal exploration for changes in object configurations. Infusions of naloxone into the dDG disrupted detection of change in both object and spatial location configurations. According to Hunsaker et al. (2007), the findings suggest that the dDG may combine spatial and nonspatial stimulus information received from the MEC and LEC to form a conjunctive contextual representation of the environment. Additionally, results from a gene knockout study conducted by Lee, Kim, Sun, and Jung (2009) found that mice with disrupted DG neural circuitry (BAX knockout mice) were impaired in the ability to integrate visual cue information with spatial representations in order to successfully navigate to a target location. Taken together, these studies suggest that the DG is important for integrating cortical inputs into spatial representations of the environment.

Given that few studies have directly tested the role of dDG in supporting conjunctive encoding of multiple sensory inputs, it is not entirely clear whether the dDG is necessary for the formation of integrated contextual representations. Therefore, the present study examined the role of the dDG in the formation of conjunctive contextual representations using a contextual associative learning task described by Luu, Pirogovsky, and Gilbert (2008; developed by Rajii et al., 2006).

Materials and Methods

Subjects

Twelve male Long-Evans rats, weighing approximately 250-350 g at the start of the experiment, were used as subjects. Each animal was housed in an individual plastic

container located in a colony room. The colony room was maintained on a 12H: 12H light/dark cycle and all testing was conducted during the light phase. All rats had unlimited access to water but were food restricted to 80-90% of their free-feed weight.

Surgical Procedures

All planned procedures and animal care were in accordance with the National Institute of Health and Institute for Animal Care and Use Committee guidelines and the Institutional Animal Care and Use Committee at the University of Utah. Each animal was randomly assigned as a control animal (n = 6) or to receive a bilateral dDG lesion (n = 6). Prior to surgery, subjects were deeply anesthetized using isoflurane gas, placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and then maintained with a continuous flow of isoflurane (2-4%) and medical air (1.5-2 L/min) and given atropine sulfate (0.54 mg/kg im). Each subject was prepared for the surgical procedure by applying a surgical drape and betadine antiseptic to the surgical site. An incision was made in the skin above the skull. The skin was retracted and small burr holes were drilled into the skull. Using a 10 µl Hamilton syringe, intracranial infusions of colchicines (2.5 mg/ml, 0.8 µl/site) were slowly infused (2.5 mg/mL, 20.0 uL/hr) into two dDG sites per hemisphere using the following coordinates: dDG: 2.7 mm posterior to bregma, 2.1 mm lateral to midline, 3.4 mm ventral from dura and 3.7 mm posterior to bregma, 2.3 mm lateral to midline, 3.0 mm ventral from dura. All lesion coordinates were based on Paxinos and Watson's (1997) stereotaxic atlas of the rat brain. For all injections, the injection cannula was left in place for at least 1 minute after the injection to allow for diffusion of the neurotoxin. Following all surgical procedures, each animal

received Children's Motrin in water as an analgesic and was given a 7-10 day recovery period prior to testing. Following recovery from surgery, each animal was tested on a contextual associative learning task. The same group of animals was tested on a two-choice olfactory discrimination task and a two- choice contextual discrimination task following the completion of the contextual associative learning task. The testing order between the olfactory and contextual discrimination tasks was counterbalanced across subjects.

Experiment 1: Contextual Associative Learning

Behavioral Apparatus

Testing was conducted in two clear Plexiglas boxes (18 x 16 x 15 inches) that represented two different contexts (Context 1 and Context 2). A context was defined by a combination of all environmental cues contained within the apparatus, including floor texture, color of walls, and visual cues on the walls. Context 1 had a black textured floor and walls with black stripes. Context 2 had a smooth white floor and each of the four walls was adorned with a single white geometric figure (i.e., triangle, square, circle, star).

Behavioral Procedures

Prior to testing, each rat was shaped in the home cage to dig in a cup filled with unscented sand to receive a food reward, a 1/2 piece of Froot Loop cereal (Kellog, Battle Creek, MI). The food reward was buried beneath the surface of the sand in order to

eliminate any potential visual cues. Once the animal consistently retrieved the food reward, the rat was assigned two pseudo-randomly selected odors (Odor A and Odor B) out of four possible odorants. Odor pairings were counterbalanced across animals and were used throughout all testing procedures. Olfactory stimuli consisted of supra-threshold powdered odorants (cinnamon, cumin, baby powder, or garlic) mixed in sand and presented in clear plastic cups (3 cm diameter and 3 cm high). Each rat received 10 trials per day (five trials in each context presented in a pseudo-random order) and was tested for 10 consecutive days. On each trial, the animal was removed from a start box and placed by the experimenter into a context box (either Context 1 or Context 2). In each context, the rat was presented with Odor A and B, positioned 6 cm apart from one another and placed against the far-facing wall of the apparatus. In Context 1, the rat received a food reward if it chose Odor A, not Odor B. In Context 2, the rat received a food reward if it chose Odor B, not Odor A. Therefore, the rat had to learn to associate Odor A with Context 1 and Odor B with Context 2. The position of Odor A and Odor B varied pseudo-randomly on each trial with respect to the left and right position in the context to eliminate position bias. A 2 min intertrial interval was used.

Experiment 2: Olfactory Discrimination

Behavioral Apparatus

The testing apparatus consisted of a rectangular nontransparent red Plexiglas box (60 cm long x 40 cm wide x 40 cm high) with one removable Plexiglas door to divide the box into two separate compartments (a start chamber and a choice chamber). The door

was manually raised and lowered by the experimenter to allow the animal to shuttle between the start chamber and the choice chamber.

Behavioral Procedures

Odor discrimination was assessed using a two-choice discrimination task described by Brushfield, Luu, Callahan, and Gilbert (2008). Odor pairings consisted of odors (Odor A and Odor B) previously assigned in the contextual association task. For each rat, one odor was pseudo-randomly assigned as the rewarded odor and the other as the nonrewarded odor. The rat began each trial in the start chamber of the apparatus with the door to the choice chamber closed. The door to the choice chamber was then raised and the rat was allowed to choose between the two odors presented side by side (6 cm apart) in the choice chamber of the apparatus. If the rat dug in the cup containing the rewarded odor, the rat received a food reward. However, if the rat dug in the cup containing the nonrewarded odor, the rat did not receive a reward and was not allowed to dig in the cup containing the rewarded odor. Therefore, the rat had to learn to dig in the rewarded odor and to avoid digging in the nonrewarded odor. The position of each odor varied pseudo-randomly on each trial, with respect to the left and right position in the choice chamber, to eliminate position bias. Each animal was tested until the animal reached a criterion of nine correct choices out of a sliding block of 10 consecutive trials. The experimenter recorded the digging response of each rat and the number of trials required to reach the criterion was used as the dependent measure. A 30 s intertrial interval was used.

Experiment 3: Contextual Discrimination

Behavioral Apparatus

Testing was conducted in Context 1 and Context 2 described in the contextual associative learning task.

Behavioral Procedures

Context discrimination was assessed using a similar procedure used to assess odor discrimination; however, two contexts were used rather than two odors. Each context contained a single clear plastic cup filled with unscented sand. For each rat, one context was pseudo-randomly assigned as the rewarded context and contained a cup filled with unscented sand and a food reward. The other context was assigned as the nonrewarded context and contained a cup filled with unscented sand that did not contain a food reward. Prior to each trial, the animal was placed in a chamber box. On each trial, the door to the chamber box was manually raised and the rat was allowed to choose between the two contexts (Context 1 and Context 2) and dig in the unscented odor cup. If the rat entered the rewarded context and made a digging response in the unscented odor cup, the rat received a food reward. However, if the rat entered the nonrewarded context and made a digging response in the unscented odor cup, the rat did not receive a reward. The position of each context varied pseudo-randomly on each trial, with respect to the left and right position, to eliminate position bias. Each animal was tested until the animal reached a criterion of nine correct choices out of a sliding block of 10 consecutive trials. The experimenter recorded the response of each rat and the number of trials required to reach the criterion was used as the dependent measure. A 30 s intertrial interval was used.

Histological Procedures

At the conclusion of all testing, each animal was deeply anesthetized with an intraperitoneal injection of 1.5 ml sodium pentobarbital (70 mg/kg), and perfused intracardially with normal saline followed by a 10% formalin solution. The brain was removed from the skull and stored in a 10% formalin/30% sucrose solution in a refrigerator (4°C) for 72 hours to equalize the tissue-shrinkage rates across brains. For dDG lesions, a tissue block (Bregma -2.0 through ~ -4.0) containing only the dorsal hippocampus was cut using coronal sections. The block was frozen and cut at 24 µm sections with every third section mounted on a glass slide (the surface-to-surface distance between collected sections = 72 µm). The sections were stained with cresyl violet and examined for histological verification of the lesion placement.

Results

Histological Results

Axon-sparing, selective bilateral lesions of the dDG were made with colchicine. A representative dDG lesion and intended target zone is shown in Figure 1. In addition, a representative vehicle-infused control lesion is shown in Figure 2.

Behavioral Results

Contextual Associative Learning Task

Figure 3 shows the mean (± SE) number of correct responses on the contextual associative learning task as a function of days (1-10) for dDG lesioned rats and control

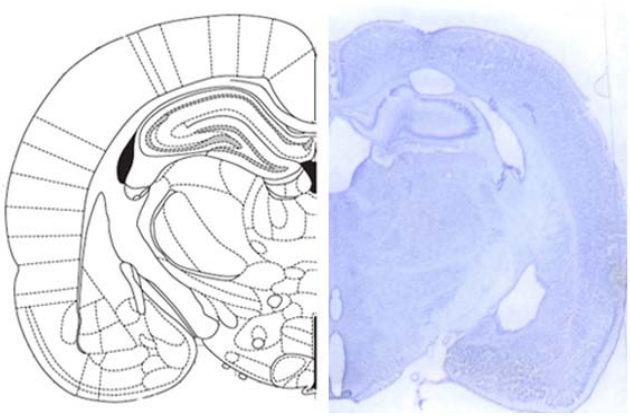

Figure 1. Histological representation of a dDG lesioned rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997).

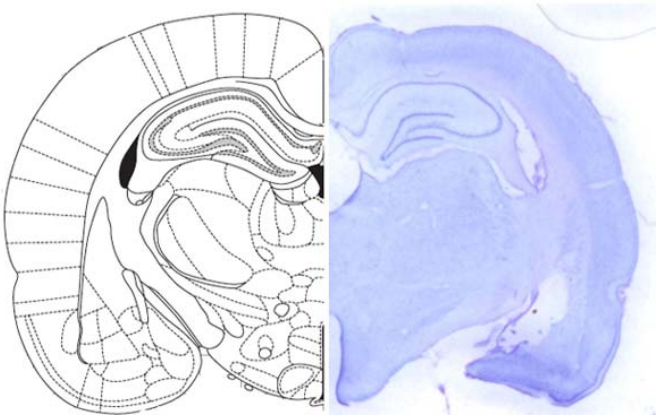

Figure 2. Histological representation of a vehicle-infused control rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997).

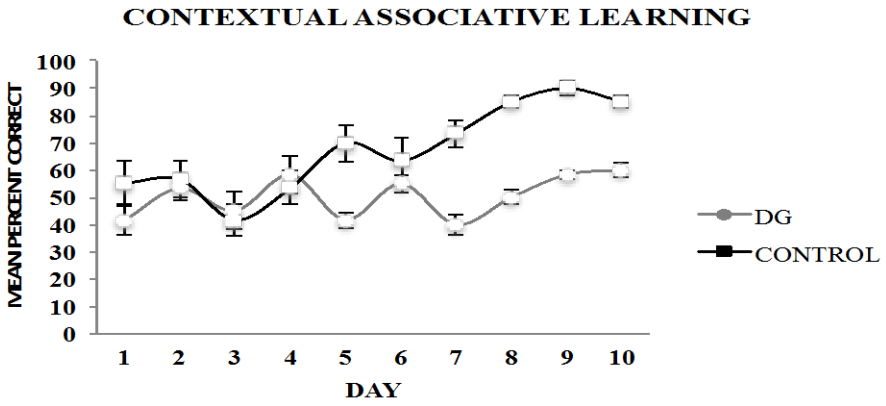

Figure 3. Mean (± SE) percent correct performance of DG lesioned rats and control rats on the contextual associative learning task as a function of days (1-10).

rats. A 2 x 10 analysis of variance (ANOVA) with group (dDG, control) as the between-group factor and days (1-10) as a within-group factor was used to analyze the data. The results revealed a significant main effect of group, $F(1, 10) = 42.36$, $p < .001$. There also was a significant main effect of day (1-10), $F(9, 90) = 7.49$, $p < .001$ and a significant group x day interaction, $F(9, 90) = 4.59$, $p < .001$.

A Newman-Keuls post hoc comparison test of the group x day interaction revealed no significant differences in performance between dDG lesioned rats and control rats during the first 4 days of testing. However, control animals significantly outperformed dDG lesioned animals on Day 5 ($p < .05$) and continued to outperform dDG lesioned rats on Day 6 and performed significantly better on Day 7-10 ($p < .05$). In addition, control rats performed significantly better on Day 5 and 7 compared to Day 3 ($p < .05$) and also performed significantly better on Day 8-10 compared to the first 6 days of testing ($p < .05$). Thus, the results showed that control animals successfully acquired

the contextual associative learning task for olfactory stimuli as indicated by improved performance across the 10 testing days. However, there were no significant differences in performance across testing days among the dDG lesioned animals, indicating an impaired ability to acquire contextual associations.

Olfactory Discrimination Task

Figure 4 shows the mean (± SE) trials to criterion on the olfactory discrimination task for dDG lesioned rats and control rats. A one-way ANOVA group (dDG, control) as a between-group factor was used to analyze the results from the odor discrimination task. There were no significant differences in acquisition rates between dDG lesioned animals and control animals, $F(1,10) = .01$, $p = .94$.

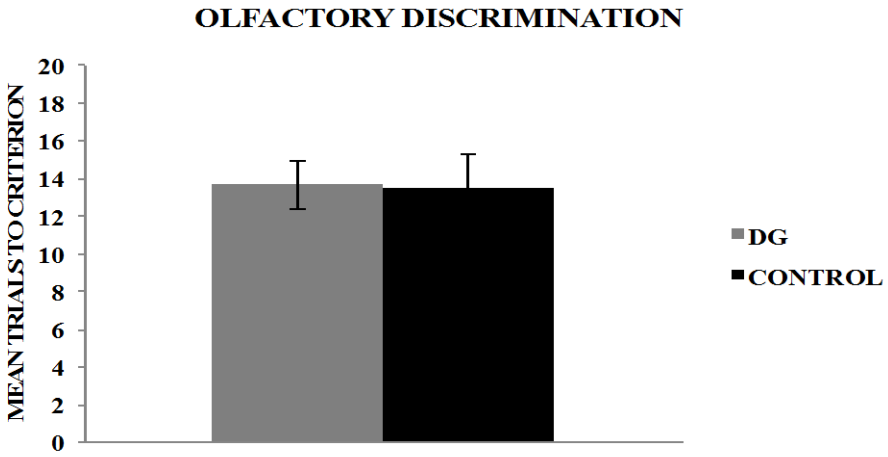

Figure 4. Mean (± SE) of dDG lesioned rats and control rats on the olfactory discrimination task.

Contextual Discrimination Task

Figure 5 shows the mean (± SE) trials to criterion on the contextual discrimination task for dDG lesioned rats and control rats. A one-way ANOVA with group (dDG, control) as a between-group factor was used to analyze the results from the context discrimination task. There were no significant differences in acquisition rates between dDG lesioned animals and control animals, $F(1, 10) = 2.39$, $p = .15$.

Discussion

The present study investigated the role of the dDG in the formation of conjunctive contextual representations using a contextual associative learning task that required animals to form an association between an odor and a context in order to receive a food reward. The results showed that control animals successfully acquired the odor-context

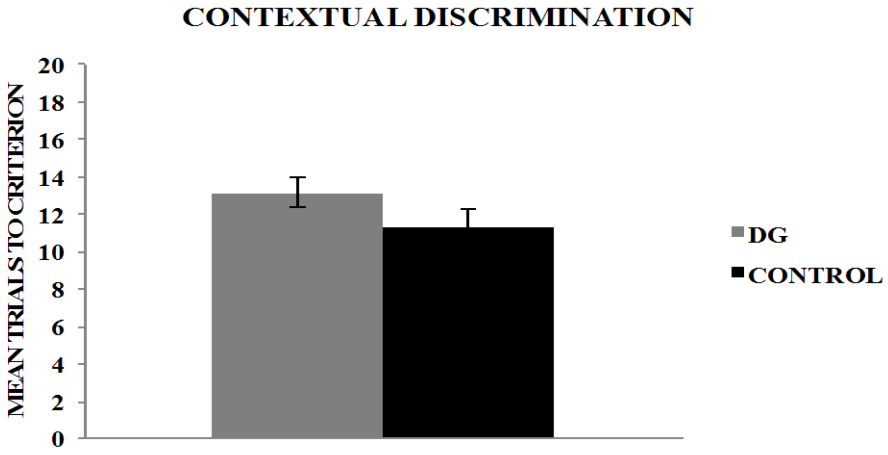

Figure 5. Mean (± SE) of dDG lesioned rats and control rats on the contextual discrimination task.

associations as indicated by improved performance across the 10 testing days. In contrast, animals with dDG lesions showed an impaired ability to acquire the odor-context associations as indicated by performance that remained near chance level across testing days. These results suggest that selective lesions of the dDG in rats disrupt acquisition of odor-context associations.

In order to rule out the possibility that impairments in contextual associative learning were attributable to a difficulty in differentiating between individual elements of the task, each animal was tested on an additional two-choice olfactory discrimination task and a two-choice contextual discrimination task. The results showed that control animals and dDG lesioned animals acquired the olfactory discrimination task at similar rates. In addition, both groups of animals acquired the contextual discriminations at similar rates. Therefore, it is unlikely that performance deficits on the contextual associative learning task were due to an inability to discriminate between odors or contexts. Consistent with prior reports of hippocampal involvement in contextual associative memory (Eichenbaum, 2004; Komorowski, Manns, & Eichenbaum, 2009; Langston & Wood, 2010; Rajii et al., 2006), the present findings suggest that the dDG hippocamapal subregion is involved in the formation of conjunctive associations between a specific stimulus and a context.

Previous research suggests that the DG plays an important role in the encoding of spatial information during new learning (Eldridge, Engel, Zeineh, Bookheimer, & Knowlton, 2005; Jerman, Kesner, & Hunsaker, 2006; Lee & Kesner, 2004; Rolls & Kesner, 2006). There also is considerable evidence to suggest that the dDG plays a critical role in spatial pattern separation, a mechanism for encoding and separating

spatially similar events into distinct representations (Gilbert, Kesner, & Lee, 2001; Kesner, Lee, & Gilbert, 2004; Leutgeb, Leutgeb, Moser, & Moser, 2007; Rolls & Kesner, 2006). More recently, it has been suggested that the dDG may support the formation of distinct representations through a conjunctive encoding process that integrates multiple sensory inputs into a highly organized contextual representation of the spatial environment (Kesner, 2007). In support of this claim, electrophysiological recording data and anatomical descriptions of the HPP demonstrate a convergence of spatial and non-spatial information from the EC onto the DG (Amaral & Witter, 1995; Hafting et al., 2005; Hargreaves et al., 2005). In addition, there is some behavioral evidence to indicate that DG disruptions in rodents impair the formation of conjunctive representations of the spatial environment (Hunsaker et al., 2007; Lee et al., 2009). Results from the present study are consistent with previous findings and provide further support for the role of the dDG in the formation of integrated contextual representations.

Although findings from the present investigation provide support for dDG involvement in the formation of conjunctive contextual associations, there is also evidence to demonstrate CA3 hippocampal subregion involvement in the acquisition of paired associations (Gilbert & Kesner, 2003; Rajii et al., 2006; Rolls & Kesner, 2006). More specifically, previous accounts of hippocampal function suggest that CA3 supports the formation of arbitrary associations, or associations formed between seemingly disparate inputs (e.g., visual input with a spatial input; Gilbert & Brushfield, 2009; Kesner, Hunsaker, & Warthen, 2008; Rolls, 1996; Rolls & Kesner, 2006), and studies have shown that selective damage to dorsal CA3 (dCA3) produces impairments on paired associate learning paradigms that require the formation of an association between a

stimulus and a spatial location (Gilbert & Kesner, 2003; Kesner et al., 2008). For example, Gilbert and Kesner (2003) tested rats with lesions of the dDG, dCA3, or dorsal CA1 (dCA1) on an odor-place and object-place paired associate task. The results showed that dCA3 lesioned animals were significantly impaired in acquisition for both paired associate tasks. However, dDG and dCA1 failed to produce disruptions in paired associate learning for object-place or odor-place associations. Although these results appear to contradict the present findings, it should be mentioned that context was not directly manipulated in the Gilbert and Kesner (2003) study. Therefore, it may be the case that the dDG is particularly sensitive to associative learning that involves the formation of associations between a stimulus and a context (defined by a combination of multimodal information) rather than between a stimulus and a location.

There also is evidence to suggest that CA3 is involved in the acquisition of novel contextual associations (Komorowski et al., 2009; Rajii et al., 2006). For example, the paradigm used in the present study was originally developed by Rajii and colleagues (2006) and was used to examine CA3 hippocampal NMDA receptor involvement in paired associate learning. The results showed that mice with CA3 NR1 gene deletions were impaired in the acquisition of odor-context associations. Additionally, an electrophysiological recording study using a similar paired associate task in rats found that CA3 neurons developed specific firing responses to odor-context pairs that corresponded to learning. In other words, as the animal learned to associate the rewarded odor with the appropriate context (e.g., Odor A with Context 1) the firing rates increased significantly.

Although results from these studies (Komorowski et al., 2009; Rajii et al., 2006) provide evidence for CA3 in contextual associative learning, they do not exclude possible dDG involvement given that the dDG was not directly examined. In addition, using the same behavioral paradigm as described above, results from the present study provide direct evidence for dDG involvement in the formation of odor-context associations. Therefore, the possibility remains that CA3 related deficits in the formation of contextual associations observed in the Rajii et al. (2006) study might be attributable to an impaired ability to receive and utilize information fed forward from the dDG. CA3 also receives a direct projection from the EC (Amaral & Witter, 1995); however, it is considered to be relatively weak in comparison to the powerful mossy fiber projections from the DG into CA3 (Rolls, 2008). Moreover, results from the BAX-KO study conducted by Lee and colleagues (2009) suggest that an intact DG is necessary for combining environmental cues with internal spatial representations whereas the EC-CA3 direct pathway alone was incapable of supporting the formation of conjunctive associations.

In conclusion, results from the present investigation suggest that the dDG hippocampal subregion is involved in contextual associative learning that requires the formation of an association between an odor and a context. These findings provide further support for dDG involvement in the formation of conjunctive contextual representations and may have important implications for understanding episodic memory formation.

Acknowledgements

The research was supported by NIH grant #MH065314 to Raymond P. Kesner. The authors would like to thank Dr. Jeanine Stefanucci for her helpful suggestions on the manuscript. We also thank Samantha Domingo and Genevieve Smith for their assistance with data collection and James Taylor for his assistance with histological procedures.

Please address all correspondence to Dr. Raymond Kesner, University of Utah, Department of Psychology, 380 South 1530 East, Room 502, University of Utah, Salt Lake City, UT 84112, USA. Email: ray.kesner@psych.utah.edu.

References

Brushfield, A. M., Luu, T., Callahan, B. D., & Gilbert, P. E. (2008). A comparison of discrimination and reversal learning for olfactory and visual stimuli in aged rats. *Behavioural Neuroscience, 122*, 54-62.

Eichenbaum, H. (2004). Hippocampus: Cognitive processes and neural representations that underlie declarative memory. *Neuron, 44*, 109-120.

Eldridge, L. L., Engel, S. A., Zeineh, M. M., Bookheimer, S. Y., & Knowlton, B. J. (2005). A dissociation of encoding and retrieval processes in the human hippocampus. *The Journal of Neuroscience, 25*(13), 3280-3286.

Gilbert, P. E., & Brushfield, A. M. (2009). The role of the CA3 hippocampal subregion in spatial memory: A process oriented behavioral assessment. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33*(5), 774-781.

Gilbert, P. E., & Kesner, R. P. (2003). Localization of function within the dorsal hippocampus: The role of the CA3 subregion in paired-associate learning. *Behavioral Neuroscience, 117*, 1385-1394.

Gilbert, P. E., Kesner, R. P., & Lee, I. (2001). Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. *Hippocampus, 11*(6), 626-636.

Hafting, T., Fyhn, M., Molden, S., Moser, M. B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature*, *436*, 801-806.

Hargreaves, E. L., Rao, G., Lee, I., & Knierim, J. J. (2005). Major dissociation between medial and lateral entorhinal input to dorsal hippocampus. *Science*, *308*, 1792-1794.

Hunsaker, M. R., Mooy, G. G., Swift, J. S., & Kesner, R. P. (2007). Dissociations of the medial and lateral perforant path projections into dorsal DG, CA3, and CA1 for spatial and nonspatial (visual object) information processing. *Behavioral Neuroscience, 121*, 742-750.

Jerman, J. T., Kesner, R. P., & Hunsaker, M. R. (2006). Disconnection analysis of CA3 and DG in mediating encoding but not retrieval in a spatial maze learning task. *Learning and Memory*, *13*(4), 458-464.

Kesner, R. P. (2007). A behavioral analysis of dentate gyrus function. *Progress in Brain Research*, *163*, 567-576.

Kesner, R. P., Hunsaker, M. R., & Warthen, M. W. (2008). The CA3 subregion of the hippocampus is critical for episodic memory processing by means of relational encoding in rats. *Behavorial Neuroscience*, *122,* 1217-1225.

Kesner, R. P., Lee, I., & Gilbert, P. (2004). A behavioral assessment of hippocampal function based on a subregional analysis. *Reviews in the Neurosciences*, *15*, 333-351.

Komorowski, R. W., Manns, J. R., & Eichenbaum, H. (2009). Robust conjunctive item-place coding by hippocampal neurons parallels learning what happens where. *Journal of Neuroscience, 29*, 9918-9929.

Langston, R. F., & Wood, E. R. (2010). Associative recognition and the hippocampus: Differential effects of hippocampal lesions on object-place, object-context and object-place-context memory. *Hippocampus, 20*, 1139-1159.

Lee, I., & Kesner, R. P. (2004). Encoding versus retrieval of spatial memory: Double dissociation between the dentate gyrus and the perforant path inputs into CA3 in the dorsal hippocampus. *Hippocampus*, *14*, 66-76.

Lee, J. W., Kim, W. R., Sun, W., & Jung, M. W. (2009). Role of dentate gyrus in aligning internal spatial map to external landmark. *Learning and Memory*, *16*, 530-536.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science, 315*(5814), 961-966.

Luu, T. T., Pirogovsky, E., & Gilbert, P. E. (2008). Age-related changes in contextual associative learning. *Neurobiology of Learning and Memory*, *89*, 81-85.

O'Reilly, R. C., & McClelland, J. L. (1994). Hippocampal conjunctive encoding, storage, and recall: Avoiding a trade-off. *Hippocampus*, *4*, 661-682.

O'Reilly, R. C., & Rudy, J. W. (2001). Conjunctive representations in learning and memory: Principles of cortical and hippocampal function. *Psychological Review, 108*(2), 311-45.

Paxinos, G., & Watson, C. (1997). *The rat brain in stereotaxic coordinates*. New York, NY: Academic Press.

Rajii, T., Chapman, D., Eichenbaum, H., & Greene, R. (2006). The role of CA3 hippocampal NMDA receptors in paired associate learning. *Journal of Neuroscience*, *26*(3), 908-915.

Rolls, E. T. (1996). A theory of hippocampal function in memory. *Hippocampus*, *6*, 601-620.

Rolls, E. T. (2008). *Memory, attention, and decision-making: A unifying computational neuroscience approach*. Oxford, UL: Oxford University Press.

Rolls, E. T., & Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Progress in Neurobiology*, *79*, 1-48.

Sutherland, R. J., & Rudy, J. W. (1989). Configural association theory: The role of the hippocampal formation in learning, memory, and amnesia. *Psychobiology, 17*, 129-144.

CHAPTER 3

STUDY TWO: SELECTIVE LESIONS OF THE DENTATE GYRUS PRODUCE DISRUPTIONS IN PLACE LEARNING FOR ADJACENT SPATIAL LOCATIONS

Abstract

The hippocampus (HPP) plays a known role in learning novel spatial information. More specifically, the dentate gyrus (DG) hippocampal subregion is thought to play an integral role in pattern separation, a mechanism for encoding and separating spatially similar events into distinct representations. Several studies have shown that lesions of the dorsal DG (dDG) in rodents result in inefficient spatial pattern separation for working memory; however, it is unclear whether selective dDG lesions disrupt spatial pattern separation for reference memory. Therefore, the current study investigated the role of the dDG in pattern separation using a spatial reference memory paradigm to determine whether the dDG is necessary for acquiring spatial discriminations for adjacent locations. Male Long-Evans rats were randomly assigned as control animals or to receive bilateral intracranial infusions of colchicine into the dDG. Following recovery from surgery, each rat was pseudo-randomly assigned to an adjacent or separate condition and subsequently tested on a place learning task using an eight-arm radial maze. Rats were trained to

discriminate between a rewarded arm and a nonrewarded arm that were adjacent to one another or separated by a distance of two arm positions. Each rat received 10 trials per day and was tested until the animal reached a criterion of nine correct choices out of 10 consecutive trials across 2 consecutive days of testing. Both groups acquired spatial discriminations for the separate condition at similar rates. However, in the adjacent condition, dDG lesioned animals required significantly more trials to reach the learning criterion than controls. The results suggest that dDG lesions decrease efficiency in pattern separation resulting in impairments in the adjacent condition. In the separate condition, there was less overlap among distal cues during encoding and less need for pattern separation. These findings provide support for a more general role for the dDG in spatial pattern separation by demonstrating the importance of a processing mechanism that is capable of reducing interference among overlapping spatial inputs across a variety of memory demands.

Introduction

The HPP plays a known role in learning and memory processes. In particular, many claim that a primary mnemonic function of the HPP is to reduce interference among similar inputs during learning allowing for more accurate encoding and retrieval of a memory event (Gilbert, Kesner, & Lee, 2001; O'Reilly & Rudy, 2001; Shapiro & Olton, 1994). The process for reducing interference among overlapping memory representations is referred to as pattern separation (Gilbert et al., 2001; Rolls & Kesner, 2006; Shapiro & Olton, 1994). Specifically, pattern separation may serve to encode and separate highly overlapping spatial information into distinct representations so that one

place can be remembered as separate from another (Gilbert & Brushfield, 2009; Rolls & Kesner, 2006).

Computational models of hippocampal function suggest that the HPP may support pattern separation based on sparse but powerful connections between DG granule cells and CA3 pyramidal cells coupled with the low probability that the same set of CA3 cells will receive inputs from a similar set of DG granule cells (Jung & McNaughton, 1993; Rolls & Kesner, 2006). The DG receives its major cortical input from the EC via the perforant pathway. Information is then fed forward to CA3 along the mossy fiber projection system (Amaral & Witter, 1995; Johnston & Amaral, 2004) and there is evidence to suggest that this pathway may play a prominent role during encoding of spatial information thereby facilitating the formation of distinct memory representations (Bakker, Kirwan, Miller, & Stark, 2008; Eldridge, Engel, Zeineh, Bookheimer, & Knowlton, 2005; Jerman, Kesner, & Hunsaker, 2006; Lee & Kesner, 2004; Rolls, 2010).

Electrophysiological recording data and evidence from behavioral studies provide additional support for hippocampal involvement in pattern separation processes (Fyhn, Hafting, Treves, Moser, & Moser, 2007; Gilbert, Kesner, & DeCouteau, 1998; Jung & McNaughton, 1993; S. Leutgeb et al., 2005; Renaudineau, Poucet, & Save, 2007). In addition, pattern separation studies have been conducted in both humans and rodents (Bakker et al., 2008; Gilbert et al., 1998; Kirwan & Stark, 2007; Lacy, Yassa, Stark, Muftuler, & Stark, 2010; McHugh et al., 2007; McTighe, Mar, Romberg, Bussey, & Saksida, 2009). For example, a functional magnetic resonance imaging (fMRI) study conducted by Kirwan and Stark (2007) tested participants on a continuous recognition task that required pattern separation to differentiate between similar visual stimuli.

Participants were shown a series of pictures of everyday objects and were asked to make "new, old, or similar" judgments when each visual object was presented. The results showed that HPP activity accurately differentiated between objects that were previously seen (old), and objects that were similar to previously seen objects. Further, there is evidence to suggest that damage to the HPP in rats results in an inability to distinguish between spatial locations with a high degree of similarity among proximal and distal cues (Gilbert et al., 1998). Taken together, findings from these studies suggest that the HPP is important for reducing interference among memory representations with a high degree of similarity.

Subregional accounts of hippocampal function suggest that the dDG plays a critical role in spatial pattern separation (Gilbert et al., 2001; Kesner, 2007; Kesner, Lee, & Gilbert, 2004; Rolls & Kesner, 2006). In support of this mnemonic processing role, several studies have shown that disruptions of the DG in rats are capable of producing functional alterations in pattern separation on spatial working memory tasks, or tasks that require use of information that is trial unique (Emerich & Walsh, 1989; Gilbert et al., 2001; Goodrich-Hunsaker, Hunsaker, & Kesner, 2008; Olton, 1978; Talpos, McTighe, Dias, Saksida, & Bussey, 2010). For example, Gilbert and colleagues (2001) tested rats with selective dDG lesions on a delayed-match-to-sample (DMTS) for spatial location task that was designed to measure the ability to discriminate between spatial locations that varied in spatial similarity. On each trial, animals were given a choice between two identical objects that were separated by one of five spatial separations (15 cm to 105 cm). The results showed that rats with dDG lesions were impaired at short separations (high degree of overlap among distal cues); however, their performance increased as the

distance between the two objects increased (lessening degree of overlap among distal cues). Similar results were obtained in a study conducted by Goodrich-Hunsaker et al. (2008). Using a spontaneous recognition task, they showed that rats with dDG lesions were incapable of detecting a change in metric distance between two identical objects on a cheeseboard maze as evidenced by a reduction in exploration for the displaced objects. Taken together, results from these studies suggest that the dDG hippocampal subregion is important for reducing interference among working memory representations with a high degree of spatial similarity. The results also indicate that the dDG may be particularly sensitive to manipulations in metric distance (Kesner, 2007).

The HPP was previously thought to support spatial working memory but not spatial reference memory, or memory for information that remains constant across time (Olton, Becker, & Handelman, 1979). Since that time, several studies have shown that HPP damage in rats produces acquisition impairments on spatial reference memory tasks (McDonald & White, 1995; McTighe et al., 2009; Morris, Garrud, Rawlins, & O'Keefe, 1982). For example, McDonald and White (1995) tested rats with fornix-fimbria lesions on an active place-learning paradigm that required animals to distinguish between spatial locations on an eight-arm radial maze with a high degree of similarity among extra-maze cues. The results showed that lesioned animals were impaired in acquiring spatial discriminations when spatial locations were adjacent to each other; however, their performance matched normal control animals when the spatial locations were widely separated. The findings from this study suggest that the HPP is necessary for acquiring spatial discriminations for proximal spatial locations.

In addition, several studies have shown that selective lesions of the DG in rodents disrupt performance on spatial reference memory tasks (McLamb, Mundy, & Tilson, 1988; Nanry, Mundy, & Tilson, 1989; Okada & Okaichi, 2009; Xavier, Oliveira-Filho, & Santos, 1999). However, the distance between spatial locations was not directly manipulated in these studies. Therefore, the present study directly examined the role of the dDG in spatial pattern separation for reference memory using an active place-learning paradigm described by McDonald and White (1995) in order to determine whether an intact dDG is necessary for acquiring spatial discriminations for proximal spatial locations. Acquisition impairments would provide support for a more general role for the dDG in the encoding and separation of distinct spatial memory representations by demonstrating the importance of a processing mechanism that is capable of reducing interference among overlapping inputs across a variety of different memory demands.

Materials and Methods

Subjects

Twenty-four male Long-Evans rats, weighing approximately 250-350 g at the start of the experiment, were used as subjects. Each animal was housed in an individual plastic container located in a colony room. The colony room was maintained on a 12H:12H light/dark cycle and all testing was conducted during the light phase. All rats had unlimited access to water but were food restricted to 80-90% of their free-feed weight.

Surgical Procedures

All planned procedures and animal care were in accordance with the National Institute of Health and Institute for Animal Care and Use Committee guidelines and the Institutional Animal Care and Use Committee at the University of Utah. Each animal was randomly assigned as a control animal (n = 12) or to receive a bilateral dDG lesion (n = 12). Prior to surgery, subjects were deeply anesthetized using isoflurane gas, placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and then maintained with a continuous flow of isoflurane (2-4%) and medical air (1.5-2 L/min) and given atropine sulfate (0.54 mg/kg im). Each subject was prepared for the surgical procedure by applying a surgical drape and betadine antiseptic to the surgical site. An incision was made in the skin above the skull. The skin was retracted and small burr holes were drilled into the skull. Using a 10 µl Hamilton syringe, intracranial infusions of colchicine (2.5 mg/ml, 0.8 µl/site) were slowly infused (2.5 mg/mL, 20.0 uL/hr) into two dDG sites per hemisphere using the following coordinates: dDG: 2.7 mm posterior to bregma, 2.1mm lateral to midline, 3.4 mm ventral from dura and 3.7 mm posterior to bregma, 2.3 mm lateral to midline, 3.0 mm ventral from dura. All lesion coordinates were based on Paxinos and Watson's (1997) stereotaxic atlas of the rat brain. For all injections, the injection cannula was left in place for at least 1 minute after the injection to allow for diffusion of the neurotoxin. Following all surgical procedures, each animal received Children's Motrin in water as an analgesic and was given a 7-10 day recovery period prior to testing.

Behavioral Apparatus

Testing was conducted in an eight-arm radial maze. The maze consisted of an octagonal central platform 42 cm in diameter with eight arms radiating from the central platform like the spokes of a wheel. Each arm was 71 cm long and 9.5 cm wide and was attached to the central platform with metal braces. Each arm had 0.3 cm-thick clear Plexiglas sides, which rose 5.7 cm above the surface of the arm. A food-well, 2.5 cm in diameter, was drilled 1.5 cm deep at the distal end of each arm. A 0.3 cm-thick Plexiglas guillotine door was located at the juncture between the platform and the arm. Each door was 10 cm wide, and when raised, extended 18 cm above the surface of the platform. The doors were manually raised and lowered by the experimenter to permit entrance to the arms. An opaque cylindrical bucket (38 cm in diameter and 75 cm in height) was positioned directly over the central platform and was manually raised and lowered by the experimenter from a room located directly outside the testing room. The maze was located in the center of a windowless room containing a variety of distal cues.

Behavioral Procedures

Prior to testing, each animal was allowed to individually explore the test apparatus for 0.25 hr. During the exploration period, Froot Loop cereal (Kellogg, Battle Creek, MI) was distributed across the surface of the apparatus (including each individual food well) and the guillotine doors were lowered to permit the animal to explore each arm of the apparatus and to retrieve the food reward. Once each rat had been acclimated to the apparatus, they were pseudo-randomly assigned to an adjacent condition

(dDG n = 6; control n = 6) or a separate condition (dDG n = 6; control n = 6) and subsequently tested on an active place-learning paradigm described by McDonald and White (1995).

For the adjacent condition, one of the eight arms of the radial maze was assigned as the rewarded arm. The arms positioned immediately to the left and right of the rewarded arm were assigned as the nonrewarded arms (see Figure 6). Prior to the beginning of each testing session, the animal was placed on the center platform and an opaque cylindrical bucket was manually lowered over the rat and the experimenter lowered the doors of the designated rewarded arm and one of the two nonrewarded arms. Two different nonrewarded arms were randomly used to ensure that the rats did not adopt a simple response strategy that could provide an accurate nonspatial solution to the task if only one nonrewarded arm was used. On each trial, the bucket was raised and the rat was allowed to choose between a designated rewarded arm and the nonrewarded arm. If the rat entered the rewarded arm, then the rat received a food reward; however, if the rat entered a nonrewarded arm, then the rat did not receive a food reward and was not allowed to enter the arm containing the food reward. Each of the two nonrewarded arms was used on 5 of the 10 daily trials in a pseudo-randomly determined order. The same arms were used throughout all testing procedures. Each rat received 10 trials per day with a 60 s intertrial interval. Testing was conducted daily and each animal was tested until the animal reached a criterion of nine correct choices out of 10 consecutive trials across two consecutive days of testing or until the animal was tested for 20 consecutive days without reaching the learning criterion.

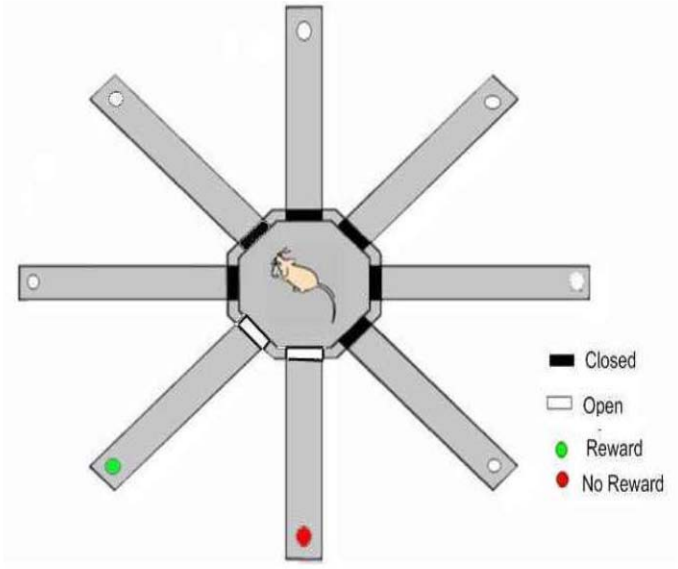

Figure 6. Schematic of eight-arm radial maze configuration for the adjacent condition of the place-learning task.

The separate condition was conducted using an identical procedure and criterion as described for the adjacent condition except that the rewarded arm was separated from the two possible nonrewarded arms by a distance of two arm positions (see Figure 7).

Histological Procedures

At the conclusion of all testing, each animal was deeply anesthetized with an intraperitoneal injection of 1.5 ml sodium pentobarbital (70 mg/kg), and perfused intracardially with normal saline followed by a 10% formalin solution. The brain was removed from the skull and stored in a 10% formalin/30% sucrose solution in a refrigerator (4°C) for 72 hours to equalize the tissue-shrinkage rates across brains. For dDG lesions, a tissue block (Bregma -2.0 through ~ -4.0) containing only the dorsal

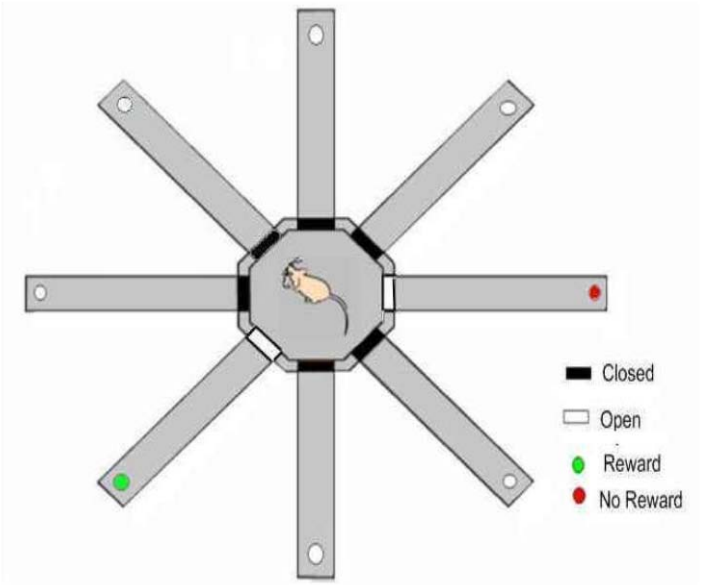

Figure 7. Schematic of eight-arm radial maze configuration for the separate condition of the place-learning task.

hippocampus was cut using coronal sections. The block was frozen and cut at 24 µm sections with every third section mounted on a glass slide (the surface-to-surface distance between collected sections = 72 µm). The sections were stained with cresyl violet and examined for histological verification of the lesion placement.

Results

Histological Results

Axon-sparing, selective bilateral lesions of the dDG were made with colchicine. A representative dDG lesion and intended target zone is shown in Figure 8. In addition, a representative vehicle-infused control lesion is shown in Figure 9.

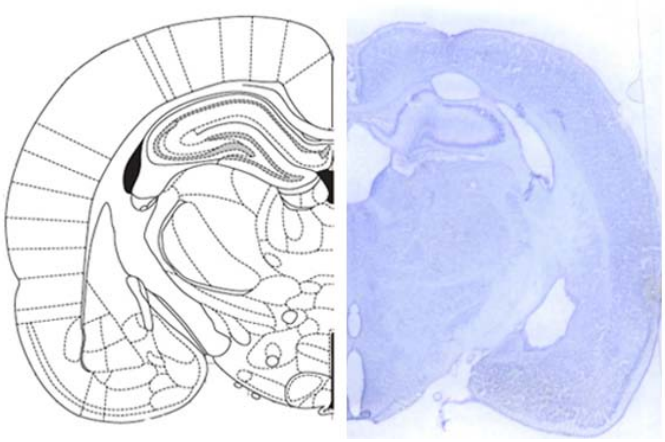

Figure 8. Histological representation of a dDG lesioned rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997).

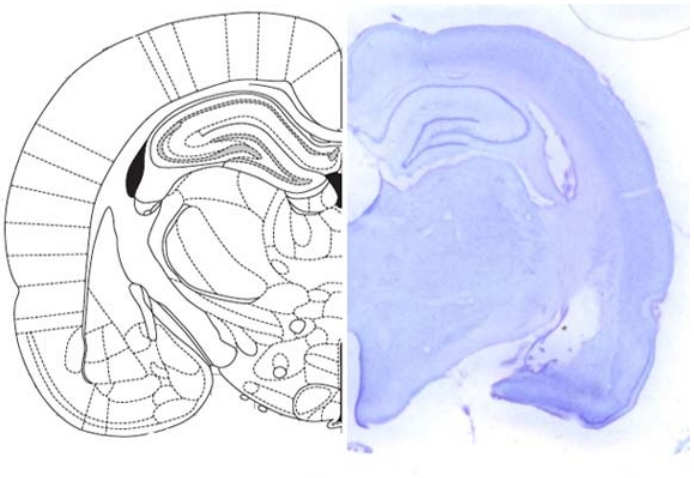

Figure 9. Histological representation of a vehicle-infused control rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997).

Behavioral Results

Figure 10 shows the mean (± SE) number of trials required by dDG lesioned rats and control rats to reach the learning criterion on the separate and adjacent conditions of the place-learning task. A 2x2 analysis of variance (ANOVA) with group (dDG, control) and condition (adjacent, separate) as between-group factors was used to analyze the data. The dependent variable was the mean number of trials required to reach the learning criterion of nine correct choices out of 10 consecutive trials across 2 consecutive days of testing. The results revealed a significant main effect of group, $F(1, 20) = 4.67$, $p = .04$, indicating that control rats outperformed dDG lesioned rats regardless of task condition. There also was a significant main effect of condition, $F(1, 20) = 18.94$, $p \leq .001$, indicating that rats acquired the spatial discriminations for the separate condition at a faster rate than the adjacent condition. In addition, there was a significant group x condition interaction, $F(1, 20) = 10.07$, $p = .01$.

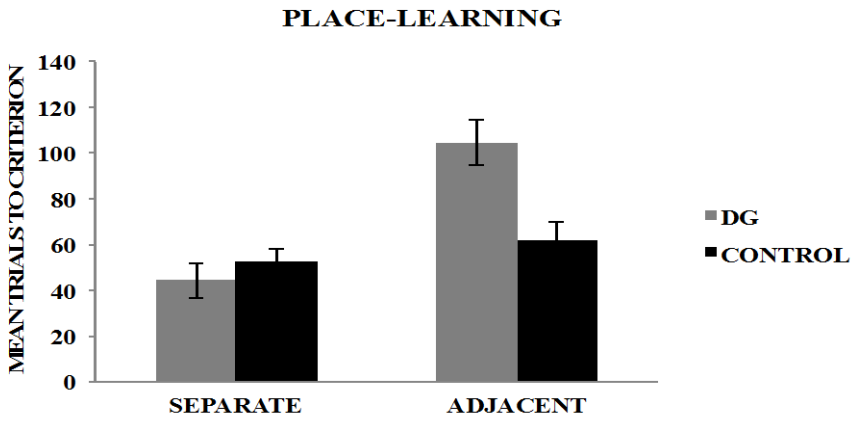

Figure 10. Mean (± SE) trials to criterion for dDG lesioned rats and control rats on the separate and adjacent conditions of the place-learning task.

A Newman-Keuls post hoc comparison test of the group x condition interaction revealed that there were no significant differences in the number of trials required by dDG lesioned and control rats to reach the learning criterion on the separate condition of the task. However, on the adjacent condition, dDG lesioned rats required significantly more trials to reach the learning criterion relative to control rats ($p < .05$). Dorsal DG lesioned rats also required more trials to reach the learning criterion on the adjacent condition compared to control animals on the separate task condition. In addition, dDG lesioned rats required significantly more trials to reach the learning criterion on the adjacent condition than the separate condition ($p < .05$). However, there were no significant differences in the number of trials required by control rats to reach learning criterion on the adjacent and separate task conditions.

Discussion

The present study investigated the role of the dDG in pattern separation during acquisition using a spatial reference memory task (McDonald & White, 1995). In this task, rats were trained to discriminate between a rewarded location and a nonrewarded location that were adjacent to one another or separated by a distance of two arm positions on an eight arm radial maze in order to receive a food reward. The results showed that dDG lesioned animals and control animals acquired spatial discriminations for the separate condition at similar rates. However, on the adjacent condition, dDG lesioned animals required significantly more trials to reach the learning criterion than controls. Animals with dDG lesions also required significantly more trials to reach the learning criterion for the adjacent condition than the separate condition. In contrast, there were no

significant differences between control animals in acquisition rates for either condition. These results suggest that dDG lesions in rats disrupt spatial pattern separation during the acquisition of spatial discriminations for proximal but not distal spatial locations.

Findings of the present investigation are consistent with results of the McDonald and White (1995) study that show that fornix-fimbria lesions result in inefficient use of place information when animals are required to discriminate between proximal spatial locations defined by a similar set of external cues. Results of the present study extend the findings of McDonald and White (1995) to include a role for the dDG hippocampal subregion in active place learning for adjacent locations.

Findings from the present investigation also are consistent with previous research that suggests that the DG plays an important role in pattern separation (Bakker et al., 2008; Gilbert et al., 2001; Goodrich-Hunsaker et al., 2008; Lacy et al., 2010; J. K. Leutgeb, Leutgeb, Moser, & Moser, 2007; Rolls & Kesner, 2006; Talpos et al., 2010). However, many of the tasks previously used to investigate pattern separation have a strong working memory component, making it difficult to determine whether performance deficits represent a selective impairment in spatial pattern separation for working memory function or whether damage to the DG results in a more general impairment in the encoding and separation of spatial representations across a variety of memory types. Therefore, the present study used an acquisition task that placed minimal demands on working memory (McDonald & White, 1995) to investigate dDG involvement in pattern separation processes for spatial reference memory. Results from the present study provide further support for the role of the dDG in spatial pattern separation and extend previous findings to include a reference memory component.

A number of behavioral studies have shown that damage to the HPP or selective DG damage in rodents disrupts acquisition learning for spatial reference processes (McDonald & White, 1995; Morris et al., 1982; Okada & Okaichi, 2009; Xavier et al., 1999); however, relatively few animal studies have directly examined hippocampal involvement in spatial pattern separation using a reference memory task (McTighe et al., 2009). Therefore, to the authors' knowledge, the present study represents the first direct investigation conducted in rats to show that selective colchicine-induced lesions of the dDG disrupt pattern separation for acquisition learning on a spatial reference memory task. However, it should be mentioned that although animals with dDG lesions were impaired in acquiring spatial discriminations on the adjacent condition, their performance matched controls on the separate condition. This finding suggests that impairments on the spatial reference memory task may be attributable to a pattern separation effect rather than a direct deficit in spatial reference memory. In support of this view, a study conducted by Hunsaker and Kesner (2008) found that animals with dDG lesions showed exploration impairments on a temporal order for spatial locations task when the metric distance between spatial locations was reduced; however, their performance matched controls when the distance between locations was increased. Taken together with prior observations that DG lesions impair pattern separation on working memory tasks, data from the present study provide support for a more general processing role for the dDG in the encoding and separation of spatial representations across a variety of memory demands.

Previous research has shown that DG lesions in rodents impair encoding processes during new learning of spatial information (Jerman et al., 2006; Lee & Kesner,

2004). Consistent with prior investigations, results from the present study show that animals with dDG lesions are impaired in acquiring spatial discriminations for locations with a high degree of spatial similarity. This finding suggests that impairments in pattern separation for spatial reference memory may be attributable to an encoding deficit. More specifically, performance deficits in the ability to distinguish between adjacent locations might be due to impaired pattern separation during encoding of the rewarded arm vs. the adjacent nonrewarded arm and a comparison with the stored representation of the rewarded arm (which may not be very accurate in the first place due to poor pattern separation at the time of encoding). In the adjacent condition, there is high overlap among the cues associated with the rewarded arm and nonrewarded arm thus requiring pattern separation. However, in the separate condition, there is less overlap among distal cues during encoding and less need for pattern separation.

It should be mentioned that, despite initial impairments in the ability to distinguish between the rewarded arm and adjacent nonrewarded arm, dDG lesioned animals were able to eventually reach the learning criterion. Improvements in performance on spatial tasks following HPP or selective DG lesions have been reported in numerous studies (Costa, Bueno, & Xavier, 2005; Jarrard, Okaichi, Steward, & Goldschmidt, 1994; Xavier et al., 1999) suggesting that animals may employ multiple response strategies in order to solve a task. It has been suggested that the HPP supports the use of place strategies to solve spatial tasks whereas the use of other response strategies may rely on systems outside of the HPP system (O'Keefe & Nadel, 1978; Xavier & Costa, 2009). Therefore, one possible explanation for the present finding is that animals used an egocentric response strategy based on body orientation (e.g., always

turn right) in order to correctly identify the rewarded arm and locate the food reward. However, two different spatial configurations were randomly used to ensure that the rats did not adopt an egocentric response strategy that could provide an accurate nonspatial solution to the task if only one nonrewarded arm was used (McDonald & White, 1995). Therefore, the data cannot be explained in terms of the adoption of a simple response strategy. Consistent with prior investigations (Xavier et al., 1999), findings from the present study suggest that dDG lesions disrupt but do not prevent acquisition on a spatial reference memory task.

In summary, results from the present study suggest that dDG lesions decrease efficiency in pattern separation during encoding resulting in impairments in the ability to discriminate between proximal spatial locations defined by a similar set of external stimuli. However, when spatial locations are widely separated, there is less overlap among distal cues during encoding and less need for pattern separation. Findings from the present study provide direct evidence for the role of the dDG hippocampal subregion in pattern separation during acquisition using a spatial reference memory task. Further, these findings provide support for a more general role for the dDG in spatial pattern separation by demonstrating the importance of a processing mechanism that is capable of reducing interference among overlapping inputs across a variety of different memory demands.

Acknowledgements

The research was supported by NIH grant #AG026505 from NIA to Paul E. Gilbert and by NIH grant #MH065314 to Raymond P. Kesner. The authors would like to

thank Dr. Jeanine Stefanucci for her helpful suggestions on the manuscript. We also thank Nora Ko and Nick Musso for their assistance with data collection, James Taylor for his assistance with histological procedures, and Christy S. Weeden for her advice and support. Please address all correspondence to Dr. Paul Gilbert, SDSU-UCSD Joint Doctoral Program in Clinical Psychology, 6363 Alvarado Court, Suite 103, San Diego, CA 92120. Tel: (619) 594-7409; Fax: (619) 594-3773;

Email: pgilbert@sciences.sdsu.edu.

References

Amaral, D. G., & Witter, M. P. (1995). Hippocampal formation. In G. Paxinos (Ed.), *The rat nervous system* (pp. 443-493). San Diego, CA: Academic Press.

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*, *319*(5870), 1640-1642.

Costa, V. C., Bueno, J. L., & Xavier, G. F. (2005). Dentate gyrus-selective colchicine lesion and performance in temporal and spatial tasks. *Behavioural Brain Research, 160*(2), 286-303.

Eldridge, L. L., Engel, S. A., Zeineh, M. M., Bookheimer, S. Y., & Knowlton, B. J. (2005). A dissociation of encoding and retrieval processes in the human hippocampus. *The Journal of Neuroscience, 25*(13), 3280-3286.

Emerich, D. F., & Walsh, T. J. (1989). Selective working memory impairments following intradentate injection of colchicine: Attenuation of the behavioral but not the neuropathological effects by gangliosides GM1 and AGF2. *Physiology Behavior, 45*, 93-101.

Fyhn, M., Hafting, T., Treves, A., Moser, M. B., & Moser, E. I. (2007). Hippocampal remapping and grid realignment in entorhinal cortex. *Nature, 446*(7132), 190-194.

Gilbert, P. E., & Brushfield, A. M. (2009). The role of the CA3 hippocampal subregion in spatial memory: A process oriented behavioral assessment. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33*(5), 774-781.

Gilbert, P. E., & Kesner, R. P. (2003). Localization of function within the dorsal hippocampus: The role of the CA3 subregion in paired-associate learning. *Behavioral Neuroscience, 117*, 1385-1394.

Gilbert, P. E., Kesner, R. P., & DeCoteau, W. E. (1998). Memory for spatial location: Role of the hippocampus in mediating spatial pattern separation. *The Journal of Neuroscience, 18*(2), 804-810.

Gilbert, P. E., Kesner, R. P., & Lee, I. (2001). Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. *Hippocampus, 11*(6), 626-636.

Goodrich-Hunsaker, N. J., Hunsaker, M. R., & Kesner, R. P. (2008). The interactions and dissociations of the dorsal hippocampus subregions: How the dentate gyrus, CA3, and CA1 process spatial information. *Behavioral Neuroscience, 122*, 16-26.

Hunsaker, M. R., & Kesner, R. P. (2008). Evaluating the differential roles of the dorsal dentate gyrus, dorsal CA3, and dorsal CA1 during a temporal ordering for spatial locations task. *Hippocampus, 18*(9), 955-964.

Jarrard, L. E., Okaichi, H., Steward, O., & Goldschmidt, R. B. (1984). On the role of hippocampal connections in the performance of place and cue tasks: Comparisons with damage to hippocampus. *Behavioral Neuroscience, 98*(6), 946-954.

Jerman, J. T., Kesner, R. P., & Hunsaker, M. R. (2006). Disconnection analysis of CA3 and DG in mediating encoding but not retrieval in a spatial maze learning task. *Learning and Memory, 13*(4), 458-464.

Johnston, D., & Amaral, D. G. (2004). Hippocampus. In G. M. Shephard (Ed.), *The synaptic organization of the brain* (pp. 455-498). Oxford, UK: Oxford University Press.

Jung, M. W., & McNaughton, B. L. (1993). Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus, 3*(2), 165-182.

Kesner, R. P. (2007). A behavioral analysis of dentate gyrus function. *Progress in Brain Research, 163*, 567-576.

Kesner, R. P., Lee, I., & Gilbert, P. (2004). A behavioral assessment of hippocampal function based on a subregional analysis. *Reviews in the Neurosciences, 15*, 333-351.

Kirwan, C. B., & Stark, C. E. (2007). Overcoming interference: An fMRI investigation of pattern separation in the medial temporal lobe. *Learning and Memory, 14*(9), 625-633.

Lacy, J. W., Yassa, M. A., Stark, S. M., Muftuler, L. T., & Stark, C. E. (2010). Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learning and Memory, 18*, 15-18.

Lee, I., & Kesner, R. P. (2004). Encoding versus retrieval of spatial memory: Double dissociation between the dentate gyrus and the perforant path inputs into CA3 in the dorsal hippocampus. *Hippocampus, 14*, 66-76.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science, 315*(5814), 961-966.

Leutgeb, S., Leutgeb, J. K., Barnes, C. A., Moser, E. L., McNaughton, B. L., & Moser, E. I. (2005). Independent codes for spatial and episodic memory in hippocampal neuronal ensembles. *Science, 309*(5734), 619-623.

McDonald, R. J., & White, N. M. (1995). Hippocampal and nonhippocampal contributions to place learning in rats. *Behavioral Neuroscience, 109*(4), 579-593.

McHugh, T. J., Jones, M. W., Quinn, J. J., Balthasar, N., Coppari, R., Elmquist, . . . Tonegawa, S. (2007). Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science, 317*(5834), 94-99.

McLamb, R. L., Mundy, W.R., & Tilson, H. A. (1988). Intradentate colchicine disrupts the acquisition and performance of a working memory task in the radial arm maze. *Neurotoxicology, 9*(3), 521-528.

McTighe, S. M., Mar, A. C., Romberg, C., Bussey, T. J., & Saksida, L. M. (2009). A new touchscreen test of pattern separation: effect of hippocampal lesions. *Neuroreport, 29*(9), 881-885.

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*(5868), 681-683.

Nanry, K. P., Mundy, W. R., & Tilson, H. A. (1989). Colchicine-induced alterations of reference memory in rats: Role of spatial versus non-spatial task components. *Behavioural Brain Research, 35*, 45-53.

Okada, K., & Okaichi, H. (2009). Functional differentiation and cooperation among the hippocampal subregions in rats to effect spatial memory processes. *Behavioural Brain Research, 200*, 181-191.

O'Keefe, J., & Nadel, L. (1978). *The hippocampus as a cognitive map.* Oxford, UK: Oxford University Press.

Olton, D. S. (1978). Characteristics of spatial memory. In S. H. Hulse, H. Fowler, & W. K. Honig (Eds.), *Cognitive processes in animal behavior* (pp. 327-342). Hillsdale, NJ: Erlbaum.

Olton, D. S., Becker, J. T., & Handelman, G. E. (1979). Hippocampus, space, and memory. *Behavioral and Brain Sciences, 2,* 313-322.

O'Reilly, R. C., & Rudy, J. W. (2001). Conjunctive representations in learning and memory: Principles of cortical and hippocampal function. *Psychological Review, 108*(2), 311-45.

Paxinos, G., & Watson, C. (1997). *The rat brain in stereotaxic coordinates*. New York, NY: Academic Press.

Renaudineau, S., Poucet, B., & Save, E. (2007). Flexible use of proximal objects and distal cues by hippocampal place cells. *Hippocampus, 17*(5), 381-395.

Rolls, E. T. (2010). A computational theory of episodic memory formation in the hippocampus. *Behavioural Brain Research, 215*(2), 1265-1274.

Rolls, E. T., & Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Progress in Neurobiology, 79,* 1-48.

Shapiro, M. L., & Olton, D. S. (1994). Hippocampal function and interference. In D. L. Schacter, & E. Tulving (Eds.), *Memory systems* (pp. 39-63). London, UK: MIT Press.

Talpos, J. C., McTighe, S. M., Dias, R., Saksida, L. M., & Bussey, T. J. (2010). Trial-unique, delayed nonmatching-to-location (TUNL): A novel, highly hippocampus-dependent automated touchscreen test of location memory and pattern separation. *Neurobiology of Learning and Memory, 94*(3), 341-352.

Xavier, G. F., & Costa, V. C. (2009). Dentate gyrus and spatial behaviour. *Progress in Neuropsychopharmacology and Biological Psychiatry, 33*(5), 762-773.

Xavier, G. F., Oliveira-Filho, F. J., & Santos, A. M. (1999). Dentate gyrus-selective colchicine lesion and disruption of performance in spatial tasks: Difficulties in "place strategy" because of lack of flexibility in the use of environmental cues? *Hippocampus, 9*(6), 668-681.

CHAPTER 4

STUDY THREE: THE ROLE OF THE DENTATE GYRUS IN THE FORMATION OF TEMPORAL ASSOCIATIONS FOR SPATIAL LOCATIONS

Abstract

The hippocampus (HPP) is involved in processing spatiotemporal information in order to form a memory representation of an episodic event. Previous research suggests that the dorsal dentate gyrus (dDG) hippocampal subregion mediates spatial processing functions. However, a novel role for the DG in temporal processing for spatial information has begun to emerge based on the development of a computational model of neurogenesis. According to this model, adult born granule cells in the DG contribute to a temporal associative integration for spatial events. Currently, there is a paucity of behavioral evidence to support the temporal integration theory. Therefore, we developed a novel behavioral paradigm to investigate the role of the dDG in temporal integration for proximal and distal spatial events. Male Long-Evans rats were randomly assigned as control animals or to receive bilateral intracranial infusions of colchicine into the dDG. Following recovery from surgery, each rat was tested on a novel cued-recall of sequence paradigm for different spatial locations. In this task, animals were allowed to explore identical objects placed in designated spatial locations on a cheeseboard maze across 2

days (e.g., Day 1: A and B; Day 2: C and D). One week later, animals were given a brief cue (A or C) followed by a preference test between spatial location B and D. Control animals showed a significant preference for the spatial location previously paired with the cue (the temporal associate), but dDG lesioned animals did not show a preference for either spatial location during the preference test. These findings suggest that selective colchicine-induced dDG lesions are capable of disrupting the formation of temporal associations between spatial events presented close in time. The results may have important implications for the selection of behavioral paradigms used to examine the effects of adult neurogenesis in the DG on temporal associative memory formation.

Introduction

The HPP is involved in processing spatiotemporal information in order to form a memory representation of an episodic event (Tulving, 1983). Specifically, previous research suggests that the DG hippocampal subregion mediates spatial processing functions (Rolls & Kesner, 2006), while the CA1 hippocampal subregion is thought to support more temporal-based processing functions (Kesner, Lee, & Gilbert, 2004). For example, a rodent lesion study conducted by Gilbert, Kesner, and Lee (2001) showed a double dissociation between the dDG and dorsal CA1 (dCA1) with respect to spatial and temporal pattern separation. Animals received posttraining dDG or dCA1 lesions and were subsequently tested on either a spatial pattern separation task or a temporal pattern separation task. Dorsal DG lesioned animals were impaired for spatial pattern separation but matched the performance of controls on the temporal pattern separation task. In contrast, dCA1 lesioned animals were impaired for temporal but not spatial pattern

separation. The results provide strong evidence for the dDG in spatial but not temporal processing.

Though the previous evidence suggests that the dDG does not mediate temporal processing of information, results from a study conducted by Hunsaker and Kesner (2008) showed that dDG lesions could disrupt performance in temporal ordering for spatial locations with high spatial similarity (increased interference). However, their performance matched controls when the degree of spatial similarity between spatial locations was reduced (decreased interference). Therefore, their results do not rule out the possibility that dDG-related impairments in temporal processing might be attributable to a spatial pattern separation effect rather than a direct deficit in temporal order processing.

More recently, a novel role for the DG in temporal processing has emerged in the literature based on the development of a computational model of neurogenesis (Aimone, Wiles, & Gage, 2006). Neurogenesis, or the proliferation of new neurons, has been shown to occur in two regions in the adult brain including the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the DG hippocampal subregion (Kempermann, Wiskott, & Gage, 2004). Thousands of granule cells (GC) are born daily; however, only a portion of these cells survive and develop into fully mature GCs (Kempermann et al.). Interestingly, immature GCs exhibit unique characteristics that differ from mature GCs. For example, immature GCs appear to be hyper-excitable and have a lower threshold for the induction of long-term potentiation (LTP) than adult GCs (Aimone, Deng, & Gage, 2010; Ge, Yang, Hsu, Ming, & Song, 2007; Schimdt-Hieber, Jonas, & Bischofberger, 2004). However, during the maturation process,

immature GCs begin to take on characteristics associated with fully developed GCs (Deng, Aimone, & Gage, 2010). Essentially, the continual influx of newly formed GCs into existing hippocampal circuitry gives rise to a continually changing population of GCs (Kempermann et al.).

Based on the unique characteristics of young GCs during different stages of maturation, Aimone et al. (2006) suggested that young GCs might make a distinct contribution to memory formation. Specifically, they proposed that young GCs may mediate a temporal integration process that operates to form associations among temporally contiguous events. In other words, events that occur close in time may be encoded by a similar set of young GCs, while events that occur farther apart in time may be encoded and represented by different cell populations allowing for the formation and separation of distinct memory representations (Aimone, Wiles, & Gage, 2009; Aimone et al., 2010; Deng et al., 2010). Evidence provided by these models indicates that adult born GCs may provide a temporal tag to sparse spatial representations formed in the DG.

Although there is computational evidence to suggest that adult neurogenesis in the DG contributes to a temporal associative process for proximal spatial events, there is a paucity of behavioral evidence to support the temporal integration theory. In addition, the role of the dDG in the formation of temporal associations for spatial events has not been directly tested. Therefore, we developed a novel behavioral paradigm to determine whether the dDG supports temporal integration for proximal spatial events. Aimone and colleagues (2009) stressed the importance of developing new behavioral paradigms to test computational models of temporal pattern integration and the formation of temporal associations. Therefore, the results of the present study may have important implications

for the selection of behavioral paradigms used to examine the effects of adult neurogenesis in the DG on temporal associative memory formation (Aimone et al., 2006).

Materials and Methods

Subjects

Thirty-two male Long-Evans rats, weighing approximately 250-350 g at the start of the experiment, were used as subjects. Each animal was housed in an individual plastic container located in a colony room. The colony room was maintained on a 12H:12H light/dark cycle and all testing was conducted during the light phase. All rats had unlimited access to water but were food restricted to 80-90% of their free-feed weight.

Surgical Procedures

All planned procedures and animal care were in accordance with the National Institute of Health and Institute for Animal Care and Use Committee guidelines and the Institutional Animal Care and Use Committee at the University of Utah. Each animal was randomly assigned to be a control animal (n = 16) or to receive a bilateral dDG lesion (n = 16). Prior to surgery, subjects were deeply anesthetized using isoflurane gas, placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and then maintained with a continuous flow of isoflurane (2-4%) and medical air (1.5-2 L/min) and given atropine sulfate (0.54 mg/kg im). Each subject was prepared for the surgical procedure by applying a surgical drape and betadine antiseptic to the surgical site. An incision was made in the skin above the skull. The skin was retracted and small burr

holes were drilled into the skull. Using a 10 µl Hamilton syringe, intracranial infusions of colchicine (2.5 mg/ml, 0.8 µl/site) were slowly infused (2.5 mg/mL, 20.0 uL/hr) into two dDG sites per hemisphere using the following coordinates: dDG: 2.7 mm posterior to bregma, 2.1 mm lateral to midline, 3.4 mm ventral from dura and 3.7 mm posterior to bregma, 2.3 mm lateral to midline, 3.0 mm ventral from dura. All lesion coordinates were based on Paxinos and Watson's (1997) stereotaxic atlas of the rat brain. For all injections, the injection cannula was left in place for at least 1 minute after the injection to allow for diffusion of the neurotoxin. Following all surgical procedures, each animal received Children's Motrin in water as an analgesic and was given a 7-10 day recovery period prior to testing. Following recovery from surgery, each rat was tested on a cued-recall of sequence for spatial location paradigm (dDG = 10; Control = 10) or a novelty detection version of the cued-recall of sequence for spatial location paradigm (dDG = 6; Control = 6).

Experiment 1: Cued Recall for Spatial Location

Behavioral Apparatus

Testing was conducted on a round cheeseboard maze (65 cm above the floor, 120 cm in diameter, and 3.5 cm in thickness) covered with a white vinyl shower curtain. The cheeseboard was kept in a well-lit room with no windows but which contained a variety of distal spatial cues (e.g., posters on the walls, only one door). A video camera was positioned directly above the maze and all testing was videotaped.

Visual stimuli used during testing procedures consisted of identical copies of objects that were approximately 15 cm in height x 9 cm in width. Visual objects were

made from nonporous materials that were heavy enough that animals were unable to displace them and were used throughout all testing procedures to represent spatial locations. Spatial locations consisted of four designated locations (A, B, C, and D) positioned 53 cm apart and 23 cm from the outer edges of the cheeseboard apparatus. The spatial locations remained constant across all behavioral testing and across animals.

Behavioral Procedures

Following recovery from surgery, each rat was tested on a novel cued recall for spatial location paradigm (see Figure 11 for schematic representation). Prior to testing, each animal was allowed to individually explore the test apparatus for 5 min. No objects were present during the habituation phase. Testing began on the following day. The task consisted of a study phase and a test phase. The study phase was conducted across 2 consecutive days (Day 1 and Day 2) and consisted of two 5-min exploration sessions separated by a 3-min intersession interval (ISI) per day. On Day 1, the animal was placed on the cheeseboard apparatus and allowed to explore the object positioned at spatial location A for 5 min. Following the exploration period, the animal was removed from the maze and placed in the home cage outside of the testing room for 3 min. After this interval, the animal was placed on the maze and allowed to explore the object positioned at spatial location B for 5 min. The same procedure was used on Day 2 of the study phase; however, the object was positioned at spatial location C and D, respectively. The test phase was conducted 7 days after the first study phase (Day 1) and was conducted across two consecutive days (Day 8 and Day 9). On the first day of the test phase (Day 8), animals were placed on the maze and allowed to explore an object positioned at

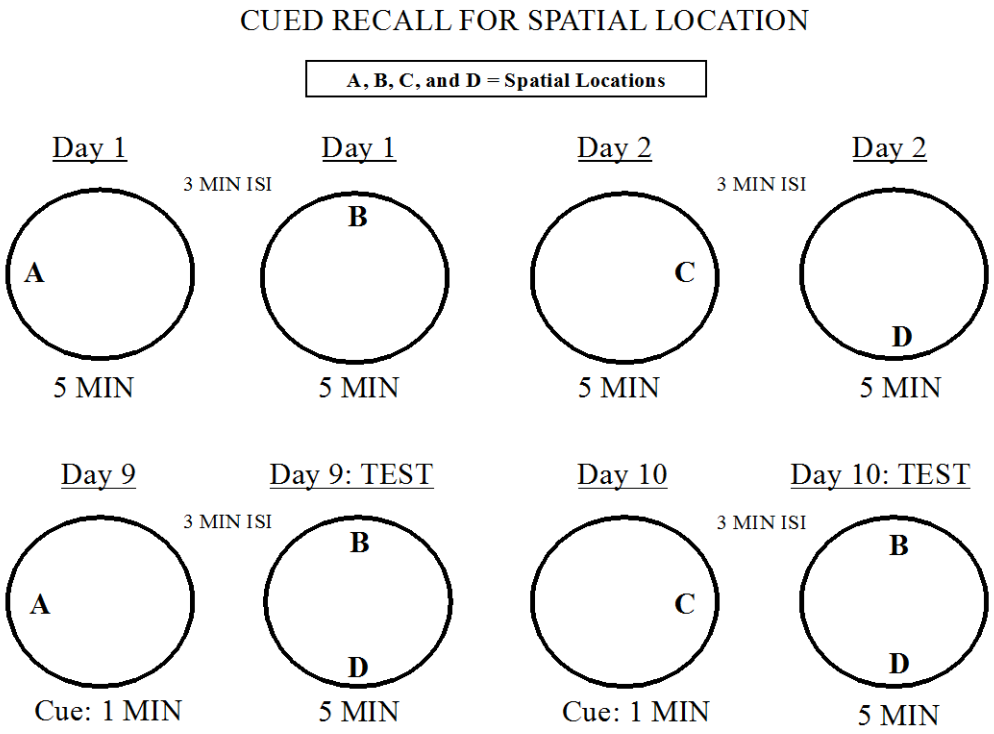

Figure 11. Schematic representation of behavioral procedures for cued recall for spatial location task.

spatial location A or C (A and C were used as cues) for 1 min followed by a 3-min ISI. After this interval, animals were given a 5-min preference test between spatial location B and D (positioned 106 cm apart). The same procedure was used on the second day of the test phase (Day 9). The presentation order of spatial location A and C was counterbalanced across subjects and across days. The start location was held constant across all sessions and phases.

In order to account for individual activity levels of each rat, a preference ratio for time spent exploring objects positioned in spatial location B and D was calculated for each animal. Exploration was defined as active and direct contact with an object such as

sniffing and pawing the objects. When cued with A, the preference ratio was (B - D / B + D). When cued with C, the preference ratio was (D-B/D+B). Positive preference ratio scores (above zero) indicated a preference for the paired temporal associate (B when cued with A; D when cued with C). Negative preference ratio scores (below zero) indicated a preference for the spatial location that was not previously paired with the cue (D when cued with A; B when cued with C). A score of zero indicated no preference for B or D.

Experiment 2: Novelty Detection for Spatial Location

Behavioral Apparatus

The apparatus used in Experiment 2 was the same apparatus described in Experiment 1. Spatial locations were the same four designated spatial locations (A, B, C, and D) used in Experiment 1. However, two additional spatial locations (E and F) were introduced during the preference tests in order to test for novelty preference. The spatial locations remained constant across all behavioral testing and across animals.

Behavioral Procedures

A novelty detection condition was used as a control measure to determine whether results from Experiment 1 could be attributed to a novelty preference rather than the formation of a temporal association. The procedure for the novelty detection condition was identical to the procedure described for Experiment 1 except that the preference test

was between a spatial location B and a novel spatial location E when cued with A or between spatial location D and a novel spatial location F when cued with C (see Figure 12 for a schematic representation).

In order to account for individual activity levels of each rat, a preference ratio of time spent exploring B or D (familiar spatial location) vs. E or F (novel spatial location) was calculated for each animal. When cued with A, the preference ratio used was (B – E / B + E). When cued with C, the preference ratio used was (D - F / D + F). Positive preference ratio scores (above zero) indicated a preference for the paired temporal

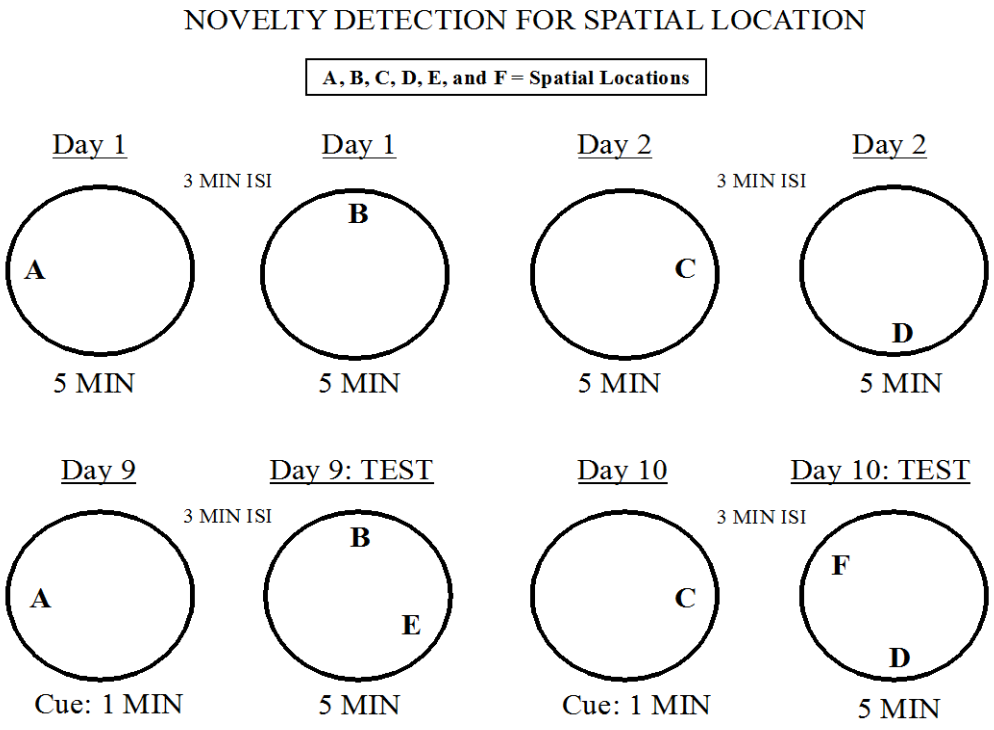

Figure 12. Schematic representation of behavioral procedures for novelty detection for spatial location task.

associate (B when cued with A; D when cued with C). Negative preference ratio scores (below zero) indicated a preference for the novel spatial location (E when cued with A; F when cued with C). A zero score reflected no preference for B or E, D or F.

Histological Procedures

At the conclusion of all testing, each animal was deeply anesthetized with an intraperitoneal injection of 1.5 ml sodium pentobarbital (70 mg/kg), and perfused intracardially with normal saline followed by a 10% formalin solution. The brain was removed from the skull and stored in a 10% formalin/30% sucrose solution in a refrigerator (4°C) for 72 hours to equalize the tissue-shrinkage rates across brains. For dDG lesions, a tissue block (Bregma -2.0 through ~ -4.0) containing only the dorsal hippocampus was cut using coronal sections. The block was frozen and cut at 24 µm sections with every third section mounted on a glass slide (the surface-to-surface distance between collected sections = 72 µm). The sections were stained with cresyl violet and examined for histological verification of the lesion placement.

Results

Histological Results

Axon-sparing, selective bilateral lesions of the dDG were made with colchicine. A representative dDG lesion and intended target zone is shown in Figure 13. In addition, a representative vehicle-infused control lesion is shown in Figure 14.

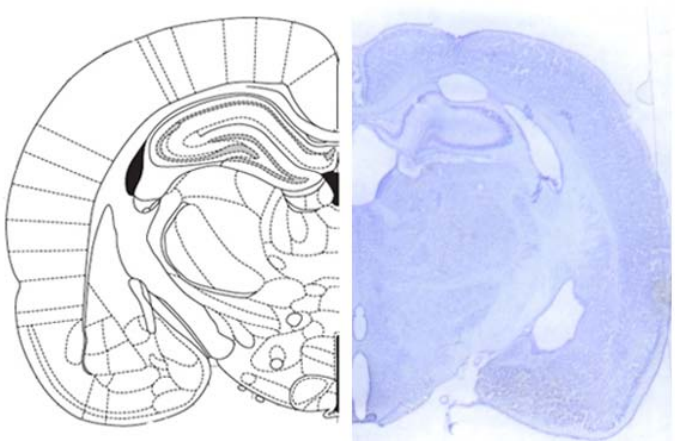

Figure 13. Histological representation of a dDG lesioned rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997).

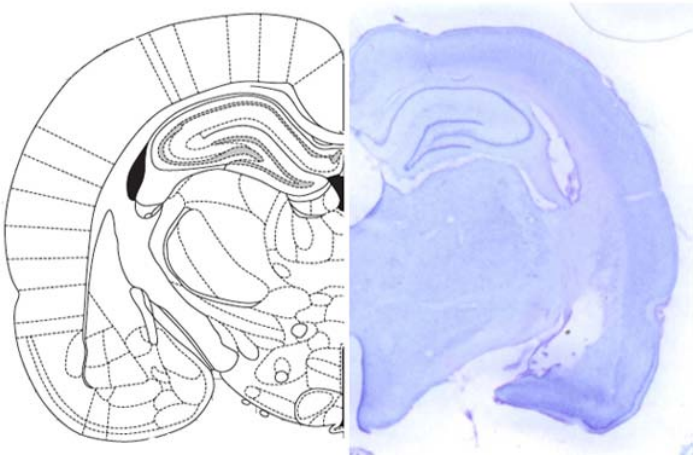

Figure 14. Histological representation of a vehicle-infused control rat brain and schematic drawing of intended target zone (adapted from Paxinos & Watson, 1997).

Behavioral Results

Cued Recall for Spatial Locations

Figure 15 shows the mean (± SE) preference ratios on the cued-recall for spatial location task for dDG lesioned rats and control rats. A one-way analysis of variance (ANOVA) with group (dDG, control) as a between-subjects factor was used to analyze the data. The dependent variable was the mean ratio score for the preference test (B -D / B + D when cued with A; D - B / D + B when cued with C). The results revealed a significant main effect of group, $F(1, 18) = 8.04$, $p = .01$. Control animals showed a significant preference for the spatial location previously paired with the cued location during the study phase compared to dDG lesioned animals, as indicated by a positive preference ratio score. More specifically, control rats spent more time exploring B than D when cued with A and more time exploring D than B when cued

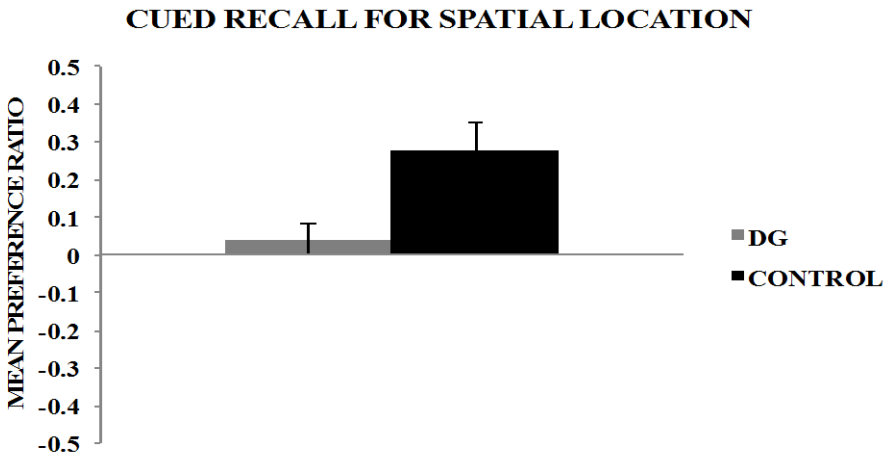

Figure 15. Mean (± SE) preference ratio of dDG lesioned rats and control rats for cued recall of temporally based spatial location associations.

with C. Dorsal DG lesioned animals did not show a preference for either B or D when cued with A or C, as indicated by a preference ratio score that was approaching zero.

Novelty Detection for Spatial Location

Figure 16 shows the mean (± SE) preference ratios on the novelty detection condition of the cued-recall for spatial locations task for dDG lesioned rats and control rats. A one-way ANOVA with group (dDG, control) as a between-group factor was used to compare preference ratio scores for familiar vs. novel spatial locations. The dependent variable was the mean ratio score for the preference test (B - E / B + E when cued with A; D - F / D + F when cued with C). The results revealed a significant main effect of group, $F(1, 10) = 30.75$, $p \leq .01$. Control animals showed a significant preference for the novel spatial location rather than the familiar spatial location compared to dDG lesioned animals, as indicated by a positive preference ratio score. More specifically, control rats spent more time exploring B than E when cued with A and more time exploring D than F when cued with C. Dorsal DG lesioned animals did not show a preference for the familiar (B or D) or novel spatial location (E or F), as indicated by a preference ratio score that was approaching zero.

Discussion

The present study sought to determine whether the dDG mediates the formation of temporal associations for proximal spatial events using a novel cued-recall of sequence paradigm for different spatial locations. In this task, animals were allowed to explore

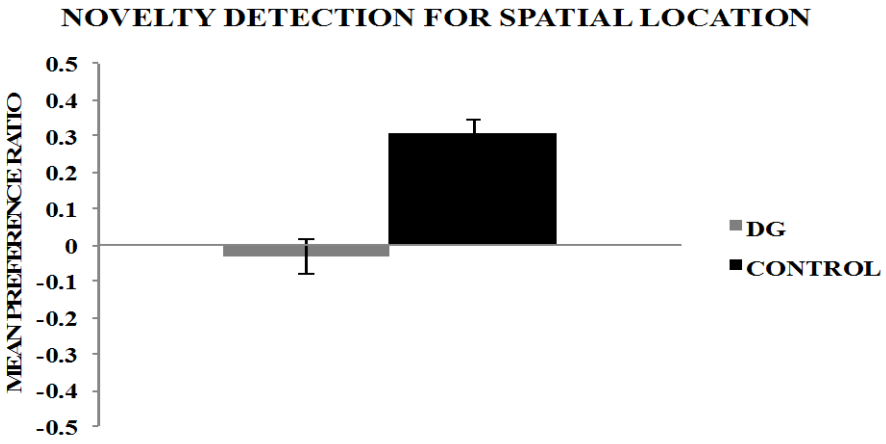

Figure 16. Mean (± SE) preference ratio of dDG lesioned rats and control rats on the novelty detection condition for cued recall of temporally based spatial location associations.

identical objects placed in designated spatial locations across 2 days (e.g., Day 1: A and B; Day 2: C and D). One week later, animals were given a brief cue (A or C) followed by a preference test between spatial location B and D. The data revealed that during the preference test, control animals showed a significant preference for the spatial location previously paired with the cue (a within day over a between day preference) suggesting that control animals formed a stronger temporal association for proximal rather than distal spatial events. In comparison to controls, animals with dDG lesions did not show a preference for either spatial location during the preference test. This finding suggests that selective colchicine induced dDG lesions are capable of disrupting the formation of temporal associations between spatial events presented closer in time.

In order to determine whether novelty preference was influencing the results for the cued recall for spatial location task, animals were tested on a novelty detection

condition to evaluate preference for a novel spatial location vs. a familiar spatial location (the temporal associate previously paired with the cue). Previous research suggests that normal rodents display a natural tendency to detect changes in the environment as evidenced by increased exploration for novel topological and metric changes compared to configurations that were previously encountered (Dix & Aggleton, 1999; Ennaceur & Delacour, 1988). In contrast to prior reports, data from the present investigation revealed that control animals showed a significant preference for the familiar spatial location (B or D) rather than the novel spatial location (E or F). This finding suggests that, for normal control rats, the formation of temporal associations for proximal spatial events may outweigh novelty preference. In comparison to controls, dDG lesioned animals did not show a preference for the novel spatial location or the familiar spatial location. Therefore, the results of the cued recall for spatial location task are not likely due to a novelty preference. Dorsal DG lesioned animals also failed to demonstrate a preference on the cued recall for spatial location task, despite previous exposure to both spatial locations (B and D) presented during the preference test. Therefore, the present findings point to a possible encoding deficit rather than a deficit in spatial novelty detection per se. These results are consistent with previous research that suggests that the DG plays an important role during encoding of spatial information (Bakker, Kirwan, Miller, & Stark, 2008; Eldridge, Engel, Zeineh, Bookheimer, & Knowlton, 2005; Jerman, Kesner, & Hunsaker, 2006; Lee & Kesner, 2004; Rolls & Kesner, 2006). The results are also consistent with descriptions of temporal pattern integration as an encoding process that serves to form associations among events that occur close in time (Deng et al., 2010).

Previous research suggests that the dDG hippocampal subregion supports spatial, not temporal, processing (Gilbert et al., 2001; Goodrich-Hunsaker, Hunsaker, & Kesner, 2008; Rolls, 1996; Rolls & Kesner, 2006). Specifically, the dDG is thought to play an important role in spatial pattern separation processes, or the encoding and separation of similar spatial inputs into distinct representations (Gilbert et al., 2001; Kesner, 2007; Leutgeb, Leutgeb, Moser, & Moser, 2007; Rolls & Kesner, 2006). There is some evidence to suggest that selective damage to the dDG in rats is capable of disrupting performance on a temporal processing task when there is high interference among spatial locations (Hunsaker & Kesner, 2008). However, based on the results of the Gilbert et al. (2001) study, the distance between spatial locations presented during the preference tests (85 cm apart) in the present study does not require spatial pattern separation. Therefore, it is unlikely that the results in the present study were attributable to a pattern separation effect. Data from the present investigation extend the role of the dDG in spatial processing to include a temporal component. Specifically, the results provide evidence for a novel mnemonic processing function in the dDG that serves to form associations between spatial locations based on the proximity of the temporal events. These results are consistent with previous research that shows that events encoded closer in time are more likely to be recalled together (Brown & Schopflocher, 1998).

In addition, data from the present investigation provide support for the temporal integration theory proposed by Aimone and colleagues (2006, 2009; Deng et al., 2010). The temporal integration theory is largely based on computational evidence that indicates that newly generated GCs, at different stages of development, may differentially contribute to hippocampal dependent learning and memory by forming associations

among temporally proximal events (Aimone et al., 2010; Deng et al., 2010). Specifically, Aimone et al. (2010) suggest that young GCs may support a pattern integration process such that temporally proximal events are encoded by a similar set of new cells and different cell populations represent temporally distal events. The neurotoxic lesion method used in the present study did not selectively target young GCs; however, colchicine-induced lesions of the DG have been shown to effectively destroy both young and mature GCs (Gilbert et al., 2001; Mundy & Tilson, 1990), thereby disrupting neurogenesis. Although results of the present investigation suggest that selective colchicine lesions of the dDG are capable of disrupting the formation of temporal associations for proximal spatial events, the authors acknowledge that a disruption of neurogenesis may be the actual mechanism that underlies this disruption. Therefore, future research with targeted ablation of young GCs needs to be conducted to directly investigate the temporal integration theory. Based on results obtained in the present study, the cued-recall of sequence paradigm for different spatial locations may serve as an appropriate behavioral measure for examining the effects of adult neurogenesis in the DG on temporal associative memory formation.

In summary, results from the present investigation suggest that the dDG hippocampal subregion supports associative encoding for spatial events presented close in time. However, the dDG is not necessary for encoding associations among spatial locations presented farther apart in time. Findings from the present study provide direct evidence for a novel role of the dDG hippocampal subregion in temporal pattern integration for spatial events. In addition, the present findings provide support for the temporal integration theory proposed by Aimone and colleagues (2006; 2009; Deng et al.,

2010) and may have important implications for the selection of behavioral paradigms used to examine the effects of adult neurogenesis in the DG on temporal associative memory formation (Aimone et al., 2006).

Acknowledgements

The research was supported by NIH grant #MH065314 to Raymond P. Kesner. The authors would like to thank Dr. Jeanine Stefanucci for her helpful suggestions on the manuscript. We also thank Tracy Hubertz for his assistance with data collection, James Taylor for his assistance with histological procedures, and Christy S. Weeden for her advice and support. Please address all correspondence to Dr. Raymond Kesner, University of Utah, Department of Psychology, 380 South 1530 East, Room 502, University of Utah, Salt Lake City, UT 84112, USA. Email: ray.kesner@psych.utah.edu.

References

Aimone, J. B., Deng, W., & Gage, F. H. (2010). Adult neurogenesis: Integrating theories and separating functions. *Trends in Cognitive Sciences, 14*(7), 325-337.

Aimone, J. B., Wiles, J., & Gage, F. H. (2006). Potential role for adult neurogenesis in the encoding of time in new memories. *Nature Neuroscience, 9*(6), 723-727.

Aimone, J. B., Wiles, J., & Gage, F. H. (2009). Computational influence of adult neurogenesis on memory encoding. *Neuron, 61,* 187-202.

Amaral, D. G., & Witter, M. P. (1995). Hippocampal formation. In G. Paxinos (Ed.), *The rat nervous system* (pp. 443-493). San Diego, CA: Academic Press.

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science, 319*(5870), 1640-1642.

Brown, N. R., & Schopflocher, D. (1998). Event cueing, event clusters, and the temporal distribution of autobiographical memories. *Applied Cognitive Psychology, 12*, 305-319.

Deng, W., Aimone, J. B., & Gage, F. H. (2010). New neurons and new memories: How does adult hippocampal neurogenesis affect learning and memory? *Nature Reviews Neuroscience, 11*(5), 339-350.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: Evidence of object-location and object-context recognition. *Behavioural Brain Research, 99,* 191-200.

Eldridge, L. L., Engel, S. A., Zeineh, M. M., Bookheimer, S. Y., & Knowlton, B. J. (2005). A dissociation of encoding and retrieval processes in the human hippocampus. *The Journal of Neuroscience, 25*(13), 3280-3286.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. *Behavioural Brain Research, 31*, 47-59.

Ge, S., Yang, C. H., Hsu, K. S., Ming, G. L., & Song, H. (2007). A critical period for enhanced synaptic plasticity in newly generated neurons in the adult brain. *Neuron, 54*, 559-566.

Gilbert, P. E., Kesner, R. P., & Lee, I. (2001). Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. *Hippocampus, 11*(6), 626-636.

Goodrich-Hunsaker, N. J., Hunsaker, M. R., & Kesner, R. P. (2008). The interactions and dissociations of the dorsal hippocampus subregions: How the dentate gyrus, CA3, and CA1 process spatial information. *Behavioral Neuroscience, 122*, 16-26.

Hunsaker, M. R., & Kesner, R. P. (2008). Evaluating the differential roles of the dorsal dentate gyrus, dorsal CA3, and dorsal CA1 during a temporal ordering for spatial locations task. *Hippocampus, 18*(9), 955-964.

Jerman, J. T., Kesner, R. P., & Hunsaker, M. R. (2006). Disconnection analysis of CA3 and DG in mediating encoding but not retrieval in a spatial maze learning task. *Learning and Memory, 13*(4), 458-464.

Kempermann, G., Wiskott, L., & Gage, F. H. (2004). Functional significance of adult neurogenesis. *Current Opinion in Neurobiology, 14*(2), 186-191.

Kesner, R. P. (2007). A behavioral analysis of dentate gyrus function. *Progress in Brain Research, 163,* 567-576.

Kesner, R. P., Lee, I., & Gilbert, P. (2004). A behavioral assessment of hippocampal function based on a subregional analysis. *Reviews in the Neurosciences, 15,* 333-351.

Lee, I., & Kesner, R. P. (2004). Encoding versus retrieval of spatial memory: Double dissociation between the dentate gyrus and the perforant path inputs into CA3 in the dorsal hippocampus. *Hippocampus, 14,* 66-76.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science, 315*(5814), 961-966.

Mundy, W. R., & Tilson, H. A. (1990). Neurotoxic effects of colchicine. *Neurotoxicology, 11,* 539-548.

Paxinos, G., & Watson, C. (1997). *The rat brain in stereotaxic coordinates*. New York, NY: Academic Press.

Rolls, E. T. (1996). A theory of hippocampal function in memory. *Hippocampus, 6,* 601-620.

Rolls, E. T., & Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Progress in Neurobiology, 79,* 1-48.

Schimdt-Hieber, C., Jonas, P., & Bischofberger, J. (2004). Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature, 429,* 184-187.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery, and Psychiatry, 20,* 11-21.

Tulving, E. (1983). *Elements of episodic memory*. London, UK: Oxford University Press.

CHAPTER 5

GENERAL DISCUSSION

The present investigations further defined the role of the dorsal dentate gyrus in spatial learning and memory. Through a series of studies that examined the role of the dDG in spatial processing, I found that the dDG supports a variety of mnemonic functions that contribute to the formation, separation, and integration of spatial representations.

The first study in this dissertation determined that the dDG supports the formation of conjunctive contextual representations of the spatial environment. Dorsal DG involvement in conjunctive encoding was evaluated using a contextual associative learning task that required rats to form an association between an odor and a context. The results showed that dDG lesions disrupted acquisition of odor-context associations. In order to determine whether deficits on the contextual associative learning task were attributable to an inability to differentiate between odors or contexts, animals were tested on an additional two-choice olfactory discrimination task and a two-choice contextual discrimination task. Dorsal DG lesioned animals and control animals acquired the discriminations at a similar rate. This finding is important because it showed that when the individual components of the task were teased apart, dDG lesioned animals were no longer impaired, suggesting that the real impairment lies in the ability to combine

olfactory information and context information in a meaningful way in order to solve the task. These results are consistent with the notion that multiple sensory inputs are bound together by a conjunctive encoding process in the dDG (Kesner, 2007).

The second study in this dissertation further defined the role of the dDG in the encoding and separation of spatial representations. Specifically, dDG involvement in spatial pattern separation was examined using an acquisition task that placed minimal demands on working memory in order to determine whether the dDG supported pattern separation processes for spatial reference memory. In the study, rats were trained to discriminate between a rewarded location and a nonrewarded location that were adjacent to one another (high spatial interference) or widely separated (low spatial interference). Both groups acquired spatial discriminations for the separate condition at similar rates. However, in the adjacent condition, dDG lesioned animals required significantly more trials to reach the learning criterion than controls. The results indicated that dDG lesions may have decreased efficiency in pattern separation during encoding resulting in impairments in the ability to discriminate between proximal spatial locations defined by a similar set of external stimuli. Importantly, the findings support a more general role for the dDG in spatial pattern separation by demonstrating the importance of a processing mechanism that is capable of reducing interference among overlapping spatial inputs across a variety of memory demands.

The third study of this dissertation found that the dDG mediates the formation of temporal associations for proximal spatial events. Dorsal DG involvement in temporal integration was evaluated using a novel cued recall of sequence paradigm for different spatial locations. The results showed that control animals formed a temporal association

between proximal spatial events. In contrast, dDG lesions disrupted the temporal integration of proximal spatial events. Similar results were obtained on a novelty preference condition. Findings from this study suggest that the dDG supports associative encoding for spatial events presented close in time. However, the dDG did not appear to be necessary for encoding associations among spatial locations presented farther apart in time. Taken together, the results provide support for a novel role for the dDG in temporal integration for spatial representations.

Consistent with previous research (Goodrich-Hunsaker et al., 2008; Kesner et al., 2004; Rolls & Kesner, 2006; Xavier & Costa, 2009), the results from the three studies in this dissertation suggest that the dDG hippocampal subregion plays an important role in spatial learning and memory. Specifically, the results provide evidence for dDG involvement in (1) the integration of multimodal information into unique representations of the spatial environment via conjunctive encoding, (2) the reduction of interference among similar spatial locations via spatial pattern separation, and (3) the formation of temporal associations among distinct spatial events via temporal integration. Collectively, the results provide evidence for a dynamic role for the dDG in spatial processing by demonstrating the importance of an intact dDG across a variety of spatial tasks and under a variety of learning and memory demands.

Previous research has emphasized a role for the DG in spatial pattern separation (Bakker et al., 2008; Gilbert et al., 2001; Goodrich-Hunsaker et al., 2008; Leutgeb, Leutgeb, Moser, & Moser, 2007; Rolls & Kesner, 2006). While the present findings support an important role for the dDG in spatial pattern separation, they also suggest that the dDG serves a critical role in integration. Although these may be distinct processes,

they appear to serve complementary rather than conflicting functions that operate in a cooperative manner to create and maintain distinct spatial representations. For example, conjunctive encoding and spatial pattern separation may act in concert to produce highly organized unique spatial representations. More specifically, conjunctive encoding may operate to integrate converging multimodal inputs into a higher-order representation of the spatial context thereby enhancing the distinctiveness of the representation. Spatial pattern separation may then serve to further enhance and maintain the distinctiveness of these newly formed conjunctive representations. Together, these processes may operate to reduce interference during learning (Shapiro & Olton, 1994) thereby increasing the likelihood of remembering one spatial event as separate from another spatial event (Kesner et al., 2004). In addition, temporal integration may provide a mechanism for linking or associating distinct representations that are experienced close in time (Aimone et al., 2006). Specifically, the formation of temporal associations among proximal spatial representations may provide a degree of similarity to the spatial events increasing the possibility that events that are encoded close in time can later be recalled together (Aimone et al., 2010). Collectively, these processing functions constitute a highly efficient information processing system that operates to form distinct temporally associated spatial representations. For illustrative purposes, consider the following scenario. While thumbing through books at a bookstore, you come across a book that you read when you were younger and suddenly recall the summer day that you sat in the shade of a maple tree reading the book. You then recall that it was the same day that you rode your bike over to your best friend's house to make cupcakes for her little sister's birthday. You also remember that it was the day before you started a new pottery class at

the recreation center down the street from your grandmother's house. These memory representations contain rich detailed information about the distinct spatial context in which the events occurred and are linked together by the time at which they were experienced. This hypothetical scenario provides an illustration of how the reactivation of a specific memory event may induce the reactivation of other distinct memories that occurred (and were formed) around the same time (Aimone et al., 2006).

In summary, the results from the present investigations provide evidence for a dynamic role for the dDG in spatial processing and the formation of unique spatial representations. Spatial representations play a critical role in navigational processes, real or imagined. Spatial representations are also an important component of episodic memory, or memory that "requires the ability to remember particular events and to distinguish them from other events" (Rolls, 2010, p. 181). The present findings contribute to our understanding of how spatial representations are formed, the neural mechanisms that are involved in this formation, and the neural structures that support these functions. They also have important implications for understanding human spatial processing and memory formation.

References

Aimone, J. B., Deng, W., & Gage, F. H. (2010). Adult neurogenesis: Integrating theories and separating functions. *Trends in Cognitive Sciences*, *14*(7), 325-337.

Aimone, J. B., Wiles, J., & Gage, F. H. (2006). Potential role for adult neurogenesis in the encoding of time in new memories. *Nature Neuroscience*, *9*(6), 723-727.

Bakker, A., Kirwan, C. B., Miller, M., & Stark, C. E. (2008). Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science*, *319*(5870), 1640-1642.

Gilbert, P. E., Kesner, R. P., & Lee, I. (2001). Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. *Hippocampus, 11*(6), 626-636.

Goodrich-Hunsaker, N. J., Hunsaker, M. R., & Kesner, R. P. (2008). The interactions and dissociations of the dorsal hippocampus subregions: How the dentate gyrus, CA3, and CA1 process spatial information. *Behavioral Neuroscience, 122,* 16-26.

Kesner, R. P. (2007). A behavioral analysis of dentate gyrus function. *Progress in Brain Research, 163,* 567-576.

Kesner, R. P., Lee, I., & Gilbert, P. (2004). A behavioral assessment of hippocampal function based on a subregional analysis. *Reviews in the Neurosciences, 15,* 333-351.

Leutgeb, J. K., Leutgeb, S., Moser, M. B., & Moser, E. I. (2007). Pattern separation in the dentate gyrus and CA3 of the hippocampus. *Science, 315*(5814), 961-966.

Rolls, E. T. (2010). A computational theory of episodic memory formation in the hippocampus. *Behavioural Brain Research, 215*(2), 1265-1274.

Rolls, E. T., & Kesner, R. P. (2006). A computational theory of hippocampal function, and empirical tests of the theory. *Progress in Neurobiology, 79,* 1-48.

Shapiro, M. L., & Olton, D. S. (1994). Hippocampal function and interference. In D. L. Schacter, & E. Tulving (Eds.), *Memory systems* (pp. 39-63). London, UK: MIT Press.

Xavier, G. F., & Costa, V. C. (2009). Dentate gyrus and spatial behaviour. *Progress in Neuropsychopharmacology and Biological Psychiatry, 33*(5), 762-773.