THE ROLE OF THE VENTRAL DENTATE GYRUS IN OLFACTORY LEARNING AND MEMORY AND ANXIETY-BASED BEHAVIORS

by

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ABSTRACT

Dorsoventral lesion studies of the hippocampus (HPP) have suggested that the dorsal axis is important for spatial processing and the ventral axis is involved in olfactory learning and memory as well as anxiety. Accrued reports have indicated that subregions along the dorsal axis play specialized roles in spatial information processes and there is some evidence to indicate that the ventral CA3 and ventral CA1 subregions are involved in cued retrieval in fear conditioning and also carry out olfactory learning and memory processes similar to dorsal axis counterparts. The current study investigated the less-understood role of the ventral DG in olfaction and anxiety. A series of odor stimuli were used that provide a range of differentiation on only one level in a matching-to-sample paradigm to investigate ventral DG involvement in working memory for similar and less similar odors, in which there was a memory-based pattern separation effect. A novelty detection paradigm was used to investigate ventral DG involvement in recognition of familiar and new social odors. Finally, an elevated-plus maze and open field maze were selected in order to investigate the role of the ventral DG in the ability to modify behavior in potentially dangerous environments. The current study has provided evidence to suggest that the ventral DG plays an important role in olfactory learning and memory processes as well as anxiety-based behaviors during exploration in anxiety-provoking environments.

I dedicate this work to my loving family. Particularly to my patient, understanding, and ever-supporting husband, Matt.

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CHAPTER 1

GENERAL INTRODUCTION

The hippocampus (HPP) has long been associated with learning and memory processes for humans, non-human primates, rodents and many other animals. And while there is a rich experimental history that supports its role in spatial processing, there is substantial evidence that the HPP is critical to other forms of learning and memory, such as odor information processing and anxiety-based behaviors (Bannerman et al., 2003; Eichenbaum, Mathews, & Cohen, 1989; Kjelstrup et al., 2002). The importance of the HPP in olfactory processing has been shown in aging adults with Alzheimer's Disease as well as rodent lesion studies (Gilbert, Barr, & Murphy, 2004; Hunsaker, Fieldsted, Rosenberg, & Kesner, 2008;Kesner, Hunsaker, & Ziegler, 2011). Many functions of the HPP have been identified as specific to particular hippocampal subregions (DG, CA3, and CA1) (Kesner, Lee, & Gilbert, 2004). However, the ventral subregions of the HPP are not as well studied as their dorsal counterparts (Fanselow & Dong, 2010). The role of the ventral DG is least understood among ventral subregions. Lesion studies of the ventral DG have been difficult to execute, in part because the location requires deep needle penetration through other brain structures, increasing risk of seizure behavior. While the anatomical location of the ventral DG is a challenge to the study of the area in isolation, differentiating anatomical characteristics have served to inform or indicate

specialized processing roles for each subregion and it is possible that specializations of the ventral DG may be indicated in a similar fashion.

Dorsoventral differentiation of processing specialty may also hold true for individual subregions. For example, the CA3 has been shown to be importantly involved in pattern completion for spatial information and imaging studies have revealed that dorsal activity shifts toward the ventral HPP when an odor has been paired with a cued experience (Gold & Kesner, 2005; Kent, Hess, Tonegawa, & Small, 2007; Rolls, Treves, Foster, & Perez-Vicente, 1997). Further, lesion studies show that the dorsal CA1 is importantly involved in temporal processing, with the dorsal CA1 important for spatial processing and the ventral CA1 necessary for processing sequences of odor information (Gilbert, Kesner, & Lee, 2001; Hunsaker, Fieldsted, Rosenberg, & Kesner, 2008). Though substantial evidence supports the idea that the ventral CA3 and CA1 are important for specialized processing of odor information and the entire ventral HPP is involved in these processes, the role of the ventral DG is poorly understood. Because of the prominent role played by the DG in processing information that is retrieved from all sense modalities, it is important to understand contributions of the ventral DG to learning and memory processes. The evidence provided suggests that there may be a parallel processing relationship across the dorsoventral axis for the CA3 and CA1 subregions. It is possible that the dorsal and ventral DG may share a similar relationship for pattern separation of spatial and olfactory information. For example, lesion studies have shown that the ventral, but not the dorsal HPP is critical for pattern separation of highly overlapping olfactory information (Kesner et al., 2011). Given the established role of the dorsal DG in pattern separation for spatial representations, it is possible that the ventral

DG shares a similar role in pattern separation for olfactory learning and memory processes (Gilbert et al., 2001). In their investigation, Gilbert and colleagues (2001) demonstrated that lesions to the dorsal DG resulted in impairments for spatial locations, but only when the locations were highly overlapping in distance. In order to investigate a possible role for the ventral DG in pattern separation for odor information, we used a non-matching-to-sample paradigm similar to that described by Kesner and colleagues (2011). The paradigm implements olfactory stimuli that vary only in the number of methyl groups, which make it possible to directly investigate pattern separation processes for odors by varying the degrees of difference between stimuli during testing (Cleland, Morse, Yue, & Linster, 2002).

In addition to olfactory processing for carbonic odorants, behavioral studies have shown that each subregion of the dorsal HPP can be importantly involved in different aspects of detecting novelty for spatial information and objects (Beselia, Maglakelidze, Chkhikvishvili, Burjanadze, & Dashniani, 2010; Hunsaker, Mooy, Swift, & Kesner, 2007; Kesner et al., 2004; Vago & Kesner, 2008). Anatomical functions have led researchers to suggest that, within the perforant pathway that projects from the entorhinal cortex to the DG, the medial pathway is important for processing object information (Hargreaves et al., 2005; Witter, Groenewegen, Lopes da Silva, & Lohman, 1989). An important study by Hunsaker and colleagues (2007) that targeted NMDA and opioid receptor activity demonstrates that although the medial pathway is important for processing spatial novelty, it is also involved in some aspects of novelty detection for objects. Likewise, the same study demonstrates that the lateral pathway can also be involved in object novelty detection (Hunsaker et al., 2007). Additional research shows

that novelty detection can be processed via direct projections from the entorhinal cortex to the CA1 subregion, which suggests that novelty detection is an important process that is carried out in different ways within the individual subregions of the HPP (Vago & Kesner, 2008).

Because the DG receives and processes most hippocampal information, it is possible that the ventral DG may also play an important role in detecting novelty for odor information, but investigations have not been conducted. Therefore, we conducted a novel olfactory paradigm similar to a spatial exploratory task described by Goodrich-Hunsaker and colleagues (2008) in order to investigate the role of the ventral DG in novelty detection for odors.

As indicated previously, the ventral HPP shares connections with structures that regulate hormones involved in feeding, motivation, stress and emotional states through hypothalamic involvement in the hypothalamic-pituitary adrenal axis (HPA) (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). Behavioral evidence shows that though rats with dorsal HPP lesions are not affected, rats with ventral and whole HPP lesions fail to demonstrate anxiety behaviors exhibited by control subjects on well-established anxiety measures such as the elevated-plus maze and tests of hyponeophagia (Bannerman et al., 2002; Kjelstrup et al., 2002). Interestingly, rats administered anti-anxiety drugs, such as midazolam, become impaired on the elevated plus maze and perform similar to those with lesions to the ventral HPP. This indicates that the elevated-plus maze is sensitive to anxiety-based behaviors (Kjelstrup et al., 2002). Behavioral evidence shows that ventral CA3 is important to retrieval of contextual fear conditioning and ventral CA1 is important for retention of trace fear conditioning, which provides support for ventral

subregional specialization in emotional behaviors (Hunsaker & Kesner, 2008; Rogers, Hunsaker, & Kesner, 2006). Due to other ventral subregion involvement in anxiety-related behavior, it is possible that the ventral DG may also be involved in processing such emotional behaviors. However, no behavioral lesion studies have been conducted to directly investigate the impact the ventral DG may have in these processes. Therefore, we used an elevated-plus maze, as originally described by Pellow, Chopin, File and Briley (1985), in order to investigate the role of the ventral DG in anxiety-based behaviors.

Because there is a lack of individual ventral subregional evidence in regard to anxiety-based behaviors, much of the premise of the current studies has been founded on reports that have involved ventral hippocampal lesions. Previous ventral investigations have reported displays of hyperactivity, but the evidence is mixed (Bannerman et al., 2002; Bannerman et al., 1999; Kjelstrup et al., 2002). Reports provide different experiences within the Morris water maze: ventral HPP lesions have been reported to result in both faster and slower swim speeds, which would indicate contradictory levels of activity, or hyperactivity (Bannerman et al., 2002; Bannerman et al., 1999). It is important to note that hyperactivity is not actively observed, but instead is calculated by comparing activity scores of the treatment group to that of the control group. For example, the hyperactivity score of a treatment group increases when control subjects' activity decreases, even if treatment group activity remains stable. Therefore, observations of hyperactivity within the treatment group are mediated by activity levels of controls. Several measures of anxiety include reduced locomotion; therefore, measures of hyperactivity taken from an anxiety-inducing task will serve to confound the relationship between exhibition of anxiety-based behaviors and assessments of

hyperactivity. In the studies above, rats with ventral HPP lesions did not exhibit hyperactivity when in the home cage. This suggests that the rats with ventral lesions were only hyperactive in specific situations. A more plausible explanation may be that controls did not exhibit anxiety in their home cages, and thus their behaviors did not vary from rats with ventral lesions. However, when in anxiety-provoking tasks, it is likely that controls demonstrated "normal" anxiety (such as freezing behavior) and therefore were more stationary than lesioned subjects that did not show anxiety (freezing). This indicates that observations of hyperactivity in anxiety-provoking environments may not provide an accurate depiction of overall locomotor activity. Clearly, a task that minimizes possible displays of anxiety yet maximizes exploration would be a better assessment of hyperactivity. The open field maze has been widely used and is considered a classic test of locomotor behaviors that minimizes anxiety provocation (Ramos, Berton, Mormède, & Chaouloff, 1997). We used the open field paradigm, described by Walsh and Cummins (1976), in order to investigate the role of the ventral DG in locomotor activity.

Though previous research has demonstrated that the HPP is important for spatial and olfactory learning and memory, as well as anxiety-based behaviors, there is little information about how individual subregions, especially along the dorsal axis, may contribute to these processes. Though a minimal amount of research supports roles for the ventral CA3 and ventral CA1 in learning and memory, there has been little evidence offered to indicate whether the ventral DG is important in these processes.

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CHAPTER 2

STUDY ONE: THE ROLE OF THE DENTATE GYRUS IN OLFACTORY LEARNING AND MEMORY

Abstract

Dorsoventral lesion studies have indicated that the dorsal axis of the hippocampus (HPP) is important for spatial processing and the ventral axis of the HPP is important for olfactory learning and memory and anxiety. There is some evidence to suggest that the ventral CA3 and ventral CA1 conduct parallel processes for pattern completion and temporal processing, respectively. Studies have suggested that the dorsal DG is importantly involved in pattern separation processes for spatial information. However, the ventral DG is less understood. The current study investigated the less-understood role of the ventral DG in olfactory learning and memory processes. A matching-to-sample paradigm was used and a series of odor stimuli that provide a range of differentiation only one level, number of methyl groups, were used in order to investigate ventral DG involvement in working memory for similar and less similar odors. A novelty detection paradigm was implemented that included conspecifics as odor stimuli in order to investigate the role of the ventral DG in novelty detection for social odors. The current data indicate that rats with ventral DG lesions were impaired on the delayed-matching-to-sample paradigm at delays of 60 seconds, but not at delays of 15 seconds. Also, a

memory-based pattern separation effect was observed in that performance was poorest with only one separation between trial odors and performance was highest when there were four separations between trial odors. The present data also indicate impairment on the novelty detection task, in which rats with ventral DG lesions failed to show an exploratory preference for novel conspecifics over familiar conspecifics. The current study results suggest that the ventral DG plays an important role in olfactory learning and memory processes that include novelty detection, especially when odors are highly similar. The results also indicate a role for the ventral DG in pattern separation processes for odor information, which may have further implications for parallel processing across the dorsoventral axis for the DG in spatial (dorsal) and olfactory (ventral) pattern separation.

Introduction

The majority of investigations involving the HPP have focused on the dorsal portion of the structure. However, behavioral and anatomical differences have been observed along the dorsoventral axis, and these distinctions have led theorists to investigate possible specializations along the dorsoventral axis. There is ample behavioral evidence that the dorsal region of the HPP plays a critical role in learning and memory for spatial information and though the ventral region can be involved, it does not appear to be necessary for spatial processing (E. Moser, Moser, & Andersen, 1993; M. Moser & Moser, 1998). Evidence does support that the ventral HPP is important for working memory processing for odor information (Kesner, Hunsaker, & Ziegler, 2011; Pentkowski, Blanchard, Lever, Litvin, & Blanchard, 2006). Anatomical connectivity

supports the concept of dorsoventral specialization across the hippocampus. For example, only the dorsal HPP shares connections with the mammillary and anterior thalamic nuclei, which are known to house navigational neurons (Dong, Swanson, Chen, Fanselow, & Toga, 2009; Taube, 2007). Place fields in the dorsal HPP are more dense, smaller and fire more reliably in particular locations than do their ventral counterparts (Jung, Wiener, & McNaughton, 1994). These and other anatomical differences make the dorsal HPP especially suitable for carrying out learning and memory processes involving spatial information. In contrast, the ventral HPP shares connections with the nucleus accumbens, amygdala, hypothalamus and olfactory bulb (Van Groen & Wyss, 1990). These structures are involved in conditioning and motivation, hormone regulation and behavioral expression of emotional states, and olfactory processing, respectively (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009; Everitt & Robbins, 2005; Gulyás, Tóth, McBain, & Freund, 1998; Hughes & Shin, 2011). The ventral HPP has a direct connection to the prefrontal cortex that is not shared with the dorsal HPP (Bannerman et al., 2004). These anatomical attributes, along with behavioral evidence, support a role for the ventral HPP in olfactory learning and memory (Kesner et al., 2011).

Computational theories and a considerable body of research support that individual dorsal subregions of the HPP, namely the dentate gyrus (DG), CA3 and CA1, are specialized for specific spatial learning and memory processes (Rolls & Kesner, 2006). The DG has been shown to be important for separation of highly overlapping spatial representations and the CA1 is critical to processing temporal spatial information (Gilbert, Kesner, & Lee, 2001). Evidence shows that the CA3 is important for rapid encoding and pattern completion for spatial information (Gold & Kesner, 2005; Rolls,

Treves, Foster, & Perez-Vicente, 1997). Though dorsal hippocampal subregions have been established to have specialized roles, ventral subregional roles in processing are not well understood, but there is some evidence to suggest that individual ventral subregions of the HPP may be importantly involved in processing odor information and that this function may parallel across the dorsoventral axis of the HPP, in which the dorsal portion processes spatial information and the ventral portion processes olfactory and emotional information. For example, the ventral CA1 has been shown to be important for temporal order processing of odors, which parallels the same temporal processing role for processing of spatial information in the dorsal CA1 (Hunsaker, Fieldsted, Rosenberg, & Kesner, 2008). Evidence also show that the ventral CA3 is important for forming associations between contexts and odors which may complement pattern completion processes that are the primary function of the dorsal CA3 for spatial information (Gold & Kesner, 2005; Kent, Hess, Tonegawa, & Small, 2007). Because substantial evidence supports that the ventral CA3 and CA1 are important for specialized processing of odor information and the entire ventral HPP is involved, it is likely that the ventral DG is also involved in specialized odor processing. But there have yet to be ventral DG investigations to complete the picture. Because the DG receives inputs from all sensory modalities which it processes then sends to other subregions, it is well-suited to make a substantial impact in olfactory learning and memory processes. Further, it is possible that a dorsoventral parallel functioning relationship exists between the role of the dorsal DG in pattern separation for highly overlapping spatial information and a possible role for the ventral DG in pattern separation of highly overlapping odor information. As previously mentioned, there is evidence to support a parallel processing relationship across the

dorsoventral axis for CA3 and CA1, which indicates a similar relationship for the DG. Indirect evidence lends support for the possibility of a dorsoventral relationship in pattern separation within the DG. For example, lesion studies have shown that the ventral, but not dorsal HPP is critical for pattern separation of highly overlapping olfactory information (Kesner et al., 2011). Given the established role of the dorsal DG in pattern separation for spatial representations, it is possible that the ventral DG, without its subregional counterparts CA3 and CA1, may serve as a specialized mechanism to provide pattern separation processes for olfactory information, yet no lesion studies have been conducted to investigate this possibility (Gilbert et al., 2001). Clearly, investigations specific to the ventral DG are needed to better understand its role in learning and memory for odors.

A collection of behavioral studies have shown that each subregion of the dorsal HPP can be importantly involved in different aspects of detecting novelty for spatial information and objects (Beselia, Maglakelidze, Chkhikvishvili, Burjanadze, & Dashniani, 2010; Hunsaker, Mooy, Swift, & Kesner, 2007; Kesner, Lee, & Gilbert, 2004; Vago & Kesner, 2008). Based on anatomical features, researchers have suggested that the medial perforant pathway processes spatial information and the lateral pathway processes object information (Hargreaves, Rao, Lee, & Knierim, 2005; Witter, Groenewegen, Lopes da Silva, & Lohman, 1989). However, studies also show that both pathways are important for processing both spatial and object novelty (Hunsaker et al., 2007). Novelty detection processing can be carried out via direct projections from the entorhinal cortex to the CA1 subregion. Taken together, these studies suggest that

novelty detection is an important process that can be carried out in different ways within individual subregions of the HPP (Vago & Kesner, 2008).

Evidence suggests that the ventral HPP plays an important role in working memory for detecting differences between odors (Kesner et al., 2011). Additionally, evidence supports the concept that the dorsal DG is importantly involved in the formation of spatial representations that are further processed by other subregions down-stream (Hunsaker et al., 2007; Kesner, 2007a). The dorsal DG is also critical for the ability to form distinctions between highly overlapping patterns of spatial information (Gilbert et al., 2001). This line of dorsoventral evidence implies that the ventral DG may also play an important role in novelty detection, but for odors. However, investigations have not been conducted.

It has been established that rats are able to detect differences in odors of conspecifics, or members of their same species and social odor tasks have frequently been used to determine if subjects are able to display behaviors to indicate familiarity (Bannerman et al., 2002). These highly overlapping features of odors among rats may be suitable to serve as odor stimuli in novelty detection studies. One caveat is that conspecific odors are also used as stimuli to study stress, fear or anxiety behaviors, which may confound behavioral tests of novelty (Bannerman et al., 2002). However, using odors of juvenile rats reduces these behavioral displays (Burman & Mendl, 2003). A number of previous studies confound novelty for spatial locations, objects and odors.

The DG subregion of the HPP plays an important role in processing information before sending it down-stream to be further processed by the CA3 and CA1 subregions (Kesner, 2007a; Witter & Amaral, 2004). Despite the DG's ability to have widespread

influence over hippocampal processing, the impact of the ventral DG on such processes is not understood. Thus, the first study was conducted to investigate the role of the ventral DG in a modified working memory task sensitive to pattern separation effects for odors (Kesner et al., 2011). Olfactory stimuli whose features make it possible to directly investigate measures of differences between olfactory stimuli on a metric (carbonic) scale, thus allowing for direct investigation of pattern separation processes for odors, were used (Cleland, Morse, Yue, & Linster, 2002). The purpose of the second study was to examine the role of the ventral DG in novelty detection for odor information in a new olfactory paradigm that was modeled after a spatial novelty detection exploratory task described by Goodrich-Hunsaker and colleagues (2008) that controls for previous study confounds of object and spatial location.

Materials and Methods

Subjects

Thirty male Long-Evans rats weighing 250-350 g were used as subjects. Twelve rats were used as subjects in Experiment 1 and 18 rats were used in Experiment 2. Rats were housed individually in plastic cages that were located in a colony room with a 12H: 12H light-dark cycle. Testing was individually conducted for each rat during the light phase of the light-dark cycle. All subjects had unlimited access to water. Subjects in Experiment 1 were food restricted to 85-90 % of their free-feed weight and subjects in Experiment 2 had unrestricted access to food.

Surgical Procedures

All procedures and animal care were in compliance with the National Institute of Health and Institute for Animal Care and Use Committee of the University of Utah. Rats were randomly assigned to control (Experiment 1, $n = 6$; Experiment 2 $n = 6$), ventral DG (Experiment 1, $n = 6$; Experiment 2, $n = 6$), or dorsal DG (Experiment 2, $n = 6$) lesion groups. Rats received either bilateral intracranial infusions of colchicine (2.5 mg/ml, 0.8 µl/site) into the dorsal DG or ventral DG or intracranial infusions of saline hydrochloride solution (2.5 mg/ml, 0.8 µl/site) into the dorsal or ventral DG. Prior to surgery, animals received atropine sulfate (0.54 mg/kg, i.m.). Subjects were anesthetized by exposure to isoflurane gas and were positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). For the duration of the procedure, subjects remained anesthetized with a continuous flow of isoflurane (2 – 4%) and medical air (≈1.5L/min) mixture. Hair covering the surgical site was removed with a rechargeable Conair trimmer (Shelton, CT). Antiseptic measures were carried out: a surgical drape was positioned to expose only the shaved area, which was swabbed three consecutive times with betadine. The skin covering the skull was incised and retracted to expose the skull. Bregma was identified and burr holes were drilled through the skull at injection sites. Injections were made by lowering a 7 µl Hamilton GasTight syringe (Hamilton Company, Reno, NV) that was attached to a micro infusion pump (Cole-Palmer, Vernon Hills, IL) to infuse colchicine (2.5mg/ml) into injection sites. The infusion system remained stationery at the location for 2 minutes post infusion to allow for even diffusion. For ventral DG lesions, the Hamilton syringe was bilaterally positioned at two locations: 5.7 mm posterior to bregma, 4.1 mm lateral to midline, 3.8 mm ventral from dura and 6.3 mm posterior to

bregma, 4.5 mm lateral to midline, 4.8 mm ventral from dura. For dorsal DG lesions, the Hamilton syringe was bilaterally positioned at two locations: 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. Following injections, retracted skin was released, pulled together and sutured. Betadine was swabbed over the stitched incision site and 100% medical air (1.5L/min) was administered as the subject is released from the stereotaxic apparatus. Each subject was returned to the home cage to receive postoperative care for 7 days that included Ibuprofen (Children's Motrin; 200 mg/100 ml water) as an analgesic and mashed food. During postoperative recovery, subjects were monitored for behavioral seizures.

Experiment 1: Odor Discrimination and Working Memory

Apparatus

The test apparatus consisted of a red Plexiglas box (48 x 27 x 30 cm). One removable guillotine door, also red Plexiglas, was positioned 25 cm from one end of the box and separated the apparatus into two distinct chambers: a start chamber (27 x 25 cm) and a choice chamber (27 x 59 cm). A magnetic strip was secured to the back wall of the choice chamber. Testing dishes (3 cm diameter and 3 cm high) were placed into magnetic cup holders that adhered to the magnetic strip to stabilize sample dishes during testing. Each sample dish was filled with reptile habitat sand (Zoo Mate, San Luis Obispo, CA) that served to eliminate visual cues for the presence of food rewards within the dish. The odor set consisted of a series of aliphatic acids with unbranched carbon chains that varied from two- to six- carbons in length. The odorants and their volumes

were based on the methods described by Cleland et al. (2002) (see Table 1). Each odorant was diluted with mineral oil and was placed around the inner-rim of a testing dish with a cotton swab.

Shaping

Twelve subjects were individually handled for approximately 20 minutes per day for the first week of training and a piece of Froot Loops cereal (Kellogg, Battle Creek, MI) was placed in the cage following the handling session to habituate the rats to both handling and the food reward that were to be used throughout shaping and testing. During the second week, each rat was shaped in its home cage to dig in a small dish of sand to retrieve a food reward. Rats initially retrieved a reward that was visible and on top of the sand. During the following training trial, the reward was partially submerged, but still partially visible. Following each successful retrieval, the reward was progressively submerged further into the sand for each trial until rats began to reliably dig to retrieve fully submerged rewards from the sand. Upon successful shaping of digging behavior, rats were placed individually in the testing apparatus and were allowed to freely explore the testing environment for 5 minutes. This exploration period took place at least 24 hours before preoperative training commenced.

Preoperative Training.

Individual rats received 16 matching-to-sample trials each day that consisted of 1 sample and 1 choice phase for each trial; each rat received 4 trials of each of the possible 4 carbon chain separations per day. All trials took place in the testing apparatus. During

the sample phase, a subject was placed in the start chamber of the apparatus with the divider in place in order to separate the rat from the choice chamber. A food reward, one piece of Froot Loops cereal, was hidden in a sand-filled test dish that had been rimmed with one of the five test acids. For each trial, there was always a reward in the sample dish (sample phase). The dish was secured to the magnetic strip on the back wall of the choice chamber. The divider was removed, allowing the subject access to the choice chamber that contained the sample dish in order to dig to retrieve the reward. The chamber divider was re-inserted and the subject was placed back in the start chamber. During an approximate delay of 15 seconds, the previous, sample dish containing the original acid and another acid-laced dish from the test group were secured to the back wall of the choice chamber. A food reward was submerged only in the acid dish that was present during the sample phase. At the end of the 15 seconds delay, the divider was removed and the subject had access to both dishes, but was not allowed to self-correct: the subject was permitted to dig in only 1 dish. Once digging in a dish occurred, the other dish was removed from the choice chamber. In order to retrieve a reward, the subject was to dig in the dish whose acid matched the sample for that trial. If the subject did dig in the dish with the matching odor, the trial was scored as correct. If the subject did dig in the dish that does not match the sample odor, the trial was scored as incorrect. The intertrial interval was 60 seconds. The sample acid varied with each trial. Odor separations of 1, 2, 3, or 4 carbons were paired against the sample acid in the choice phase of every trial. Training persisted daily until rats reached criterion performance of about 80 – 90 % on the last 16 trials.

Postoperative Testing.

Following pretraining, rats underwent stereotaxic surgical procedures to receive ventral DG (colchicine; $n = 6$) or control (saline; $n = 6$) lesions. Following 7 – 10 days of postoperative rest, rats began postoperative testing that was similar to pre-operative training trials. Subjects received 4 trials of 4 possible acid separations per day for 5 days at a delay of 15 seconds. Subjects received 4 trials each of 4 possible acid separations per day for 4 days at a delay of 60 seconds. All trials were scored in the same manner as in pre-operative training. Postoperative calculations for each separation (1, 2, 3, 4), averages were obtained by summing all the scores within one separation and dividing that total by the total number of trials for that separation.

Experiment 2: Novelty Detection of Social Odors

Apparatus

The exploratory surface was 122 cm in diameter and 64 cm above the floor. Two juvenile rats were present for each session and served as stimuli, from a pool of four juveniles. The juvenile stimuli were placed under mesh wire cages (23 x 17 x 15 cm) that served to protect the juvenile rats from mature test rats. The apparatus was located in a small testing room: sessions were recorded by a researcher in a different room.

Procedural Methods

Wire cages were positioned 38 cm apart, in the center of the testing platform, and were secured to the floor of the platform with magnets. Rats were allowed to explore the testing environment for 5 minutes at least 20 minutes prior to testing. Rats were returned

to the home cage before and between trials. During the first session (sample phase), each protective wire cage contained a juvenile rat (Pup A, Pup B). The test subject was removed from the home cage and placed on the testing platform. Exploration was recorded for 5 minutes. Following the sample phase, the test subject was returned to the home cage for a 10 minute delay period. The platform and cages were cleaned during the interval. Two juveniles were placed under the wire cages: 1 juvenile from the sample phase (Pup A or Pup B) was placed under a wire cage and a novel juvenile (Pup C) was placed under the other cage. During the test phase, the subject was placed in the middle of the platform and exploration was recorded for 5 minutes (see Figure 1). Selection of the returning juvenile for the test phase (Pup A or Pup B) and which cage it would occupy was counterbalanced. Also, the roles of juveniles from the juvenile pool were pseudorandomized across subjects.

Histological Procedures

Following behavioral tests, subjects were deeply anesthetized with sodium pentobarbital (1.5 ml, 70 mg/kg, i.p.). Subjects were intracardially perfused with phosphate buffered solution (PBS) then with a formalin solution (10 mg/kg). The brain was extracted and stored in a 10% formalin/30% sucrose cryoprotectant solution for 72 hours at ≈ 4°C. A tissue block containing the hippocampus was frozen and sliced on a cryostat at a thickness of 24 µm. Ventral DG lesioned brains were cut on the horizontal plane and dorsal DG lesioned brains were cut on the coronal plane. Every third section was mounted on a gelatin coated glass slide and Nissl stained with cresyl violet. Slides were examined microscopically in order to verify lesion placement.

Results

Histological Results

Bilateral lesions using axon-sparing colchicine were made to the ventral or dorsal DG, depending on surgical condition. Figure 2 shows a schematic that represents the locations of ventral DG lesions, and Figure 3 shows a schematic that represents the location of dorsal DG lesions.

Behavioral Results

Odor Discrimination and Working Memory.

Pre-surgery at a Delay of 15 Seconds

Figure 4 provides the mean (±SE) percent correct average performance for pre-surgery that reflects the most recent block of 16 trials with a delay of 15 seconds for each of the four possible aliphatic separations. A repeated-measures analysis of variance (ANOVA) with lesion (control, ventral DG) as the between group factor and separation (1, 2, 3, 4) as the within group factor was used to analyze the data. The analysis indicated a significant effect of separation $F(3, 30) = 20.01$, $p < .001$. However, there was no significant effect for surgery $F(1, 10) = 1.08$, $p = .324$ and no significant interaction. A Newman-Keuls post hoc comparison indicated that performance for a separation of one was significantly lower than performance for separations of two, three, and four ($p < .05$).

Postsurgery at a Delay of 15 Seconds

Figure 5 provides the mean (±SE) average percent correct for a block of 20 trials with a delay of 15 seconds for each of the 4 aliphatic separations. A two-way repeated

measures analysis of variance (ANOVA) with lesion (ventral, control) as the between group and separation (1, 2, 3, 4) as the within group factor was conducted to analyze the data. The analysis indicated no significant effect for lesion F (1, 10) = .79, p = .396, no significant effect of separation F (3, 30) = 2.10, p = .121, and no significant interaction. The results suggest that there were no statistically significant differences in performance between rats with ventral or control lesions across all four separations. These results indicate that additional matching-to-sample trials after surgery may result in an increase in performance when compared to performance before surgery.

Postsurgery at a Delay of 60 Seconds

Figure 6 provides the mean (±SE) average percent correct for a block of 16 trials with a delay of 60 seconds for each of the four aliphatic separations. A two-way repeated measures analysis of variance (ANOVA) with lesion (ventral DG, control) as the between group and separation (1, 2, 3, 4) as the within group factor was conducted to analyze the data. The analysis indicated a significant effect of lesion F (1, 10) = 9.97, p = .0102, and a significant effect of separation F(3, 30) = 18.22, $p < .001$. There was also a significant lesion x surgery interaction F (3, 30) = 3.00, p = .046. A Newman-Keuls post hoc comparison test indicated that for ventral DG lesioned rats, separation 1 was significantly different from separations 2, 3, and 4, ($p < .05$). But there was no significant effect for controls. For separations 1 and 2, rats with ventral DG lesions were significantly different from controls ($p < .05$). There were no significant difference effects for separations 3 and 4. These results indicate that rats with ventral DG lesions demonstrated

significant impairment when there were less separations between odorants, when compared to controls.

Novelty Detection of Social Odors

Figure 7 provides the mean (±SE) novelty preference ratio for exploration of novel versus familiar juveniles. The novelty preference ratio was calculated by subtracting the time spent exploring the familiar juvenile from the time spent exploring the novel juvenile, divided by the sum of time spent exploring either juvenile [(novel – familiar) / (novel + familiar)]. A positive preference ratio indicates that the subject explored the novel juvenile for longer than the familiar juvenile. A preference ratio of zero indicates that each juvenile was explored for the same amount of time, and a negative preference ratio indicates that the subject explored the familiar juvenile for longer than the novel juvenile. A one-way analysis of variance (ANOVA) with lesion (ventral DG, dorsal DG, control) as the between group factor was conducted to analyze the data. The analysis revealed a significant effect of lesion for novelty preference at the $p < .05$ level, $F(2, 15) = 12.56$, $p = 0.002$. A Newman-Keuls post hoc comparison indicated no significant differences in the preference ratios of controls and dorsal DG lesioned rats. However, rats with ventral DG lesions demonstrated significantly lower novelty preference compared to controls and dorsal DG lesioned rats ($p < .05$). The results suggest that the ventral DG is important for highly overlapping odors, such as social odors in rats.

Discussion

Though the HPP has been shown to be involved in processing odor information, previous investigations have revealed that the ventral, but not dorsal, HPP is critical to these processes (Kesner et al., 2011). Subregional specialization of spatial processing function in dorsal areas has been reported in several studies (Hunsaker et al., 2008; Kesner, 2007a, 2007b; Kesner et al., 2004). Previous investigations have shown that the dorsal DG is important in pattern separation for highly overlapping spatial representations; the dorsal CA3 is important for rapid encoding, arbitrary associations, and pattern completion for spatial information; and the dorsal CA1 is critical for the temporal processing of information (Gold & Kesner, 2005; Hunsaker et al., 2008; Kesner, 2007a). It has also been suggested that some ventral subregions of the HPP carry out specialized roles in processing odor information that can parallel functions of dorsal subregions along the dorsoventral axis of the HPP. For example, investigations have revealed that the ventral CA1 is important for temporal processing of odor information, and imaging studies have suggested that the ventral CA3 may be important in formation of associations between contexts that include odor information (Hunsaker et al., 2008; Kent et al., 2007) . These reports indicate that the ventral DG may also hold a specialized role in odor memory processes. Therefore, the purpose of the present study was to investigate the role of the ventral DG in processing olfactory information.

The current investigation has provided evidence that the ventral DG, and not the dorsal DG, plays an important role in olfactory learning and memory processes. A delayed-matching-to-sample task was implemented with two time frames: a delay of 15 seconds and a delay of 60 seconds. The current investigation did not report impairment

at a delay of 15 seconds, but impairments were apparent at a delay of 60 seconds. Rats with ventral DG lesions were able to discriminate between odors without impairment when the matching-to-sample delay was 15 seconds, and performance after lesion implementation was similar to pre-training criteria. Therefore, it is unlikely that deficits observed at a delay of 60 seconds were the result of an inability to discriminate between odors after lesion implementation.

Previous research indicates that like-subregions across the dorsoventral axis of the HPP may provide parallel processing specialization. For example, the dorsal CA1 has been shown to be important for temporal processing of spatial information and the ventral CA1 is importantly involved in this same processing function for sequences of odors (Hunsaker et al., 2008). The dorsal CA3 has been shown to be important for spatial pattern completion, or the ability to recall a location when partial cues are present (Kesner, 2007b). While there is no direct ventral CA3 evidence of pattern completion, imaging studies have revealed that activity shifts to the ventral portion of the structure when a cue has previously been associated with an odorant, which suggests that partial cues involving odor may rely on the ventral CA3 for pattern completion when odor information is a component (Kent et al., 2007). Previous research indicates that the dorsal DG is important for spatial pattern separation, or the formation of separate representations for highly overlapping spatial information (Kesner, 2007a). Therefore, it may be possible that the ventral DG is important for the formation of representations of highly overlapping odor information.

In order to investigate a possible pattern separation effect, a group of acid odorants that vary on only one characteristic were used (Cleland et al., 2002). The olfactory

stimuli vary only in the number of methyl groups. These unique stimuli provided a metric of number-of-methyl-group-differences between sample and test stimuli and provided a method to compare performance in the delayed-matching-to-sample task in terms of number of separations between the sample and choice odorants, which would indicate any separation effects(Cleland et al., 2002; Kesner et al., 2011). Though rats with ventral DG lesions demonstrated overall poorer performance when compared to controls, performance was poorer when stimuli were very similar (methyl group separations of one or two) and performance rates were higher when the sample and choice stimuli were less-similar (methyl group separations of three or four). Therefore, the current results of a pattern separation effect for odor information is supported by previous research that has indicated a parallel processing relationship for roles across the dorsoventral axis.

Previous research has indicated that novelty detection, or the ability to make distinctions between new and familiar information, is not specific to any one subregion of the HPP and instead impairment has been reported in most subregions (Kesner et al., 2004; Lee, Hunsaker, & Kesner, 2005). For example, a subregional study of the dorsal HPP revealed that dorsal DG lesioned and dorsal CA3 lesioned rats are impaired in detecting spatial novelty, or the movement of a object to a new location, and that dorsal CA1 lesioned rats showed only mild impairment (Lee et al., 2005). The authors suggested that all subregions of the hippocampus may be involved in spatial novelty detection to some degree. Therefore, we devised a novelty detection paradigm to investigate the role of the ventral DG in novelty detection for odor information. The current results indicate that the ventral DG is importantly involved in novelty detection

for odors. These results are in agreement with previous subregional studies of novelty detection (Lee et al., 2005). It is not clear if the observed impairments resulted from impairment in novelty detection in general, or if impairments were due to the highly overlapping nature of the odors of conspecifics that were used as odor stimuli in the study. Further investigation is necessary to clarify the mechanism of impairment, though the present results of the previous, matching, task indicate an impairment in novelty detection. Specifically, the present study data have revealed that ventral DG lesions did not impair discrimination among odor stimuli in the delayed-matching-to-sample task with a delay of 15 seconds. Therefore, it would be suggested that discrimination among highly overlapping social odors may be intact and that impairments observed in the novelty detection task are likely a novelty-specific deficit.

There is substantial experimental evidence to support specific roles for subregions of the dorsal HPP in spatial learning and memory processes. However, less is understood about individual subregions of the ventral HPP and more studies should be carried out to better understand odor and memory processing. Anatomical studies have indicated that the dorsal region of the HPP shares connections with several structures that are involved in spatial processing. For example, the perirhinal cortex, which receives highly processed spatial and visual information shares connections with the dorsal HPP and has been indicated to play an important role in spatial learning and memory for humans, non-human primates, and rodents (Squire & Zola-Morgan, 1991; Witter, Van Hoesen, & Amaral, 1989). The ventral HPP shares connections with the olfactory bulb which, besides the obvious implications of olfaction, has been shown to drive hippocampal activity patterns when there is an expectation of the next stimulus in a series to be an

odorant (Gourévitch, Kay, & Martin, 2010). Connections that are dorsal-specific and are related to spatial processing and connections that are ventral-specific that are related to olfactory processing provide important anatomical evidence that a division of processing is possible. The current study provides support for the parallel processing concept, as it was shown that the ventral DG is important to pattern separation processes for odor information, and this complements previous findings for the parallel role for dorsal DG in spatial processing (Kesner, 2007a). As mentioned, the current study results indicate a role for the ventral DG in pattern separation for odor information that parallels the same process for the dorsal DG in spatial pattern separation processes comes from previous studies that have indicated parallel dorsoventral processes for the CA1 and CA3 (Hunsaker et al., 2008; Kent et al., 2007). The dorsal CA3 has been shown to be important for spatial pattern completion and, while the ventral CA3 has been implicated in odor pattern completion in imaging studies, future lesion studies that investigate necessity of the ventral CA3 in pattern completion processes for odor information are needed to fully support specialized parallel processing across all three major subregions of the HPP.

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Table 1

Experiment 1: Odors and Their Percentage (Vol/Vol) Dilutions

Odor				
Acetic Acid	Propionic Acid	Butyric Acid	Valeric Acid	Caproic Acid
0.014	0.069	0.214	2.326	12.866

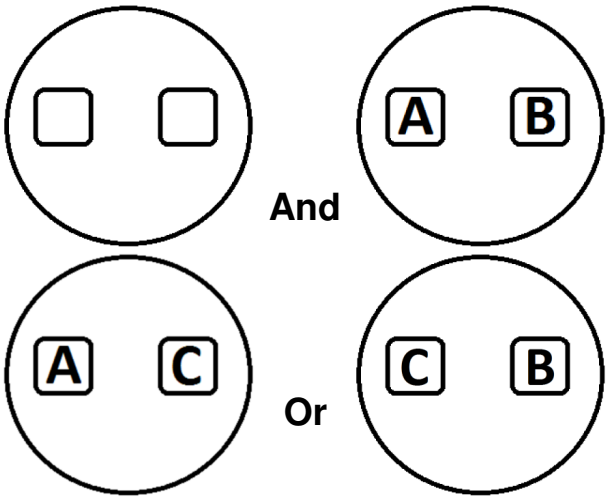

Figure 1. Schematic of novelty detection for social odor task. Clockwise, from top: the first image represents the initial exploration of corrals without stimuli (Pup A and Pup B). Next represents an exploration session with stimuli. The third and fourth images represent possible test conditions: one of the previous stimuli was returned to the same corral but novel stimuli (Pup C) replaced the other familiar stimuli.

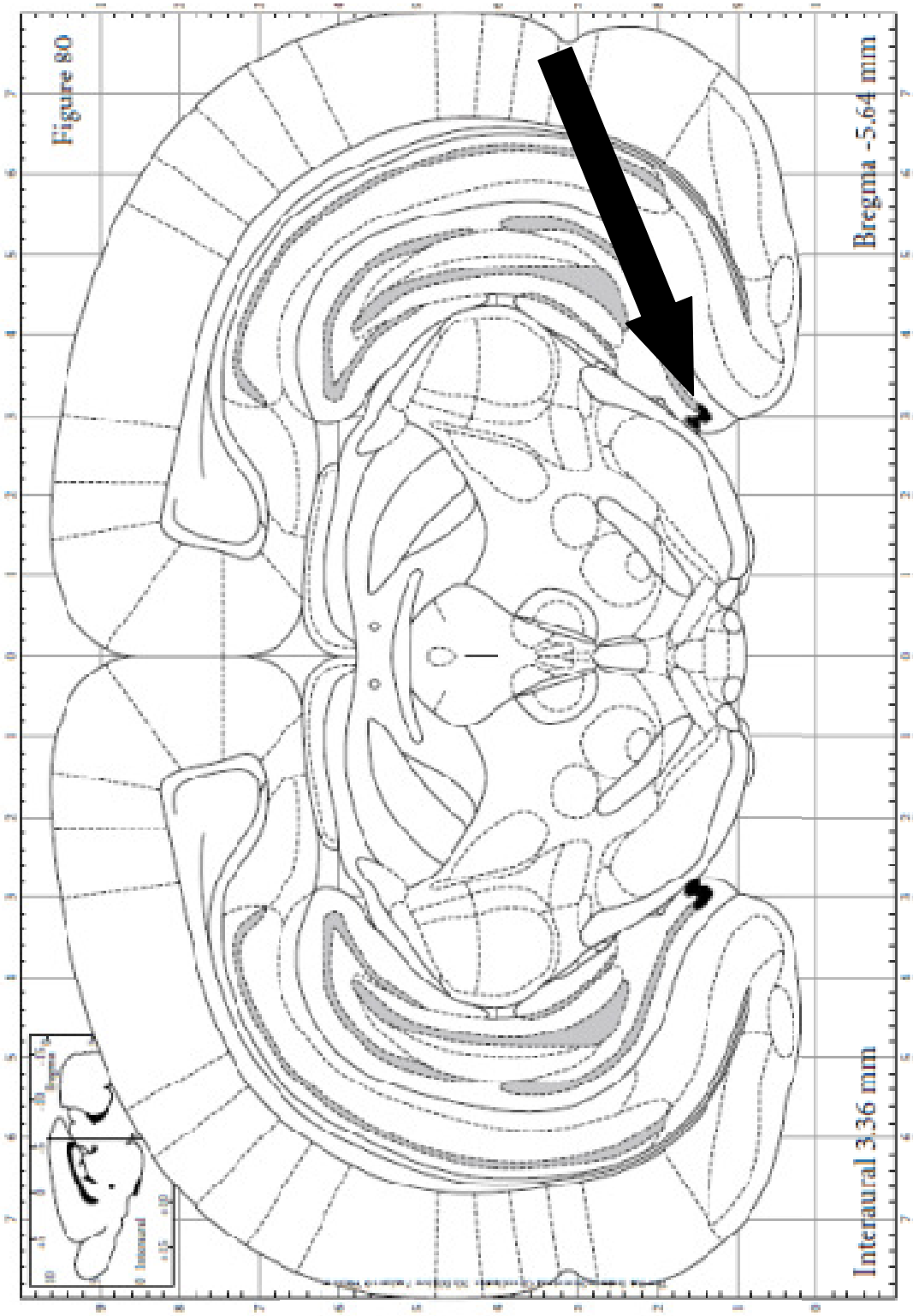

Figure 2. Schematic representation of ventral DG lesion target areas. Arrow indicates targeted ventral DG location.

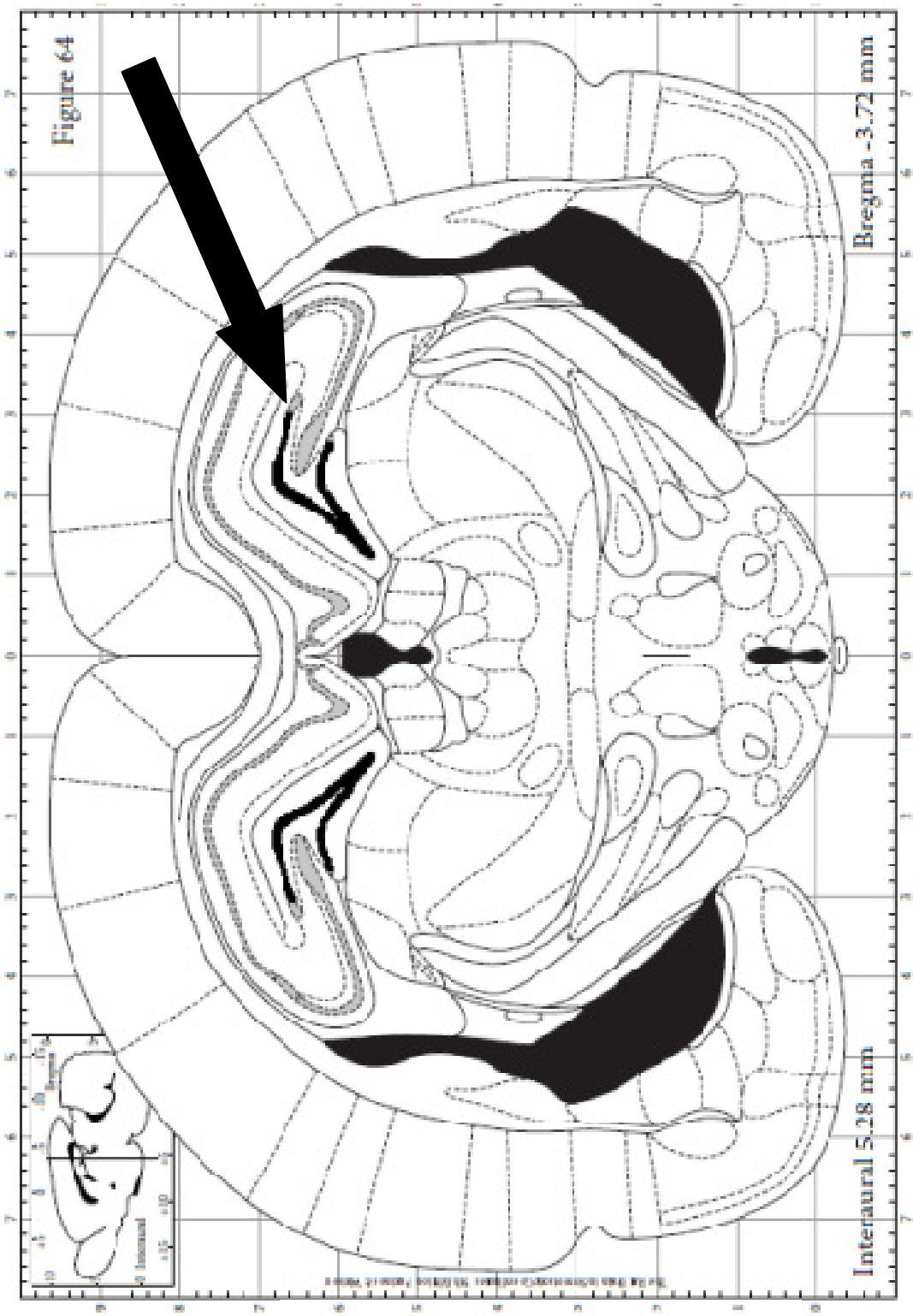

Figure 3. Schematic representation of dorsal DG lesion target areas. Arrow indicates targeted ventral DG location.

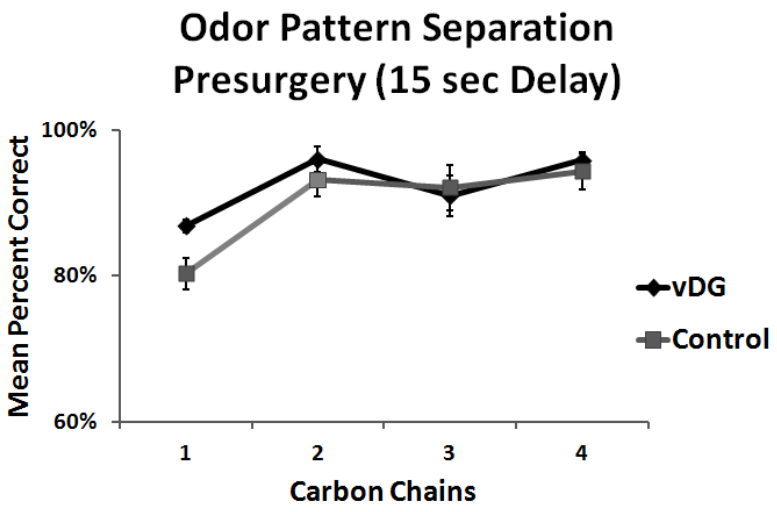

Figure 4. Mean (±SE) performance of pre-lesion subjects for levels of odor separation. Data consist of performance levels at criteria, which was 80 – 90 percent correct on the most recent block of 16 trials.

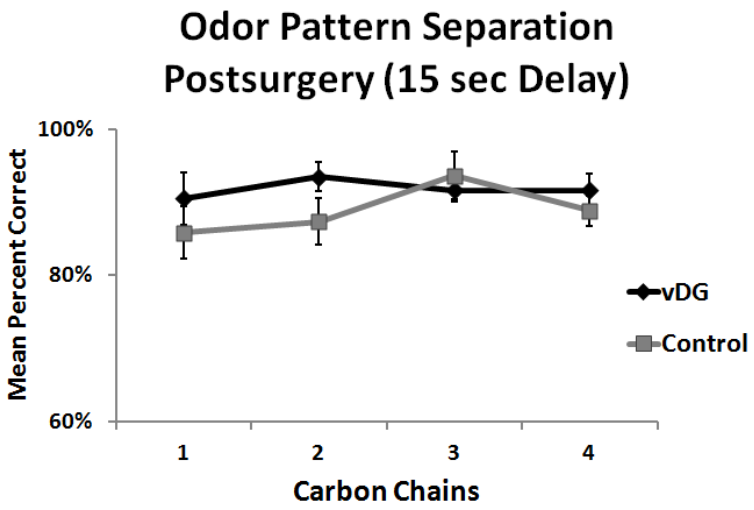

Figure 5. Mean (±SE) performance of ventral DG, dorsal DG, and control rats for levels of odor separation with a delay of 15 seconds for a block of 20 trials.

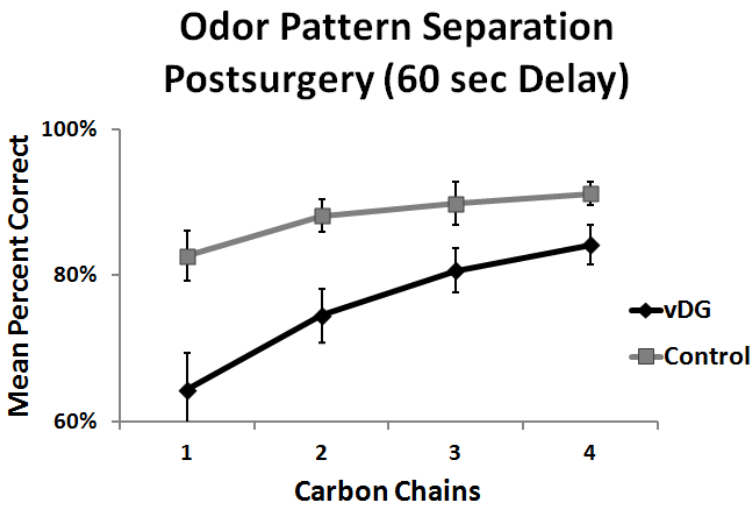

Figure 6. Mean (±SE) performance of ventral DG, dorsal DG, and control rats for levels of odor separation with a delay of 60 seconds for a block of 16 trials.

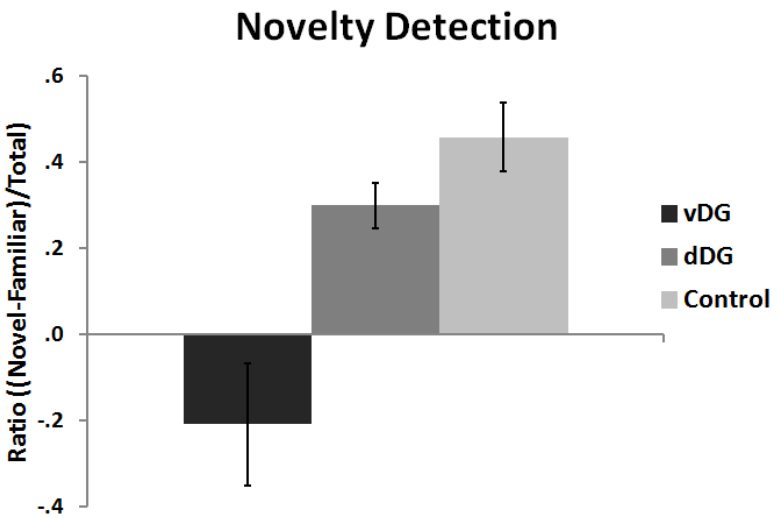

Figure 7. Mean (±SE) average of ventral DG, dorsal DG, and control rats for novel versus familiar conspecifics. A positive ratio reflects novelty preference, a ratio of zero reflects no preference, and a negative ratio reflects preference for the familiar conspecific.

CHAPTER 3

STUDY TWO: THE ROLE OF THE DENTATE GYRUS IN ANXIETY-BASED BEHAVIORS

Abstract

Dorsoventral lesion studies of the hippocampus (HPP) have indicated that the dorsal axis is important for spatial processing and the ventral axis is important for olfactory learning and memory and anxiety-based behaviors. Previous studies have suggested that there is some evidence to indicate the ventral CA3 and ventral CA1 subregions of the HPP are involved in cued retrieval in fear conditioning. The role of the ventral dentate gyrus (DG) in anxiety-based behaviors is less understood. An elevated-plus maze and an open field maze were used to investigate the role of the ventral DG in the ability to modify behavior in potentially dangerous conditions and to investigate a few previous reports that ventral HPP lesions induced hyperactivity. Preference to explore zones and the number of grid lines crossed were measured for individual exploratory sessions on both mazes for dorsal DG, ventral DG and control rats. The data indicate that rats with dorsal DG lesions behaved similar to controls and are not importantly involved in anxiety-based behaviors. However, rats with ventral DG lesions spent significantly larger percentages of time in the open arms of the elevated plus maze than did controls. The current data for number of grid lines crossed indicate that all rats

traveled at a similar rate in the open arms and that rats with ventral DG lesions traveled at a similar rate in both open and closed arms, though controls and rats with dorsal DG lesions reduced exploratory rates when in open arms of the maze. Interestingly, our data indicate that all groups crossed a similar number of grid lines in the open field maze, but rats with ventral DG lesions spent a large amount of time in the open, exposed, center of the maze than controls. The results suggest that the ventral DG plays an important role in anxiety-based behaviors and that the dorsal DG is not importantly involved in anxiety. Specifically, the ventral DG is importantly involved in preference for safer environments and is also important in the ability to modify or slow exploration when in potentially dangerous environments.

Introduction

Moser and colleagues (1998) demonstrated that the dorsal hippocampus (HPP) is important for spatial information processing. Subsequent investigations have revealed that the ventral portion of the HPP is important for processing odor information and anxiety-based behaviors (Bannerman et al., 2002; Bannerman et al., 1999; Bannerman et al., 2004; Kesner, Hunsaker, & Ziegler, 2011). While the ventral HPP can be involved in spatial processing, it is not critical, especially when learning takes place after lesions or inactivations (Bannerman et al., 2002; Bannerman et al., 1999; Bannerman et al., 2003; Bannerman et al., 2004).

Gray and McNaughton (2000) have provided a rich context and extensive experimental repertoire of lesion studies to demonstrate hippocampal involvement in anxiety and to show that anxiety is distinctly different from fear. Fear has been well-

established as an important function of the amygdala and described as a near-instinctual, behavioral response to flee and move away from the source of explicit danger (Gray & McNaughton, 2000). Anxiety has been described as a state that results from cognitive management of competing goals to avoid possible (but not directly present) danger and to engage in and explore the environment. For example, when a rat encounters a cat, it demonstrates a near instinctual response to get away from the cat. However, when a rat encounters the potential (but not immediate) presence of a cat, such as cat urine, anxiety ensues and is more complex than an instinctual fleeing behavior. Gray and McNaughton (2000) suggest that the role of the HPP is to magnify potential danger in order to maintain safety and that this magnification, along with goal conflict to avoid danger or to continue engaging the environment, creates anxiety-like states that are observed as anxious behaviors. Behavioral drug and lesion studies reveal that lesions to the ventral HPP and amygdala result in different behaviors when observed in anxiety-provoking paradigms, such as the elevated-plus maze in that rats with amygdala lesions demonstrate behaviors similar to that of controls. (Bannerman et al., 2002; Bannerman et al., 2004; Kjelstrup et al., 2002). However, when administered anti-anxiety drugs, such as midazolam, rats with amygdala lesions no longer demonstrate "normal" anxiety behaviors and instead behave similar to rats with lesions to the ventral HPP (Gray & McNaughton, 2000; Kjelstrup et al., 2002). Taken together, these studies establish strong support that anxiety and fear are distinct emotional behaviors and are mediated by different brain structures. Distinctions between anxiety and fear are not historically clear in the literature, and there is debate over what constitutes "fear" in foot-shock studies (especially for random reinforcement schedules in which the shock can be anticipated to occur, but prediction is not possible).

Although there are questions about other paradigms, the elevated-plus maze has been validated as a credible and sensitive measure for anxiety-based behaviors in rodents (Pellow, Chopin, File, & Briley, 1985).

Connections exclusive to the ventral HPP indicate that the structure is well-suited to support anxiety-based behaviors. Direct connections between the ventral HPP and amygdala may facilitate a close relationship between fear and anxiety behaviors (Van Groen & Wyss, 1990b). Distinctly ventral projections of the HPP connect with the hypothalamus, which provides impact on the hypothalamic-pituitary adrenal axis (HPA) (Dedovic, Duchesne, Andrews, Engert, & Pruessner, 2009). The importance of the HPA in regulation of feeding, motivation, stress and emotional states underscores the importance of ventral hippocampal connections and influence on anxiety. The ventral HPP also shares an exclusive connection to the prefrontal cortex, which is important for many higher-order cognitive functions (Bannerman et al., 2004). Exclusive connections with structures that are involved in such functions make the ventral HPP a likely candidate to be involved in demonstration of emotional behaviors (Dedovic et al., 2009).

Behavioral evidence indicates that individual subregions of the ventral HPP may be involved in anxiety-based behaviors. For example, lesion studies show that the ventral CA3 is important to retrieval of contextual fear conditioning and the ventral CA1 is important for retention of trace fear conditioning (Hunsaker & Kesner, 2008; Rogers, Hunsaker, & Kesner, 2006). Additionally, all of the dorsal subregions of the HPP have been shown to be important for individual processing roles, including the dorsal DG for pattern separation of highly overlapping spatial information (Gilbert, Kesner, & Lee, 2001; Kesner, 2007a; Kesner, Lee, & Gilbert, 2004). Because the ventral CA3 and CA1

carry out specialized roles in anxiety-based behaviors, it is likely that the ventral DG also carries out a specialized processing role for anxiety-based behaviors. Given the impact the DG has on hippocampal information and evidence that all other dorsal and ventral subregions support specialized processing functions, there is a credible basis to suggest that the ventral DG plays a critical role in demonstration of anxiety-based behaviors. Despite these indications, no behavioral lesion studies have been conducted to directly explore involvement in these processes. In order to investigate a potential role for the ventral DG in anxiety-based behaviors, an elevated-plus maze which has been shown to be sensitive specifically to anxiety-based behaviors in previous reports of hippocampal lesion studies related to anxiety was used (Bannerman et al., 1999; Bannerman et al., 2003; Bannerman et al., 2004; Kjelstrup et al., 2002; Pellow et al., 1985).

In the studies mentioned above, there were conflicting reports of hyperactivity in animals with lesions to the ventral HPP or entire HPP (Bannerman et al., 2002; Bannerman et al., 1999; Kjelstrup et al., 2002). For example, reports provide two different experiences with the Morris water maze: in one experiment, rats with lesions to the ventral HPP had faster swim speeds but in a subsequent water maze experiment, did not (Bannerman et al., 2002; Bannerman et al., 1999). To measure hyperactivity, the behaviors of a control group are collected and represent a baseline for "normal" activity. Because hyperactivity is calculated by control subjects' behaviors, the hyperactivity score for a treatment group fluctuates based on control behaviors – even if treatment group behaviors remain stable. Several measures of anxiety involve reduced locomotion; therefore, measures of hyperactivity in anxiety-inducing tasks serve to confound the relationship between anxiety-based behaviors and assessments of locomotion. It is quite

possible that previous mixed reports of hyperactivity reflect changes in the behaviors of controls when moved from a non-anxiety situation (e.g., home cage) to one that is designed to provoke anxiety (e.g., elevated-plus maze). In fact, subjects from those reports failed to demonstrate hyperactivity in the home cage, which further supports the notion that measures of anxiety are confounded with measures of hyperactivity, rather than actual increases in locomotion in treatment subjects in anxiety-provoking tasks (Bannerman et al., 1999; Bannerman et al., 2003). Therefore, a better measure of locomotion may be gained from a task that minimizes possible displays of anxiety yet maximizes exploration. The open field maze has been used for decades and is widely accepted as a classic test of locomotor behaviors that minimizes anxiety provocation (Ramos, Berton, Mormède, & Chaouloff, 1997). Although we have provided a plausible explanation as to why hyperactivity is likely not a characteristic of rats with ventral lesions to the hippocampus, the possibility that ventral DG lesions induce hyperactivity remains. Therefore, we utilized an open field maze in order to measure locomotion with the aim to un-bind measures of these behaviors from context reactivity (Walsh & Cummins, 1976).

Materials and Methods

Subjects

Eighteen male Long-Evans rats weighing 250-350 g were used as subjects. Rats were housed individually in plastic cages that were located in a colony room with a 12 hours light and 12 hours dark cycle. Testing was individually conducted for each rat during the light phase of the light-dark cycle. All subjects had unlimited access to food

and water. Dorsal DG lesioned subjects had previously been used for spatial tasks. All subjects were used in both experiments; first in the elevated-plus maze experiment (Experiment 1) followed by the open field experiment (Experiment 2).

Surgical Procedures

All procedures and animal care were in compliance with the National Institute of Health and Institute for Animal Care and Use Committee of the University of Utah. Rats were randomly assigned to control (n = 6), ventral DG (n = 6), or dorsal DG (n = 6) lesion groups. Rats in the ventral DG and dorsal DG lesion groups received bilateral intracranial infusions of colchicine (2.5 mg/ml, 0.8 µl/site) into the ventral DG or dorsal DG. Half of the control subjects (n = 3) received bilateral intracranial infusions of saline-hydrochloride solution (2.5 mg/ml, 0.8 µl/site) into the ventral DG and the other half of control subjects (n = 3) received the same solution into the dorsal DG. Prior to surgery, animals were administered atropine sulfate (0.54 mg/kg, i.m.). Subjects were anesthetized by exposure to isoflurane gas and positioned in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). For the duration of the procedure, subjects remained anesthetized with a continuous flow of isoflurane (2 – 4%) and medical air (≈1.5L/min) mixture. Hair covering the surgical site was removed with a rechargeable Conair trimmer (Shelton, CT). Antiseptic measures were carried out: a surgical drape was positioned to expose only the shaved area, which was swabbed three consecutive times with betadine. The skin covering the skull was incised and retracted to expose the skull. Bregma was identified and burr holes were drilled through the skull at injection sites. Injections were made by lowering a 7 µl Hamilton GasTight syringe (Hamilton

Company, Reno, NV) that was attached to a micro infusion pump (Cole-Palmer, Vernon Hills, IL) to infuse colchicine (2.5mg/ml) into injection sites. The infusion system remained stationery at the location for 2 minutes after infusion to allow for even diffusion. For ventral DG lesions, the Hamilton syringe was bilaterally positioned at two locations: 5.7 mm posterior to bregma, 4.1 mm lateral to midline, 3.8 mm ventral from dura and 6.3 mm posterior to bregma, 4.5 mm lateral to midline, 4.8 ventral from dura. For dorsal DG lesions, the Hamilton syringe was bilaterally positioned at two locations: 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 ventral from dura. Following injections, retracted skin was released, pulled together and sutured. Betadine was swabbed over the stitched incision site and 100% medical air (1.5L/min) was administered as the subject was released from the stereotaxic apparatus. Each subject was returned to the home cage to receive postoperative care for 7 days that included Ibuprofen (Children's Motrin; 200 mg/100 ml water) as an analgesic and mashed food. During postoperative recovery, subjects were monitored for behavioral seizures.

Experiment 1: Elevated-plus Maze Exploration

Apparatus

The test apparatus consisted of a central platform (10 x 10 cm) from which 4 arms radiated outward (50 x 10 cm) to form the shape of a "plus" symbol. The platform and arms were 50 cm above the floor. Two adjacent arms of the maze consisted of enclosed walls (40 cm high) that followed the length of the arms. The remaining two arms were enclosed and had a small ledge (1.3 cm) that extended the length of the arms and served

as a grip to prevent rats from falling off of the maze during testing. The maze was housed in a well-lit testing room with posters and stuffed animals attached to the walls that served as extramaze cues.

Procedural Methods

Rats were placed individually on the central platform of the apparatus and activity was recorded for 5 minutes. After 5 minutes of recording, the session ended and the rat was returned to the home cage. For each individual minute of the 5 minute exploration session, the experimenter recorded time spent in open and closed arms of the apparatus, as well as the number of zone crossings into and out of specific zones (see Figure 1). Rats met criteria for crossing into a boundary when the entire body (without regard for tail) was inside the boundary.

Experiment 2: Open Field Maze Exploration

Apparatus

The test apparatus consisted of a modified construction of the classic open field maze (Walsh & Cummins, 1976). The apparatus was comprised of a wooden box with a flat bottom surface (100 x 100 cm) and four vertical walls (40 cm). All surfaces were painted white and black pieces of tape were positioned across the floor of the maze so that the tape created 16 squares of equal size (see Figure 2). The top of the apparatus remained open; posters and stuffed animals were attached to the walls of a well-lit testing room to provide extramaze cues.

Procedural Methods

Rats were individually placed near the center of the apparatus platform. Activity was recorded for 5 minutes, at which point the session ended and the subject was returned to the home cage. The experimenter tallied the number of grid crossings in 1 minute increments. A gridline was considered crossed when the entire body (without regard for tail) crossed over the demarcation. In addition, time spent in the inner versus outer area of the apparatus was recorded for each minute of the session. The inner zone was comprised of the four, inner-most grid boxes and the remainder of the parameter was defined as the outer zone (see Figure 2).

Histological Procedures

Following behavioral tests, subjects were deeply anesthetized with sodium pentobarbital (1.5 ml, 70 mg/kg, i.p.). Subjects were intracardially perfused with phosphate buffered solution (PBS) then with a formalin solution (10 mg/kg). The brain was extracted and stored in a 10% formalin/30% sucrose cryoprotectant solution for 72 hours at ≈ 4°C. A tissue block containing the hippocampus was frozen and sliced on a cryostat at a thickness of 24 µm. Ventral DG lesioned brains were cut on the horizontal plane and dorsal DG lesioned brains were cut on the coronal plane. Every third section was mounted on a gelatin coated glass slide and Nissl stained with cresyl violet. Slides were examined microscopically in order to verify lesion placement.

Results

Histological Results

Bilateral lesions using axon-sparing colchicine were made to the ventral DG or dorsal DG, depending on surgical condition. Figure 3 shows a schematic that represents the location of ventral DG lesions, and Figure 4 shows a schematic that represents the location of dorsal DG lesions.

Behavioral Results

Elevated-plus Maze

Time

Figure 5 provides the mean (± SE) percentage of time spent in open arms during exploration of the elevated-plus maze for rats with ventral DG lesions, dorsal DG lesions, and controls. Percentage calculations were made by subtracting the amount of time in open arms (in seconds) from the total time of 300 seconds, and multiplying that sum by 100 [(300 – seconds in open arms) * 100]. A one-way analysis of variance (ANOVA) with lesion (ventral DG, dorsal DG, control) as the between group factor was conducted to analyze data. The analysis revealed a significant effect of lesion on percent of time spent in open arms, $F(2, 15) = 5.46$, $p = .017$. A Newman-Keuls post hoc comparison test indicated no significant differences in the percent of time controls and dorsal DG lesioned rats spent in open arms. However, ventral DG lesioned rats spent a significantly longer amount of time in open arms relative to controls and dorsal DG lesioned rats ($p < .05$). The results indicate that ventral DG, but not dorsal DG, is importantly involved in avoiding dangerous environments, or influencing preference toward safer environments.

Zone

Figure 6 provides the mean (± SE) number of grid lines crossed by control, ventral DG, and dorsal DG lesioned groups in the open and closed arms of the elevated-plus maze. A 2-way, 3 x 2 analysis of variance (ANOVA) with lesion (control, ventral DG, and dorsal DG) as the between group factor and maze arm (open, closed) as the within group factor was conducted to analyze the data. The results indicated a significant main effect for lesion, $F(2, 30) = 4.38$, $p = .021$, a significant main effect for maze arm, $F(1, 30) = 6.21$, $p = .018$, and a significant interaction, $F(2, 30) = 6.22$, $p = .005$. A Newman-Keuls post hoc comparison test indicated no significant differences in lines crossed by control, ventral DG and dorsal DG lesioned rats in closed arms. However, there was a significant difference in ventral DG line crossings compared to control and dorsal DG lesioned rats in open arms of the maze ($p < .05$). There was a significant difference in line crossings for both controls and dorsal DG lesioned rats on closed and open arms ($p < .05$), however there was no significant difference in ventral DG lesioned rat crossings in closed and open arms. These results indicate that the ventral DG, but not dorsal DG, is important in demonstrating anxiety-based behaviors when in potentially dangerous environments.

Open field maze

Grid crossings

Figure 7 provides the mean (±SE) number of gridlines of the open field maze crossed by rats with control, ventral DG, and dorsal DG lesions. A one-way analysis of variance (ANOVA) with lesion (control, ventral DG, dorsal DG) as the between group

factor was conducted to analyze the data. The analysis indicated no significant differences between groups for number of gridlines crossed in the open field maze, $F(2, 15) = 1.68$, $p = 0.220$. The results suggest that rats with ventral DG lesions do not reduce/increase basic locomotion in novel environments. Thus, differences in exploration may be attributed to a factor other than hyperactivity.

Zones

Figure 8 provides the mean (±SE) amount of time rats with control, ventral DG, and dorsal DG lesions spent in the Inner zone of the open field maze. Time in the Inner zone was calculated by dividing the amount of time that rats spent in the four, inner-most squares during the exploratory session. A one-way analysis (ANOVA) with lesion (control, ventral DG, dorsal DG) as the between group factor was conducted to analyze the data. The analysis indicated a significant difference between groups in the amount of time spent in the Inner zone $F(2, 15) = 8.39$, $p = .004$. A Newman-Keuls post hoc comparison test indicated no significant differences in the amount of time controls and dorsal DG lesioned rats spent in open arms. However, rats with ventral DG lesions spent a significantly longer amount of time within the Inner zone relative to controls and dorsal DG lesioned rats ($p < .05$). These results suggest that the ventral, not dorsal, DG is important for influencing exploration of novel and potentially dangerous environments.

Discussion

Previous research has indicated a dorsoventral differentiation of function across the HPP in that the dorsal HPP is important for spatial processing and the ventral HPP is

important for odor memory and anxiety-based behaviors (Bannerman et al., 1999; Gray & McNaughton, 2000; Moser & Moser, 1998). Lesion studies have also revealed that individual subregions of the dorsal HPP (DG, CA3, and CA1) carry out specialized roles in spatial processing (Kesner et al., 2004; Rolls, 1996; Rolls & Kesner, 2006). Subregional specificity has also been reported for some studies of the HPP. For example, it has been suggested that ventral CA3 and ventral CA1 are importantly involved in fear conditioning, which involves anxiety-based behaviors, such as freezing (Hunsaker & Kesner, 2008). This evidence indicated that ventral subregions may carry out specialized roles, and that the ventral DG may also be important for anxiety-based behaviors. Therefore, the present study was conducted to investigate the role of the ventral DG in anxiety-based behaviors using two exploratory paradigms that have been established to induce such behaviors. Results from the current study suggest that the ventral DG plays an important role in the expression of anxiety. Specifically, the ventral DG may influence preference to explore safer areas over potentially dangerous environments, and may also impact the manner of exploration when in less-safe situations.

Previous research lends support for a role for the ventral DG in anxiety-based behavior. For example, anatomical studies have revealed that, despite many common pathways along the dorsoventral axis, there are dorsal-specific and ventral-specific projections to structures that are involved in spatial navigation (dorsal) and emotion regulation (ventral). Specifically, studies have shown that the dorsal axis of the HPP has been shown to project to the perirhinal cortex, which receives highly processed visual and spatial information and has been shown to play a critical role in memory for rodents as well as humans and nonhuman primates (Squire & Zola-Morgan, 1991; Witter, Van

Hoesen, & Amaral, 1989). Previous studies have indicated that dorsal CA1 sends information that has also been processed by the dorsal DG and dorsal CA3 to the subiculum, which projects to mammillary and anterior thalamic nuclei before sending information back to the HPP (Dong, Swanson, Chen, Fanselow, & Toga, 2009). Previous research has suggested that mammillary and anterior thalamic nuclei house navigational neurons, which would provide support for the dorsal HPP in spatial processing (Taube, 2007). Additional anatomical support comes from previous research that has suggested dorsal projections to the perirhinal cortex, which receives highly processed visual and spatial information, plays a critical role in spatial learning and memory in rodents as well as humans and non-human primates (Squire & Zola-Morgan, 1991; van Groen & Wyss, 1990a). Previous research has indicated that the ventral axis of the HPP shares connections with the nucleus accumbens, which has been indicated as the neural substrate of drug addiction and has been shown to exert powerful conditioning effects (Everitt & Robbins, 2005). Additional studies have indicated that the ventral HPP shares connections with the amygdala, which has been indicated to mediate fear (Gray & McNaughton, 2000). Research suggests that fear and anxiety may be displayed together, but are individual emotions that are thought to be mediated by two different structures. (Gray & McNaughton, 2000; Van Groen & Wyss, 1990b). Anatomical studies have revealed that connections to the hypothalamus and its important role in hormone regulation via the hypothalamic-pituitary adrenal axis (HPA) provide the HPP with the potential influence over HPA axis processes, such as hormone, hunger, feeding, and stress regulation (Dedovic et al., 2009). Anatomical connection studies have also shown a projection between the intermediate to ventral HPP and the prefrontal cortex, which has

been suggested to allow rapid and direct sharing of information (Bannerman et al., 2004; Chiba, 2000). These anatomical characteristics lend support to the current data which showed that rats with dorsal DG lesions demonstrated a preference to explore the closed arms that is similar to control rat exploratory preferences, which suggests that emotionally based exploratory choices are not a function of the dorsal DG. Lesion studies have not established a role for the dorsal DG in emotional behaviors and instead have provided evidence which suggested that the dorsal DG is involved in spatial learning and memory processes. However, anatomical studies have suggested that the ventral HPP connects with structures that have been shown to be involved in emotion and stress. Therefore, the current study findings that the ventral DG is involved in anxiety-based behaviors and that the dorsal DG is not importantly involved in these processes are in line with previous anatomical and behavioral lesion studies.

In the current study, rats with ventral DG lesions spent a significantly larger percentage of time exploring open, exposed arms of the elevated-plus maze than both controls and dorsal DG lesioned rats. The current preference results are similar to previous investigations which reported that lesions to the HPP or ventral HPP also resulted longer exploration times on open arms of the maze when compared to controls, and that rats with dorsal HPP lesions behaved similar to controls (Bannerman et al., 2003; Kjelstrup et al., 2002). Thus, the current results are congruent with previous studies that suggested ventral, but not dorsal, axis involvement in preference to explore safer environments.

Previous studies have provided conflicting reports that lesions to the ventral HPP have resulted in hyperactivity in some cases, but not others, and not in the home cage

(Bannerman et al., 2002; Bannerman et al., 1999). This would be a concern, as locomotor exploration is the primary measure to determine arm preference. A confounded measure of behavior may explain why reports of hyperactivity in rats with lesions to the ventral HPP have not been consistent (Bannerman et al., 2002; Bannerman et al., 1999; Kjelstrup et al., 2002). It is possible that environment was a factor for behavioral displays of hyperactivity or that the effects of ventral lesions on locomotion are not well understood. Therefore, we also measured the number of grid lines crossed during the elevated-plus maze exploration session for open and closed arm activity. The current data indicate that control, dorsal DG, and ventral DG lesioned rats explored at similar rates when in the enclosed arms. However, both controls and dorsal DG lesioned rats crossed significantly less grid lines in the open arms. The data also reveal ventral DG lesioned rats crossed a similar number of grid lines in both open and closed arms of the maze. It is important to note that hyperactivity has traditionally been established based on the behaviors of controls, in that treatment groups are usually compared to control groups assess activity levels. Because the data indicate that controls and dorsal DG lesioned rats crossed fewer gridlines in the open arms, higher rates of ventral DG grid crossing could be characterized as "hyperactive" exploration. However, because activity levels of ventral DG lesioned rats remained steady across both open and closed arm exploration, it may be more appropriate to interpret the data as suggestive of an impairment in the ability to *reduce* exploration in potentially dangerous environments when compared to controls and dorsal DG lesioned rats. An important note is that the current measures separated and compared the number of grid lines crossed in the two arm environments. A traditional measure may have included overall locomotor rates for each group, but without arm

distinction. Based on these limited measures, results similar to the current data may have suggested ventral DG lesioned rats crossed more grid lines in general, which would have indicated hyperactivity. However, our current comparison of arm environment data suggest that ventral DG lesioned rats did not change rates of exploration. Therefore, it is possible that previous conflicted reports of hyperactivity may also support the current findings that there may be an impairment in the ability to reduce activity- rather than an increase in activity. Together, analyses of data from the elevated-plus maze suggest that the ventral DG may be important for alterations in behavior when in anxiety-provoking environments, and that the ventral DG may also serve to reduce exposure to potential dangers as it may exude influence to prefer safe over potentially dangerous environments.

The current elevated-plus maze data indicate that hyperactivity is likely not a factor, and may be contributed to impairment in exploratory reduction rather than increase, as explained previously. However, the measure of motion in an anxiety-sensitive paradigm such as the elevated-plus maze, may still be called to question: locomotor exploratory behavioral measures are confounded with the anxiety-inducing environment of the apparatus. Therefore, it is important to collect locomotor measures in an environment that was not designed to provoke anxiety. We used an open field maze to measure exploration in a novel environment that has been established as sensitive to hyperactivity and a wide range of other behaviors (Walsh & Cummins, 1976). The current data indicate that control, dorsal DG and ventral DG lesioned rats crossed a similar number of grid lines, which suggests that they traveled at similar rates during exploration and that hyperactivity was not detected. These data are consistent with and further support our own elevated-plus maze findings: all groups demonstrate similar

exploratory behaviors when in closed, or safer, arms of the maze. Thus, hyperactivity may be ruled out as an explanation for other behavioral differences observed in the open field maze experiment of this study (Walsh & Cummins, 1976).

Patterns of exploration in the open field have been shown to be relatively stereotyped: naïve and control rats have been shown to explore the periphery in great detail before spending time in the center, exposed, area of the maze. It is possible that an impairment in the ability to behaviorally express anxiety would result in deviations from characteristic, initial exploration. Therefore, we measured the amount of time subjects spent within the inner-most four grid squares to investigate the role of the ventral DG in characteristic exploration of the open field maze. The current data reveal that rats with ventral DG lesions spent more time within the inner-most grid squares than controls or dorsal DG lesioned rats. The exploratory patterns of controls and dorsal DG lesioned rats match activity patterns of naïve rats in other studies, which indicates that sessions were conducted correctly for this well-established paradigm (Walsh & Cummins, 1976). Exploratory behaviors of dorsal DG lesioned rats align with previous studies that have suggested the dorsal axis of the HPP is not involved in emotional behavior (Bannerman et al., 1999; Kesner et al., 2004; Walsh & Cummins, 1976). The results provide evidence to suggest that the ventral DG plays an important role in exploration even when there appears to be no overt potential for danger, which is similar to the current results of the elevated-plus maze. The current study indicates that exploration is different than controls in rats with ventral DG lesions. Even when hyperactivity may be ruled out, rats with ventral DG lesions maintained an impairment for cautious exploration that is characteristic and well-established in the open field maze. The current open field results

appear to rule out the possibility that exploratory preference rates are confounded by exploratory speeds in open versus closed arms of the elevated-plus maze. Therefore, the results suggest that the ventral DG plays an important role in mediation of anxiety-based behaviors that include preference for safer environments, alterations to exploratory behavior when in potentially dangerous environments and the manner in which novel environments are explored. Further, the current results clarify that ventral DG lesions do not result in hyperactivity, but rather an inability to modify and reduce speed when in potentially dangerous environments.

Lesion studies have been conducted on the major subregions across the dorsoventral axis of the HPP, except for the ventral DG. The current methods provide an important tool that may advance studies of the dorsoventral axis of the DG. Previous lesion studies have indicated that the dorsal axis of the HPP is important for spatial information processing and that the ventral axis of the HPP is important for emotional behaviors and olfactory information processing. Although the rest of the of the dorsal and ventral major subregions have been indicated to have impact on an individual basis, there have been no lesion studies to verify ventral DG-specific impact until now. The present evidence suggests that the ventral DG carries out a specialized role in anxiety-based behaviors. Future studies are needed to provide ventral subregion impact on anxiety-based behaviors by direct comparison. The current studies represent initial result of ventral DG involvement in emotional processes and a wider range of behavioral studies should be conducted to explore other potential roles for the structure. For example, a temporal, parallel processing relationship for spatial (dorsal) and odor (ventral) information has been shown for CA1, and similar relationships for the same

modalities have been inferred for CA3 in arbitrary association and pattern completion processes (Hunsaker, Fieldsted, Rosenberg, & Kesner, 2008; Kent, Hess, Tonegawa, & Small, 2007; Kesner, 2007b; Lacy, Yassa, Stark, Muftuler, & Stark, 2011). Studies have established that the dorsal DG is important for the formation of separate representations for highly overlapping spatial information (Gilbert, Kesner, & Decoteau, 1998; Gilbert et al., 2001; Goodrich-Hunsaker, Hunsaker, & Kesner, 2008; Kesner, 2007a). A role for the ventral DG in pattern separation would be an interesting next step to further define the impact of the ventral DG and further investigate the concept of parallel processing roles across the dorsoventral axis of the HPP.

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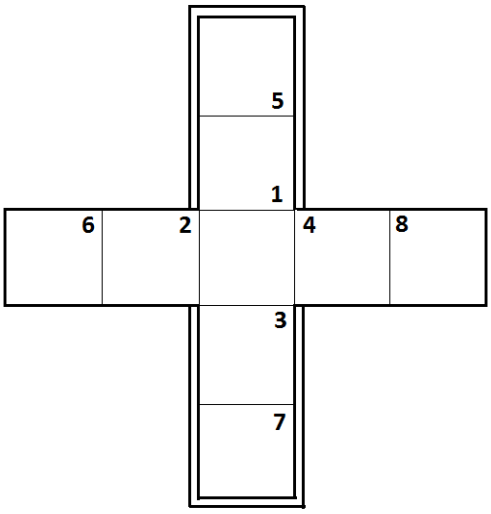

Figure 8. Elevated-plus maze. Double lines represent arms with walls and single represent open arms. Information about activity levels was collected by measuring the number of gridlines crossed within both open and closed arms of the maze.

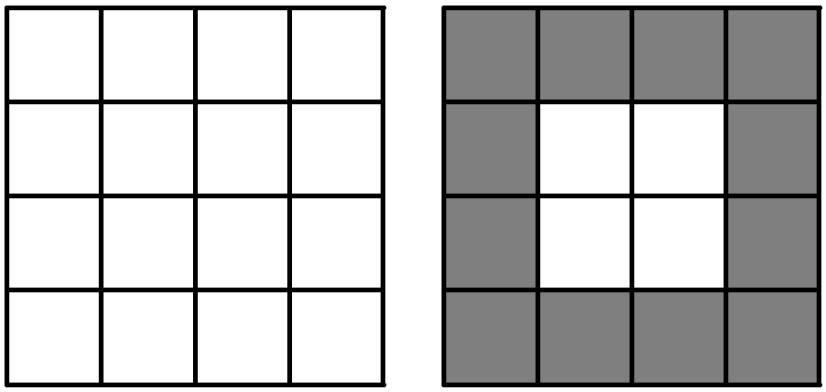

Figure 9. Open field maze. Left: Open field with lines that were used to measure activity levels. Right: Open field with outer (shaded) and inner (non-shaded) zones that were used to measure exploratory patterns.

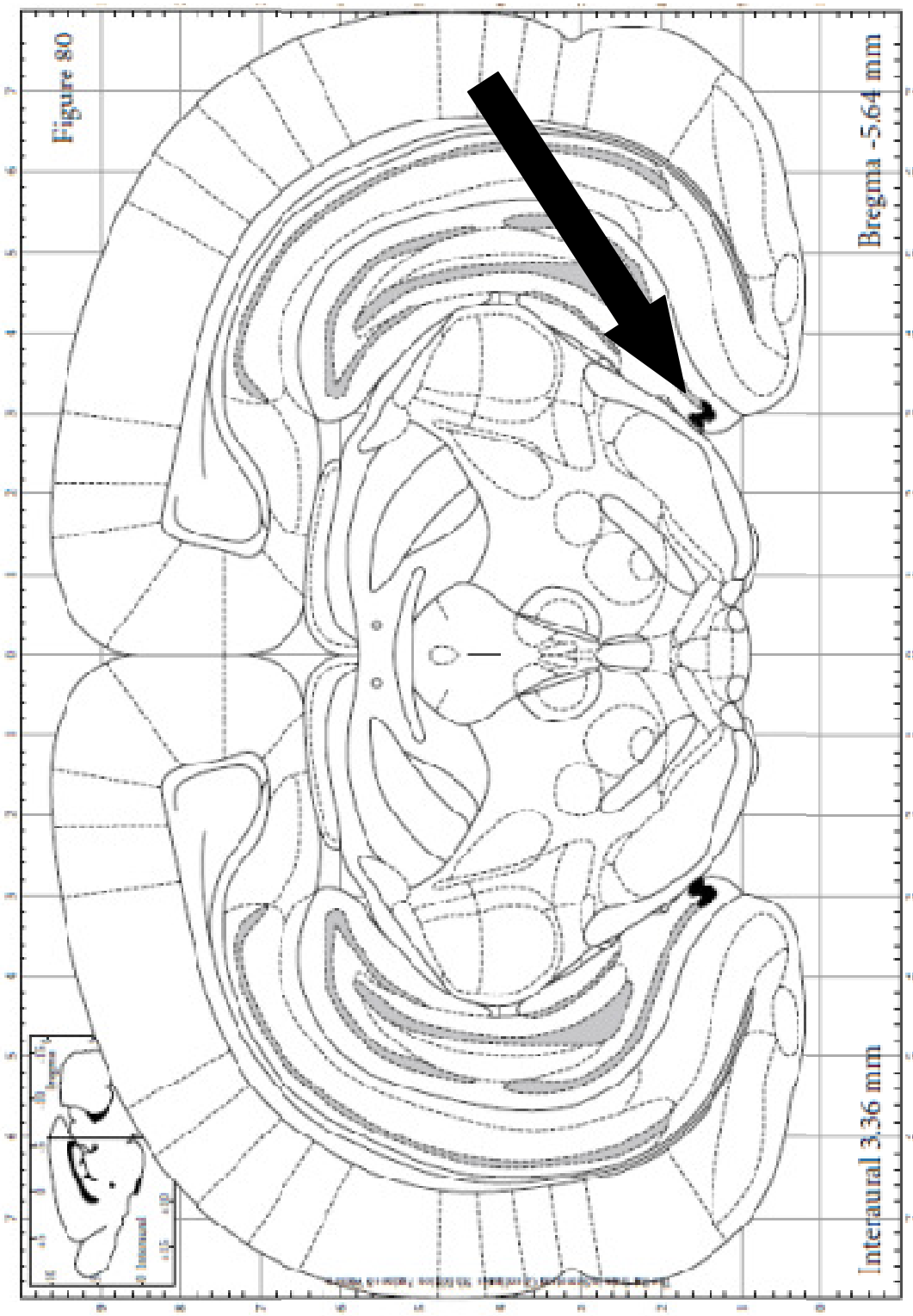

Figure 10. Schematic representation of ventral DG lesion target areas. Arrow indicates targeted ventral DG location.

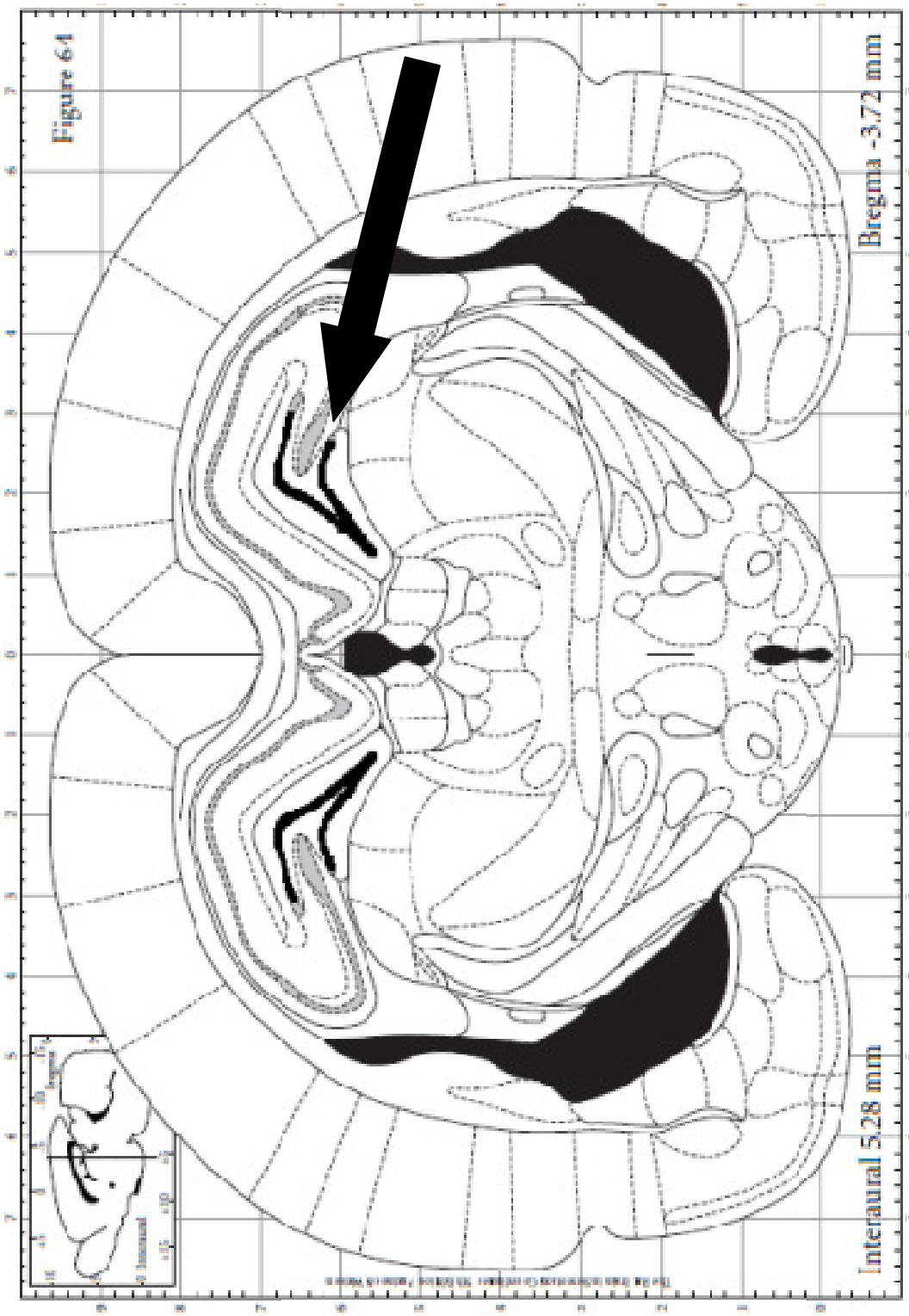

Figure 11. Schematic representation of dorsal DG lesion target areas. Arrow indicates targeted ventral DG location.

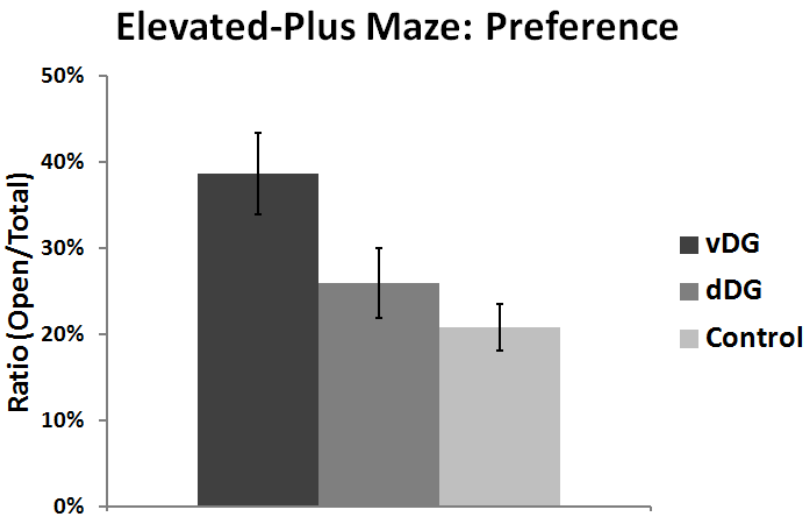

Figure 12. Mean (±SE) percentage of time ventral DG, dorsal DG, and control rats spent in the open arms of the elevated-plus maze.

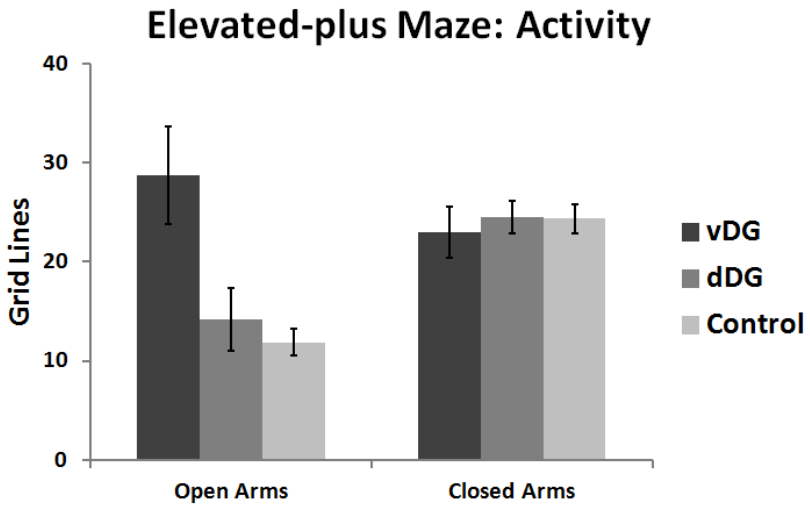

Figure 13. Mean (±SE) number of grid lines ventral DG, dorsal DG, and control rats crossed in open and closed arms of the elevated-plus maze.

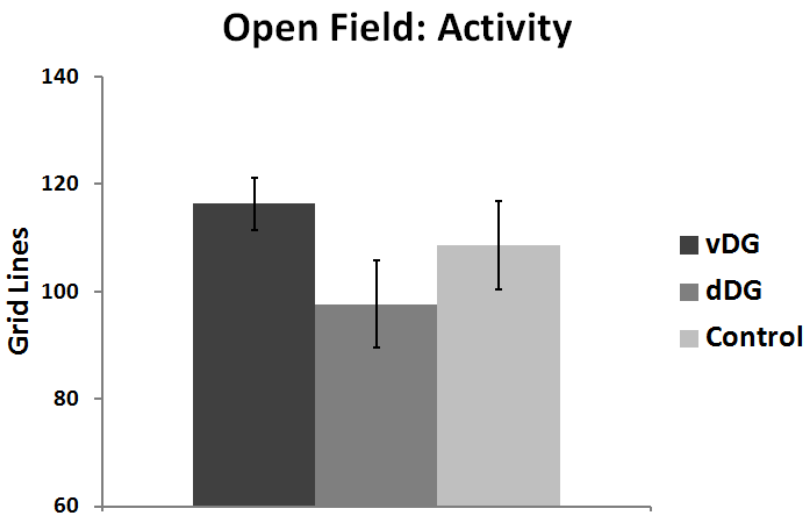

Figure 14. Mean (±SE) number of grid lines ventral DG, dorsal DG, and control rats crossed in the open field maze.

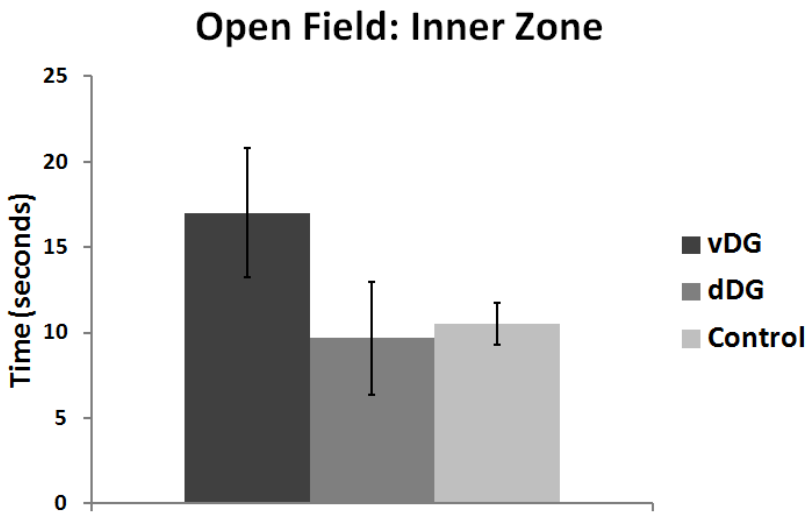

Figure 15. Mean (±SE) amount of time that ventral DG, dorsal DG, and control rats spent in the inner zone of the open field maze.

CHAPTER 4

GENERAL DISCUSSION

Previous investigations have suggested that the ventral region of the HPP may be involved in memory for odors and anxiety and that the dorsal region is not importantly involved in these processes but instead plays a critical role in processing spatial information (Bannerman et al., 1999; Bannerman et al., 2003; Fanselow & Dong, 2010; Kesner, Hunsaker, & Ziegler, 2011). Previous research also suggested that hippocampal subregions conduct specialized processing for spatial and odor information, as well as anxiety-based behaviors; however, little is known about specialized functions of the ventral DG (Bannerman et al., 2004; Goodrich-Hunsaker, Hunsaker, & Kesner, 2008; Kesner, 2007). Therefore, the current studies were conducted to investigate roles of the ventral DG. The current investigations revealed that the ventral DG is important for memory of odor information. In addition, the ventral DG may have a specialized role in pattern separation processes for highly similar odor information, which parallels the role of the dorsal DG in spatial processing. The current investigation also indicated that the ventral DG is important for exploratory preference for safer environments and careful exploration when in less-safe environments.

Anatomical connections may serve to provide support for a role of the ventral DG in anxiety and olfactory processes. The hippocampus receives the majority of cortical

input from the entorhinal cortex through the perforant pathway (Amaral & Witter, 1995). The perforant pathway is comprised of two information streams. Specifically, the medial perforant stream projects spatial information and the lateral perforant stream projects non-spatial, sensory information (Hargreaves, Rao, Lee, & Knierim, 2005). Though the CA3 and CA1 each receive minimal projections from the perforant path, the DG receives the bulk of information (Amaral & Witter, 1995). Because the DG sends that large bulk of information forward to the CA3 and CA1 after DG processing, it has been suggested that the DG – CA3 – CA1 processing route is the primary manner in which hippocampal learning and memory information is processed (Amaral, Scharfman, & Lavenex, 2007; Witter, 1993). The hilus, an excitatory cellular layer within the DG, projects information to an inhibitory layer of interneurons, which provides a recurrent pathway within the DG and allows substantial processing to be carried out in the DG (Witter, 1993). Information is then fed forward to the CA3 pyramidal cells via sparse, mossy fibers. Pyramidal cells interact through recurrent fibers, which provide a recurrent feedback system, or autoassociative network in the CA3 subregion (Amaral & Witter, 1995). Information from the CA3 then projects onto the CA1 through the Schaffer collateral system, and efferent projections mainly from the CA1 and to a lesser extent the CA3, project to the subiculum (Witter, 1993). The subiculum projects information to the entorhinal cortex. Additionally, a small amount of information is projected to the septum from the CA3, which is then sent to the subiculum, then entorhinal cortex. Ultimately information in the entorhinal cortex is routed to parahippocampal structures, completing the main information processing route of the HPP (Amaral & Witter, 1995).

Based on such anatomical characteristics, an updated computational model of subregional specificity of processing has been provided by Rolls and Kesner (2006). They suggest that sparse output of the DG onto the CA3 implies that the DG is well suited for the formation of distinct representations of highly overlapping information, or pattern separation. The recurrent collateral system of the CA3 is well suited for rapid encoding, formation of arbitrary associations and pattern completion, the feed-forward circuitry of the CA1 inputs of processed information from the other subregions as well as the fact that the CA1 is the major post processing information output, suggests that the CA1 is well suited for processing sequences of information. Rolls and Kesner (2006) provide ample behavioral evidence that supports specialization of processing function among subregions of the HPP.

Evidence also supports subregional processing roles for spatial information, and though there is substantial evidence that the HPP is also important for processing odor information and anxiety-based behaviors, these mechanisms are less understood than their spatial counterparts. Anatomy may again serve to inform about specific processing roles of individual subregions of the HPP. Despite similar processing routes, there are some notable differences in connectivity across the dorsoventral axis of the HPP. For example, while both the dorsal and ventral hippocampal streams project to the subiculum, parasubiculum, entorhinal cortex, and lateral septal nucleus, the dorsal HPP sends additional information to the subiculum, mammillary, and anterior thalamic nuclei, then the information is projected back to the dorsal HPP (Dong, Swanson, Chen, Fanselow, & Toga, 2009). Mammillary and thalamic nuclei contain navigational neurons, which implicate that they are well suited to process spatial information, which has been

supported with rodent lesion studies (Taube, 2007). This specialized connectivity also occurs in the ventral HPP. For example, projections from the ventral HPP to the olfactory bulb support an important role for the ventral HPP in processing odor information and this concept has been supported by behavioral lesion studies in rodents (Kesner, Hunsaker, & Ziegler, 2011; van Groen & Wyss, 1990). The ventral HPP also selectively projects to the nucleus accumbens, hypothalamus and amygdala, which are structures known to be important for reward and behavioral conditioning, motivational, hormonal and stress activity, and fear-based behaviors (Everitt & Robbins, 2005; Gray & McNaughton, 2000; van Groen & Wyss, 1990). It is important to clarify that there is a substantial body of evidence to support that anxiety and fear are distinct emotional behaviors that arise from different brain structures, the hippocampus and amygdala respectively, though theorists acknowledge that anxiety and fear can be experienced in alternating sequences and even simultaneously (Gray & McNaughton, 2000). Despite many processing similarities along the dorsoventral axis of the HPP, it is the difference between the dorsal and ventral afferent and efferent projection pathways that clarify the role of each region, and the anatomical characteristics such as cellular assembly that further define subregional roles- in this regard, behavioral research is largely in agreement (Kesner et al., 2011; E. Moser, Moser, & Andersen, 1993; M. Moser & Moser, 1998).

The first study revealed that the ventral DG plays an important role in olfactory learning and memory processes, and is critical to these processes when odors are very similar. The study also revealed that the ventral DG plays an important role in distinction between new and familiar odors. Memory for odors was investigated using a delayed-

matching-to-sample task and a novelty detection paradigm. Odor stimuli for the initial experiment consisted of five acids that differed only in the number of methyl groups in their structure, which allowed for further assessment of possible pattern separation effects. The results showed that rats with ventral DG lesions were impaired in correctly performing the delayed-matching-to-sample task when the delay was 60 seconds, but no impairment was observed when the delay was 15 seconds. Though rats with ventral DG lesions were overall impaired at a delay of 60 seconds, the results suggest a pattern separation effect. Specifically, performance scores were increasingly lower with fewer separations between the odor stimuli. These results are consistent with lesion studies that have shown that lesions of the ventral HPP impair working memory for odor information, and the results are consistent with previous research in showing that the dorsal DG is not necessary for odor processing (Kesner, 2007; Kesner et al., 2011). The current results are also consistent with the concept of parallel processing roles for subregions across the dorsoventral axis of the HPP. For example, previous research has shown that the dorsal and ventral CA1 subregions are both importantly involved in temporal processing for spatial and olfactory information (Hunsaker, Fieldsted, Rosenberg, & Kesner, 2008). The current findings are important; prior to the current study, evidence for parallel processing was limited. Stronger evidence for parallel processes may motivate additional dorsoventral investigation.

The second study revealed that the ventral DG plays an important role in novelty detection for odors. A novelty detection paradigm was used, and conspecifics (juvenile rats) were used as odor stimuli in order to determine ventral DG involvement in distinguishing novel and familiar social odors. The data showed that controls and rats

with dorsal DG lesions spent more time exploring a novel conspecific over a familiar one. However, the results revealed that ventral DG lesioned rats showed no preference, and instead explored familiar and novel conspecifics for a similar amount of time. The current results showed that the ventral DG is importantly involved in novelty detection for social odors. The current results are supported by previous research that has suggested that the ventral HPP is important for learning and memory for odor information and the dorsal HPP is involved in similar processes for spatial information (Kesner et al., 2011; Moser & Moser, 1998). Previous dorsal lesion investigations have shown that the dorsal DG, Dorsal CA3, and to a lesser extent dorsal CA1 are all involved in the processes of spatial novelty detection (Lee, Hunsaker, & Kesner, 2005). According to the concept of parallel processing, all ventral subregions would be predicted to be importantly involved in novelty detection for olfactory information. Further, studies have shown that the dorsal HPP is not importantly involved in processing odor information, but is important in spatial processing (Bannerman et al., 1999; Bannerman et al., 2004; Kesner et al., 2011; Kesner, Lee, & Gilbert, 2004). The current results are in agreement with hippocampal processing studies and confirm, with behavioral lesion studies, that the ventral DG plays an active role in odor processing. In addition, the current results extend important information about subregional processing in odor novelty detection, which is less understood than spatial novelty. Finally, the current results have revealed a new function of the ventral DG- novelty detection for odor information.

The HPP has been shown to also be involved in anxiety, and previous investigations revealed that the ventral region was the critical component (Bannerman et al., 1999; Bannerman et al., 2003; Kjelstrup et al., 2002; Moser & Moser, 1998).

Therefore, the second group of experiments were conducted to investigate a role for the ventral DG in anxiety. The current results suggest that there is ventral DG involvement in anxiety-based behaviors. An elevated-plus maze and open field maze were used to investigate exploratory behavior in anxiety- and non-anxiety provoking environments. Results from individual exploratory sessions on the elevated-plus maze showed that rats with ventral DG lesions had a higher preference for open arms than did controls and dorsal DG lesioned rats. A secondary measure of line crossings were compared for open and closed exploration rates and the data revealed that, although all subjects explored at similar rates in the closed arms of the maze, rats with ventral DG lesions failed to reduce exploration rates when in the open arms, as compared to controls and dorsal DG lesioned animals. Previous investigations that also used the elevated-plus maze to investigate anxiety-based behaviors have suggested that rats with ventral HPP lesions spent more time in the open arms when compared to controls and dorsal HPP lesioned rats (Bannerman et al., 1999; Bannerman et al., 2003; Bannerman et al., 2004). Though previous studies utilized larger lesions, the studies above support the current findings that ventral lesions of the HPP result in impairment for a preference to explore safer environments and that the dorsal HPP is not critical to this preference.

The number of line crossings was measured to investigate the possibility of hyperactive locomotion because there have been mixed reports of hyperactivity in rats with ventral HPP lesions (Bannerman et al., 2002; Bannerman et al., 1999; Bannerman et al., 2003; Kjelstrup et al., 2002). The current results did not indicate hyperactivity and further implies that inconsistencies in previous studies may result from the manner that data had been analyzed, rather than variations of activity level within subjects across

behavioral tasks. The grid line crossing data was organized by open or closed arm status and treatment group status. If the data had instead been analyzed based on treatment group only, our data might have reflected hyperactivity in ventral DG lesioned rats when compared to locomotor speed of controls. Such a finding would have been supported by previous reports that rats with ventral HPP lesions were hyperactive when participating in behavioral tests (Bannerman et al., 1999). However, the current data were also analyzed by open or closed arm status and it was revealed that all groups explored closed arms at a similar rate, but the data indicated that rats with control and dorsal DG lesions reduced exploration rates in the open arms of the maze and rats with ventral DG lesions continued to explore at the same rate when in open and closed arms. Rather than demonstrating hyperactivity, the current results suggested that rats with ventral DG lesions were impaired in that they did not *reduce* exploratory behavior when in open arms. Similarly, previous reports that ventral DG lesioned rats displayed hyperactive behavior indicated that this behavior was not seen in the home cage. Another interpretation may be that controls and rats with dorsal HPP lesions displayed slower rates of activity when performing in behavioral tests that may induce anxiety (less-safe environment) than when in the home cage (safer environment), and that rats with ventral DG lesions were impaired in reducing activity in potentially dangerous environments but otherwise displayed locomotion similar to controls. Although evaluation of hyperactivity based on comparisons for open and closed arms may shift the idea of hyperactivity to one of impairment to reduce activity like controls does not fit previous research, the current results can nevertheless be re-consolidated to show emulation of previous studies. Regardless of calculation differences, the current results suggest that the ventral DG

plays an important role in choice to explore safer environments, and is important for slowing rates of exploration when in potentially dangerous environments. The current results also indicated that the dorsal DG does not play a critical role in demonstration of anxiety-based behaviors, which is supported by previous studies in which rats with dorsal HPP lesions did not demonstrate impairments on the elevated-plus maze(Bannerman et al., 1999; Kjelstrup et al., 2002). The current results provided important evidence that the ventral DG is necessary for anxiety-based behaviors. In addition, the current study design analyzed arms individually by group for locomotion and these methods may have provided an important explanation for previously inconsistent reports of hyperactivity.

Although the current results of the elevated-plus maze have indicated that hyperactivity is not present in ventral DG lesioned rats (or dorsal DG, or control), there is nevertheless a possible confound between the measures of hyperactivity (fast movement) and the fact that the testing apparatus has been shown to be anxiety-provoking (slow or non-movement). Therefore, a study of activity with reduced or no anxiety-provoking features was necessary. We used an open field maze, which has been well-established to be sensitive to locomotion in order to assess exploration behaviors for ventral DG and dorsal DG lesioned rats and controls (Walsh & Cummins, 1976). The current data indicated that all groups crossed a similar number of gridlines. The current data also indicated that rats with ventral DG lesions spent a larger amount of time in the inner, open, area of the maze than did controls and dorsal DG lesioned rats. Together, the current results suggest that ventral DG lesions do not cause hyperactivity in rats, and that the ventral DG is necessary to modify exploratory behavior in less-safe environments. Observations for patterns of exploration (inner vs. outer zones) are supported by previous

research that suggested the ventral HPP is important for demonstration of anxiety-based behavior, but that the dorsal HPP is not critically involved (Bannerman et al., 1999). Current results from the elevated-plus maze also support results from the open field maze in that data from both paradigms suggested that the ventral DG is important for behavior modification in less-safe environments and that hyperactivity was not observed. Given that other reports showed no hyperactivity in the home cage (less dangerous environment) but did report hyperactivity when on mazes, it is not likely that hyperactivity was induced in particular places, but not others. A more likely explanation would be that control subjects' behaviors change based on safety level of the environment and ventral DG lesioned rats' behaviors do not change based on safety level of the environment. These findings have important implications because a finding of general hyperactivity would have indicated that behavioral observations would be tainted by a factor other than an intended variable.

Together, the four current studies suggest that the ventral DG is importantly involved in learning and memory processes for odors, which includes novelty detection, and is especially important when odors are very similar. The current results also suggest that the ventral DG plays an important role in selection of safer environments and to adjust exploratory behavior when in potentially dangerous settings. Finally, the current results indicate ventral DG lesions do not induce hyperactivity in rodents. These findings all have strong implications of parallel processing across the dorsoventral axis of the HPP in regard to spatial and olfactory processing. However, the ventral DG may impact other functions and further investigation is needed. For example, the purview of this study did not include spatial learning and memory processes. Though the current results add to

previous studies to suggest that there is a dorsoventral differentiation, additional studies should be carried out to fully understand the functions of the ventral DG.

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