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The Role of the Dentate Gyrus, CA3a,b, and CA3c for Detecting Spatial and Environmental Novelty

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ABSTRACT: It has been suggested that the dentate gyrus (DG) and CA3 cooperate to efficiently process spatial information. The DG has been proposed to be important for fine spatial discrimination, and the CA3 has been proposed to mediate larger scale spatial information processing. To evaluate the roles of the DG and CA3a,b for spatial processing, we developed a task that measures responses to either overall environmental novelty or a response to more subtle changes within the environment. Animals with lesions to the DG showed impaired novelty detection for both environment as well as smaller changes in the environment, whereas animals with lesions to CA3a,b showed no such deficits. A closer look at the lesions suggested that the CA3 lesions included only CA3a and CA3b, but spared CA3c. To test the role of the spared CA3c region, animals with selective lesions to CA3c that spared CA3a,b were run on the same task and showed an intermediate pattern of deficits. These results suggest that the DG is critical for spatial information processing. These data also suggest that CA3 is a heterogeneous structure, with CA3c lesioned animals showing greater spatial processing deficits than CA3a,b lesioned animals. These findings extend our knowledge of hippocampal function and need to be accounted for in future computational models. © **2008 Wiley-Liss, Inc.**

KEY WORDS: CA3c; CA3a,b; dentate gyrus; environmental geometry; metric relationships

INTRODUCTION

It has been proposed that the dentate gyrus (DG) and CA3 interact via the mossy fibers to process spatial information with high spatial resolution (Morris and McNaughton, 1987; Rolls and Treves, 1998; Rolls and Kesner, 2006). Further, the DG has been proposed to mediate spatial pattern separation of similar spaces, a mechanism necessary for novelty detection of small changes within an environment. CA3, however, has been proposed to mediate a similar pattern separation process, but on much larger-scale spaces, such as changes in overall environmental geometry (Rolls and Treves, 1998; i.e., referred as "charts" by Rolls and Kesner, 2006).

To evaluate the role of the DG and CA3 for spatial novelty detection, we developed a task based loosely on paradigms used during place cell recording (cf. Leutgeb and Leutgeb, 2007). In this task, there are 2 possible spatial changes the animal could encounter: (1) a change to the overall environmental geometry (i.e., from a circle to a square) with all local cues held constant or (2) a change to the distance between two objects in the environment (i.e., metric relationships; Goodrich-Hunsaker et al., 2008) with the overall environment held constant. We hypothesized that detection of a change to the environmental geometry would depend on both the DG and CA3. We further hypothesized that the detection of the changed metric relationship between the objects would require only the DG (cf. Gilbert et al., 2001; Goodrich-Hunsaker et al., 2008).

The results of this experiment, however, were intriguing in that lesions to CA3 did not result in deficits for environmental novelty detection, whereas lesions to the DG did result in deficits. To account for these results we reanalyzed the CA3 lesions and noticed that CA3a,b (the bend of CA3 and the ventral portion between the bend and the lateral end of the DG) was consistently lesioned, but CA3c (the portion encapsulated by the blades of the DG; cf. Lorente de No, 1934) was not lesioned.

The CA3 subregion of the hippocampus has traditionally been modeled as a homogeneous auto-associative network (Morris and McNaughton, 1987; cf. the anatomical architectures referred in the models of Levy, 1989; Rolls, 1996; Samsonovich and McNaughton, 1997; Rolls and Treves, 1998; Rolls and Kesner, 2006). This approximation has stemmed from the work of Marr (1971) who proposed that a generic auto-associative network could underlie simple memory formation, although he did not posit CA3 as providing that system by name. In recent years, the homogeneity of the CA3 system has been brought into question because it has been shown that CA3a (the portion of CA3 adjacent to CA2 encompassing the curve of CA3), CA3b (the region between the curve of CA3 and the blades of the DG), and CA3c (the portion of CA3 adjacent to the CA4/hilus region and surrounded by the blades of the DG; cf. Lorente de No, 1934 for the delineations/borders we will use throughout this report) have dissimilar connection matrices and exhibit distinct neurophysiological properties (albeit subtle at times; Li et al., 1994; Scharfman, 1993, 1994, 1996a,b, 2007; Witter, 2007). Of increasing interest is the role of the backprojections from CA3c pyramidal cells to mossy cells in the region of the CA4/hilus (called hilus hereafter) that

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project onto granule cells in the DG. These backprojections polysynaptically inhibit dentate granule cell activity via the mossy cells (i.e., CA3c activation of the mossy cells in the hilus acts to inhibit the granule cells in the DG) and may act to "gate" or modulate DG activity, perhaps even facilitating orthogonal encoding of incoming sensory stimuli (cf. Li et al., 1994; Scharfman, 2007; Witter, 2007; Wittner et al., 2007). Interestingly, these backprojections are increasingly prevalent at more ventral portions of the hippocampus, but they also are present even though in lower numbers in the dorsal hippocampus. Additionally, it has been shown that CA3c pyramidal cells send and receive only a few recurrent collateral fibers, but maintain a robust Schaffer collateral projection to CA1 (Li et al., 1994; cf. Wittner et al., 2007 for a three-dimensional reconstruction of an individual CA3c pyramid filled in vivo from the dorsal-intermediate portion of the hippocampus).

In light of these findings, Lisman and coworkers have developed models emphasizing the reciprocal CA3c-DG interactions and proposing that these interactions may underlie spatial sequence learning (and potentially retrieval) and may play a role in the learning processes underlying theta phase precession (Huerta and Lisman, 1996; Lisman, 1999; Lisman and Otmakhova, 2001; Jensen and Lisman, 1996, 2005; Lisman and Grace, 2005; Lisman et al., 2005; de Almeida et al., 2007). Although these backprojections are more prevalent in the ventral hippocampus, it has been suggested that the ventral hippocampus does not contribute greatly to spatial processing (Moser and Moer, 1998; Bannerman et al., 2002; but also cf. de Hoz et al., 2003; Ferbinteanu et al., 2003; Hunsaker et al., 2008a,b for studies emphasizing the role of ventral hippocampal contributions to spatial memory), so the present analysis focuses on dorsal CA3c to emphasize the frequently modeled processing of spatial information To date, there have been no experiments that have evaluated the role of the CA3c system for spatial learning and memory.

In light of these findings, we then repeated the experiment with animals that received CA3c lesions that spared CA3a,b (with their own control group). We found that CA3c lesioned animals showed deficits for the detection of an altered metric relationship, but no deficits for detection of a change to the environment as a whole. These results suggest that CA3 is not a homogenous structure, and this functional heterogeneity needs to be taken into account in future models of hippocampal function.

MATERIALS AND METHODS

Subjects

Twenty-four, 3-month-old male Long Evans rats from a commercial supplier (Simonsen Labs, Gilroy, CA) and weighing 340–400 g at the beginning of the experiment served as subjects. All animals were kept on a 12-h light/dark cycle. All experimentation was carried out during the light portion of the light/dark cycle. Animals also had ad libitum access to water and were reduced to 90% of their free-feeding weight prior to experimentation. All animal care and experimental protocols conformed to NIH, IACUC, and AAALAC standards and protocols and a veterinarian assessed the health of all animals weekly.

Surgery

Experimentally naive rats were randomly assigned to a surgery group (dorsal DG $n = 6$, dorsal CA3a,b $n = 6$; CA3c $n = 6$; DG control $n = 2$; CA3a,b control $n = 2$; CA3c control $n = 2$). Rats were anesthetized and maintained with isoflurane (2–4% (vol/vol) in 2 l/m medical air) and given atropine sulfate (0.2 mg/kg i.m.) as a prophylactic and placed in a stereotaxic apparatus (David Kopf Instruments, Tunjana, CA). Lesions of dorsal DG granule cells were made by infusing 0.8-μl colchicine (σ-Aldrich; St. Louis, MO; 2.5 mg/ml phosphate buffer vehicle) into each of two sites bilaterally at an infusion rate of 20 μl/h. Excitotoxic lesions of dorsal CA3a,b and CA3c were made by infusing ibotenic acid (Ascent Scientific; Bristol, UK; 6 mg/ml phosphate buffer), at a rate of 6 μl/h and a volume of 0.05–0.15 μl depending on the site, bilaterally, into three sites. After infusion, the injection cannula remained in each site for at least 1 min to allow diffusion of the injected excitotoxin prior to retraction from the brain.

The coordinates for dorsal DG lesions ($n = 6$) were as follows: (1) 2.7 mm posterior to bregma, 2.1 mm lateral to midline, and 3.4 mm ventral to the dura mater (0.8 μl colchicine infused), and (2) 3.7 mm posterior to bregma, 2.3 mm lateral to the midline, and 3.0 mm ventral to the dura mater (0.8 μl colchicine infused). Dorsal CA3c lesions ($n = 6$) were made using ibotenic acid, which was infused into three sites bilaterally located (1) 2.5 mm posterior to bregma, 2.0 mm lateral to midline, and 4.2 mm ventral to dura (0.05 μl of ibotenic acid injected); (2) 3.3 mm posterior to bregma, 2.0 mm lateral to midline, and 4.3 mm ventral to dura (0.08 μl infused); (3) 4.2 mm posterior to bregma, 2.1 mm lateral to the midline, and 3.6 mm ventral to dura (0.10 μl infused). Dorsal CA3a,b lesions ($n = 6$) were made using ibotenic acid, which was infused into 3 sites bilaterally located (1) 2.5 mm posterior to bregma, 2.6 mm lateral to midline, and 3.2 mm ventral to dura (0.05 μl of ibotenic acid injected); (2) 3.3 mm posterior to bregma, 3.3 mm lateral to midline, and 3.2 mm ventral to dura (0.08 μl infused); (3) 4.2 mm posterior to bregma, 4.2 mm lateral to the midline, and 3.1 mm ventral to dura (0.15 μl infused). Note that dorsal CA3c lesions were made at the same anteroposterior coordinates as the dorsal CA3a,b lesions to facilitate comparisons between lesions without possible confounds of laminar differences (cf. Witter, 2007). Vehicle control lesions (DG vehicle $n = 2$; CA3a,b vehicle $n = 2$; CA3c vehicle $n = 2$) were made using the same coordinates and procedures; however, equivalent volumes of phosphate buffer vehicle were infused instead of colchicine or ibotenic acid.

Following surgery, the incision was sutured, 1.5 ml of physiological saline was injected into each thigh subcutaneously to hydrate the animal, and the rats were allowed to recover on a

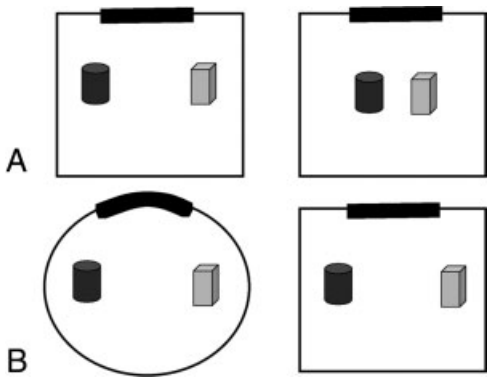

FIGURE 1. Behavioral apparatus. (A) Metric change in object location. The animals receive three, 5 min trials in the box with the objects 60 cm apart separated by 3 min intersession intervals. The animals then receive a single test session during which the box stays the same but the distance between the objects changes. (B) Environmental change. The animals receive three, 5 min trials in the circular box with the objects 60 cm apart separated by 3 min intersession intervals. The animals then receive a single test session during which the box changes to the square box and the distance between the objects stays the same. All diagrams depict a white box with a black cue card, but there were also black boxes with white cue cards.

heating pad before returning to their home cage. In addition, rats received acetaminophen (Children's Tylenol; 200 mg/100 ml water) in their drinking water as an analgesic and were provided with sweetened, powdered food for 3 days following surgery. The behavior of all animals was visually monitored for epileptiform activity for 7 days postsurgery: no behavioral seizures were observed in any cases.

Experimental Apparatus

The apparatus for this experiment consisted of four large boxes and 20 distinct objects ranging in size from 5 × 5 cm^2 to 10 × 5 cm^2. There were two large (78 cm diameter) circular aluminum boxes with 50 cm high walls, one painted white and had a black cue card (30 cm wide) and the other circular box was painted black and had a white cue card affixed to the wall. There were also two 70 cm × 70 cm × 50 cm square boxes that had approximately the same internal area as the circular boxes. One was painted white and had a black cue card and the other was painted black and had a white cue card affixed to the wall and covering the weld seam. The cue card was located in the middle of one of the walls. See Figure 1 for diagrams of the experimental apparatus and experimental design. All animals were subjected to each task in a counterbalanced order. There were always at least 3 days between tasks.

Behavioral Methods

After receiving lesions, all animals were given a 14-day recovery period prior to experimentation to allow full recovery. In all cases, during the experiment the behavior of the animals was recorded with an overhead video camera and the data scored were taken from videocassettes by experimenters blind to the experimental conditions. It also must be noted that the test session for both conditions was in a square box to facilitate comparison of the data. The order of experimentation was counterbalanced so that task order did not contribute to task performance.

In adddition, the individual groups were run in the following order: the DG lesioned and CA3a,b lesioned animals and four control animals were run as an initial experiment, then the CA3c lesioned animals and an additional two control animals were run as a secondary experiment. Because the performance for the control animals did not differ, the control data were collapsed into one group and the data are all presented together. Since the control data were the same, we can assume that the animals were treated similarly, and the same experimenter (JSR) ran the experiments on all the different groups.

Metric change in object location task

For the metric change in the object location task, the animals were placed in one of the square boxes (black or white was counterbalanced) with two objects placed 60 cm apart (Fig. 1). The animals were given three 5 min exploration sessions with the box and objects unchanged separated by 3 min intersession intervals. The box and objects were wiped down with a dilute cleaning solution between each session to prevent any odor cues left by the rat during one session to influence behavior during subsequent sessions. After the animals habituated to the objects and environment, the objects were moved closer together, to 40 cm separation, and the animals were again given 5 min to explore. An experimenter blind to the experimental conditions recorded rearing, active object exploration, and general locomotor activity as dependent measures (Poucet, 1993; cf. Lever et al., 2006). The square box was used in this experiment because preliminary experiments showed that control animals had a considerably more difficult time detecting the changed distance between the objects (metric shift) in the circular environment compared to the square environment.

Environmental change

For the environmental change detection task, the animals were placed in one of the circular boxes (black or white was counterbalanced) with two objects placed 60 cm apart (Fig. 1). The animals were given three 5 min exploration sessions with the box and objects unchanged separated by 3 min intersession intervals. The box and objects were wiped down with a dilute cleaning solution between each presentation to prevent any odor cues left by the rat during one session to influence behavior during subsequent sessions. After the animals were habituated to the objects and environment, the environment was changed to the square box that was the same color as the circular box used and the animals were given 5 min to explore. The distance between the two objects remained fixed (at 60 cm). An experimenter blind to the experimental conditions recorded rearing, active object exploration, and general locomotor activity

as dependent measures. Preliminary experiments suggested there was no difference in control animals' reactions to the environmental change when the animals went from a circle to a square or from a square to a circle. Animals were run on both conditions, but to compare the data with that from the metric change in the object location task, the data presented here are all from the condition where the environment was changed from a circular to a square environment.

Histology

After experimentation, all animals were deeply anesthetized with sodium pentobarbitol (1 ml at 70 mg/ml i.p.) and were intracardially perfused with phosphate buffer for 5 min followed by 4% formalin in phosphate buffer for 2 min. The brains were extracted and stored for 72 h in a 30% (w/v) sucrose formalin solution. Forty micrometer sections were taken on a freezing stage microtome and mounted on gelatin coated glass slides. After 3 days, the sections were Nissl stained with Cresyl violet for microscopic visualization of the lesion. All sections were photographed and imported into Image J (v.1.35j; National Institutes of Health; Bethesda, MD) for lesion quantification. All lesions were quantified at 40× magnification to better characterize the specificity of damage, because DG and CA3a,b (simply called CA3 in previous reports) lesions have previously been characterized using this procedure (Goodrich-Hunsaker et al., 2008).

In short, the lesion regions of interest were traced using the freehand selection tool on ImageJ. The number of pixels contained within the traced region was recorded. The total number of pixels showing Cresyl violet staining (>50% darker than background-this was a conservative measure as the background was always clear) were calculated and recorded. The ratio of remaining stained pixels to the total area was calculated and used to compute the percent of tissue unlesioned in the region of interest. This value was then used to ascertain percent damage (i.e., 100 – % stained tissue remaining = % tissue ablated). We calculated percent damage this way rather than by measuring unstained pixels to be as conservative as possible. We also measured damage to the other hippocampal subregions to verify that we did not obtain lesions from unintended spread of the neurotoxin. We also limited the analyses to the dorsal hippocampus as defined by the anterior half of the hippocampus (Moser and Moer, 1998; Bannerman et al., 2002). When there was damage in the ventral hippocampus (in only a single CA3a,b lesion was this the case), we quantified it as a percentage of the entire ventral hippocampus.

Dependent Measures and Statistical Analysis

For the present experiment, both object exploration and rearing were dependent measures. It has been shown previously that animals react to spatial rearrangements of familiar objects by selective re-exploration (Lee et al., 2005a,b; Goodrich-Hunsaker et al., 2005, 2008; Hunsaker et al., 2007a,b). Object exploration was defined as the rat having its nose within 1 cm of the object for greater than 0.5 s. The videotaped data was scored in duplicate by experimenters blind to the experimental conditions whose scores did not differ, and when they did, it was by less than 0.5 s, and the two scores were averaged prior to statistical analysis. Rearing was chosen as it has been shown to correlate with detection or recognition of environmental changes (Anderson et al., 2006; Lever et al., 2006), and was defined as the animal having both forepaws lifted from the floor for greater than 0.5 s. Most rearing events occurred when the animals reared and supported their forelimbs on the exterior walls of the box for 0.5–1.5 s. The videotaped data was scored in duplicate by experimenters blind to the experimental conditions whose scores did not differ (they always reported the same number of rearing events). Rearing and object exploration during the test session (e.g., after changes have been made) were compared to the immediately preceding study session and a simple difference score was taken [(test session)-(last study session)]. It must be noted that normalizing the data into ratio scores to constrain the data range between −1 and 1 did not change the results of any analyses; so raw difference scores are presented in the figures and analyzed to make the data more transparent and easier to interpret.

General locomotor activity was also measured as reported by Goodrich-Hunsaker et al. (2008) previously. A 3 × 3 grid was drawn on the monitor used to score the exploration data and whenever an animal crossed with all four paws into a new grid, it was recorded. The identity of the grids entered was recorded to verify that animals were not exploring only a small proportion of the maze (e.g., the left half or the top right quadrant only), but this was never the case. Animals explored the entire environment-so only the raw number of grids crossed is reported here. These data were recorded in duplicate by the same experimenters who recorded the other behavioral measures. There were no discrepancies between the two scores.

For each test (metric change in object location and environmental change) the difference scores were imported into a matrix and the open source statistical package, R (Vienna, Austria; http://www.r-project.org) was used to perform a one way analysis of variance (ANOVA) with lesion (dorsal DG, dorsal CA3a,b, control) as the grouping factor. Locomotor activity was analyzed by a two way repeated-measures ANOVA with lesion as the grouping factor and session as the within subject repeated factor. To better characterize any significant effects, Tukey's honestly significant difference (HSD) post hoc paired comparisons were performed.

RESULTS

Histology

Damage to the dorsal DG, dorsal CA3c, and dorsal CA3a,b was independently evaluated, as well as damage to dorsal CA1. We also analyzed damage in the ventral hippocampus, but less than 1% damage was observed and, even then, only in a single CA3 lesioned animal. See Figure 2 for representative photomicrographs of the lesions and controls as well as for diagrams of

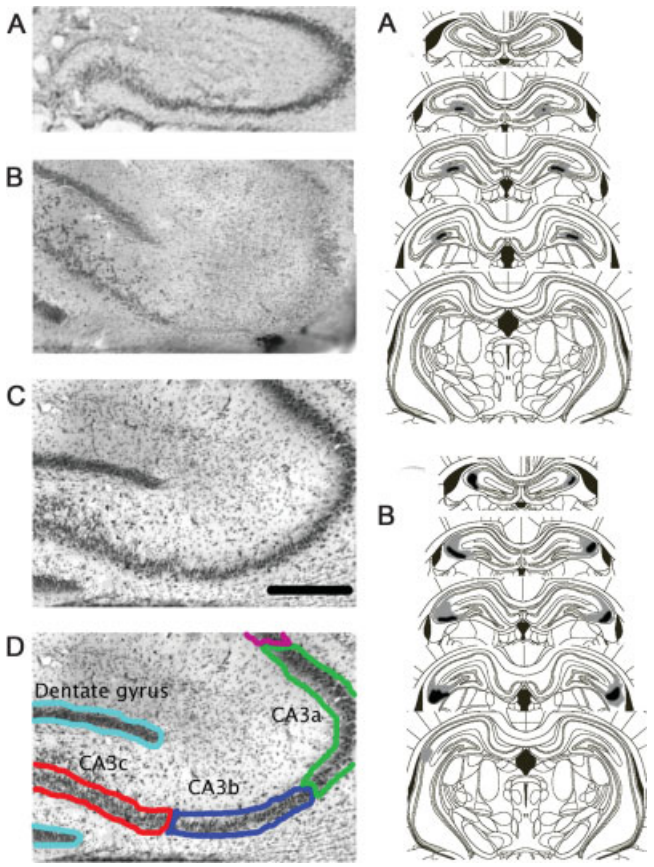

FIGURE 2. Histological results. (A) Dorsal DG lesion. Note that dorsal CA3a,b and dorsal CA3c are spared. Note the near complete elimination of dentate granule cells without a prominent disruption of dorsal CA3c or dorsal CA3a,b. (B) Dorsal CA3a,b lesion. Note that dorsal CA3c and the dorsal DG are spared. Also note the break in cellular elimination in dorsal CA3b in the middle of dorsal CA3b. (C) Vehicle control lesion. (D) Labeled version of C. to facilitate rapid identification of cell thinning in A and B. The scale bar in C applies to all plates and represents 200 μm. On the right half of the figure are line diagrams of the lesions on plates [From Paxinos and Watson, The Rat Brain: In Stereotaxic Coordinates, 2005, San Diego, © Academic Press, reproduced by permission]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the full dorsal ventral extent of the lesions. For dorsal DG lesions (Fig. 2A), there was approximately (mean ± standard error of the mean) 88% ± 2.5% damage to the dorsal DG granule cell layer, 5% ± 1.5% damage to dorsal CA3c, 0% damage to dorsal CA3a,b, and 5% ± 3.5% damage to dorsal CA1 pyramidal cells immediately dorsal to the DG lesion injection sites. An additional qualitative evaluation suggested that there was also damage to cells in the hilus. For dorsal CA3a,b lesions (Fig. 2B), there was 90% ± 12.1% damage to dorsal CA3a,b (~99% of CA3a and ~85% of CA3b), 0% damage to the dorsal DG, 5% ± 1.2% damage to dorsal CA3c, and 0% damage to dorsal CA1. Dorsal CA2 was damaged in some cases, but as it is difficult, if not controversial, to define dorsal CA2 as separate from either dorsal CA1 or dorsal CA3, it was not specifically analyzed. There was no damage observed in the hilus. Because the CA3 lesions traditionally used in our lab only damage CA3a,b, these lesions will be called CA3a,b lesions from here on. Figure 2C depicts an average vehicle control to provide the baseline condition. Figure 2D is a labeled version of Figure 2C with the approximate regions of interest outlined to facilitate quick evaluation of the location of cell thinning seen after the individual subregional lesions.

For dorsal CA3c lesions (Fig. 3A, the largest of these lesions and the one with the greatest DG damage), there was 75% ± 6.9% damage to dorsal CA3c, 10% ± 2.8% damage to the most lateral aspect of the upper blade of the dorsal DG, 15% ± 4.3% damage to dorsal CA3b (CA3b adjacent to CA3c), no damage to dorsal CA3a, and 0% damage to dorsal CA1. There was no apparent damage within the hilus and the portions of dorsal CA3c that were spared were those adjacent to the hilus. Figures 3B,C depicts an average vehicle control to provide a baseline condition. It must be noted that after the pyramidal or granule cells were ablated, glial cells infiltrated the ablated region. The scale bar in Figure 3C applies to all plates and represents 200 μm. Note that in all cases the cell loss is primarily (and almost exclusively) in the subregion targeted.

Behavior

For all behavioral tasks, there were no between group differences for locomotor activity during study or the test sessions

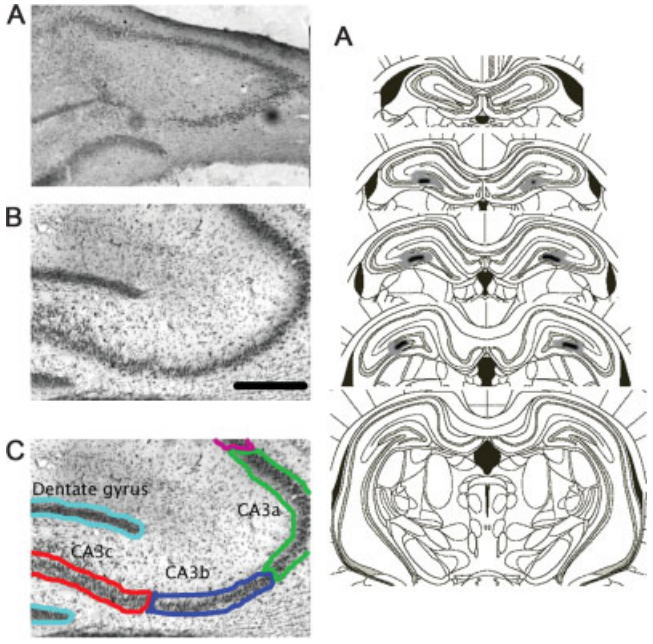

FIGURE 3. Histological results. (A) Dorsal CA3c lesion. Note that dorsal CA3a,b and dorsal the dorsal DG are spared. (B) Vehicle control lesion. (C) Labeled version of B. to facilitate rapid identification of cell thinning in A. The scale bar in B applies to all plates and represents 200 μm. On the right half of the figure are line diagrams of the CA3c lesion on plates. [From Paxinos and Watson, The Rat Brain: In Stereotaxic Coordinates, 2005, San Diego, © Academic Press, reproduced by permission]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

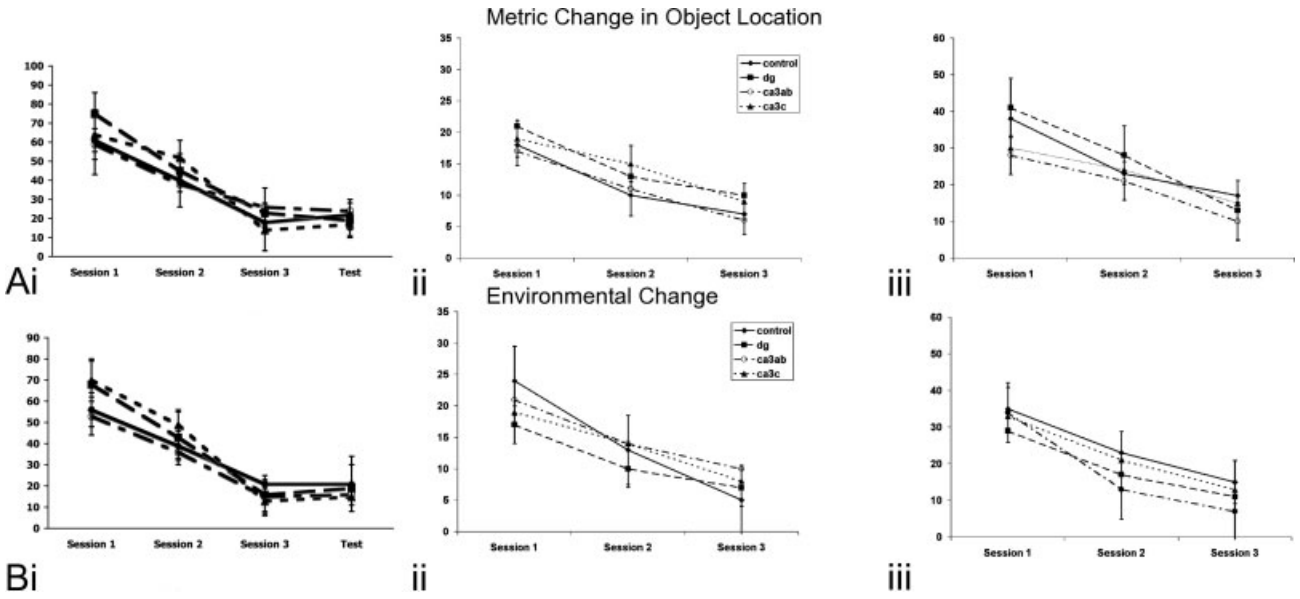

FIGURE 4. Habituation measures. (A) Metric shift in object location. (i) Locomotor activity measured by grid crossings (Grid crossings on the vertical axis). (ii) Habituation of object exploration (Exploration in seconds on the vertical axis). (iii) Habituation of rearing (number of rearing events on the vertical axis). (B) Environmental change. (i) Locomotor activity measured by grid crossings (grid crossings on the vertical axis). (ii) Habituation of object exploration (exploration in seconds on the vertical axis). (iii) Habituation of rearing (number of rearing events on the vertical axis). All horizontal axes are session. Notice that no groups differed under any condition and all groups consistently habituated as a function of session.

(Fig. 4; (all two-way repeated measures ANOVA) *P*s >0.1). This was reflected in similar level of grid crossings during the individual sessions. Figure 4 also shows that all groups showed habituation of both object exploration and rearing behavior as a function of session. There were no group differences in habituation for either object exploration or rearing (all two-way repeated measures ANOVA $P > 0.1$). Because there were no group differences for any of these measures, the raw values were used for statistical analyses and the data were not normalized in any manner prior to analysis. For each experiment, object and rearing analyses are presented in turn.

Detection of a Metric Change in Object Location

Object exploration

Figure 5A shows group differences for object exploration after a metric change in the object location. To characterize these differences, one-way ANOVA were performed with groups (control, DG, CA3a,b, and CA3c) as the between group factor. There was a significant group effect ($F(3,23) = 8.23$, $P = 0.0007$). A subsequent Tukey's HSD post hoc paired comparisons test revealed that the DG lesioned animals explored the metric change less than the CA3a,b, and control groups ($P < 0.001$), as well as less than the CA3c group ($P < 0.05$). The CA3c group also explored the metric change less than control and CA3a,b lesion groups ($P < 0.05$).

Rearing

Figure 5A shows group differences for rearing after a metric change in the object location. To characterize these differences, one-way ANOVA were performed with groups (control, DG, CA3a,b, and CA3c) as the between group factor. There was a significant group effect ($F(2,23) = 4.31$, $P = 0.015$). A subsequent Tukey's HSD post hoc paired comparisons test revealed that the DG lesioned animals reared less after the metric change less than the control group ($P < 0.001$), as well as CA3a,b and CA3c ($P < 0.05$). CA3a,b, and CA3c, differed from the control group ($P < 0.05$), but not each other ($P > 0.1$).

Detection of an Environmental Change

Object exploration

Figure 5B shows group differences for object exploration after a change in the environmental geometry. To characterize these differences, one-way ANOVA were performed with groups (control, DG, CA3a,b, and CA3c) as the between group factor. There was a significant group effect ($F(3,23) = 5.19$, $P = 0.007$). A subsequent Tukey's HSD post hoc paired comparisons test revealed that the DG lesioned animals explored the change in environmental geometry less than all the other groups ($P < 0.001$), which did not differ ($P > 0.1$).

Rearing

Figure 5B shows group differences for rearing after a change in the environmental geometry. To characterize these differences, one-way ANOVA were performed with group (control, DG, CA3a,b, and CA3c) as the between groups factor. There was a significant group effect ($F(3,23) = 7.72$, $P = 0.001$). A subsequent Tukey's HSD post hoc paired comparisons test revealed that the DG lesioned animals reared less after the

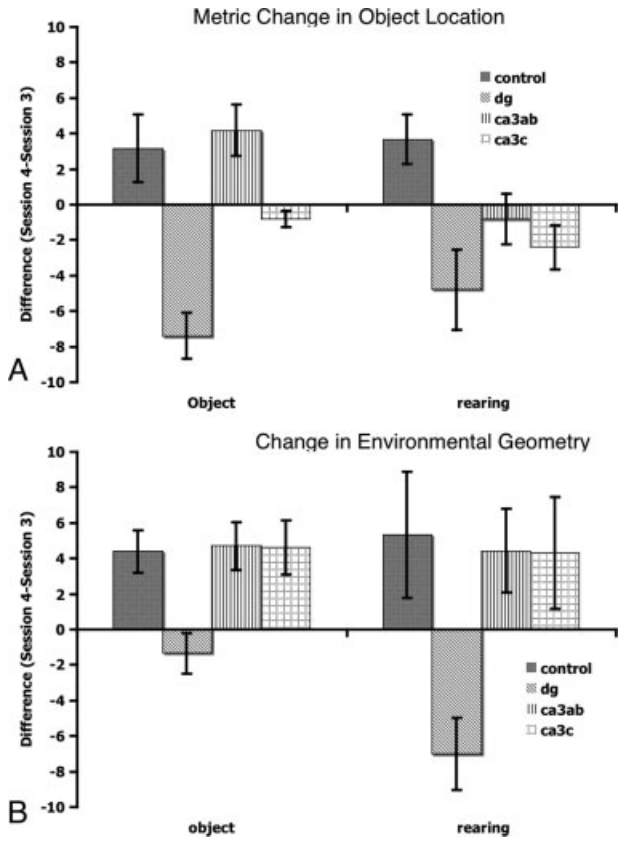

FIGURE 5. Behavioral results. (A) Metric shift in object location. Notice that for object exploration, the DG lesioned and CA3c lesioned animals show deficits relative to controls and CA3a,b lesioned animals. All lesion groups show deficits for rearing. (B) Environmental change. Note that only the DG lesion group showed an object exploration deficit. Only the dorsal DG lesioned animals showed deficits for rearing.

change in environmental geometry less than all other groups ($P < 0.001$). The control, CA3a,b, and CA3c groups did not differ ($P > 0.1$).

DISCUSSION

Dorsal Dentate Gyrus

The results of the present experiment suggest that the dorsal DG is the critical substrate underlying spatial pattern separation as measured by a reaction to spatial change, both for environmental geometry and detection of metric changes in relationships between objects. Animals with lesions to the dorsal DG did not re-explore the objects or the environment (via rearing) after changes to either the metric relationships between stimuli or after changes to the overall environment. This was reflected in the negative value of the difference score displayed in Figure 5. This result suggests that the animals with dorsal DG lesions were incapable of discriminating between the original and changed environmental conditions. These data support previous reports showing the dorsal DG is more highly sensitive to subtle environmental change than dorsal CA3, and that dorsal DG dysfunction is sufficient to disrupt spatial processing and pattern separation processes (Xavier et al., 1999; Gilbert et al., 2001; Costa et al., 2005; Lee et al., 2005a,b; Kesner, 2007a; Leutgeb and Moser, 2007; Leutgeb et al., 2007; McHugh et al., 2007; Goodrich-Hunsaker et al., 2008).

Dorsal CA3a,b

The results of the present experiment suggest that dorsal CA3a,b is not as critically involved for the detection of an environmental change as the dorsal DG. In fact, the only effect seen after dorsal CA3a,b lesions in the present experiment was an impaired reaction to the metric change in object location, but only the rearing measure was affected. There were no effects of dorsal CA3a,b lesions for detecting a change in spatial geometry (Fig. 5). This result, although unexpected, suggest that dorsal CA3a,b does not play a critical role in pattern separation processes compared to the dorsal DG, a finding supported by previous behavioral and neurophysiological research (Lee et al., 2004; Gilbert and Kesner, 2006; Leutgeb and Moser, 2007; Leutgeb et al., 2006, 2007; Goodrich-Hunsaker et al., 2008). The lack of a CA3a,b lesion effect for rearing to a change in environmental geometry is unexpected, but can be explained by the preeminent role of the DG for the detection of spatial change (or pattern separation) and the fact that our lesions spared CA3c, which receives DG input via the mossy fibers and also maintains a robust Shaffer collateral projection to CA1. The CA3c animals, interestingly, showed a deficit for rearing after a metric change in the object locations, but not for a change in spatial geometry.

Our laboratory has published numerous reports of dorsal CA3 excitotoxic lesions (Kesner et al., 2004; Jerman et al., 2005; Kesner et al., 2005; Lee et al., 2005a,b; Rolls and Kesner, 2006 for reviews of those behavioral studies; cf. Gilbert and Kesner, 2006; cf. Kesner, 2007b; for photomicrographic examples of dorsal CA3 lesions and Hunsaker and Kesner, 2008 for an example of a ventral CA3 lesion); however, a meta-analysis of these lesions suggests that dorsal CA3a and dorsal CA3b are ablated with relative precision, but dorsal CA3c is only partially ablated, and even then only occasionally. Especially noteworthy is the paper by Jerman et al. (2005) that tracked cell death after excitotoxic lesions using fluorojade staining. After dorsal CA3 lesions, dorsal CA3a,b show very robust cell damage, but dorsal CA3c does not. Interestingly, dorsal CA3c damage was present initially (at 2 days after the toxin infusion), but was absent at 4 and 7 days, suggesting that any CA3c damage was limited to a low number of cells. Concordant with this data, the previous excitotoxic dorsal CA3 lesions from Kesner's labs are considered dorsal CA3a,b lesions in the present report. We reviewed the lesions effected in our own lab because no other labs to our knowledge have specialized in these lesions and published as widely using these types of lesions; also because we have the tissue and were able to verify the lesions directly. These data suggest that the DG is more critical than CA3a,b for the detection of environmental novelty as well as for the

detection of a change in the metric relationships between stimuli. What is unclear, however, is whether the spared portion of CA3 (CA3c) may participate in these processes.

Dorsal CA3c

The results of the present experiment suggest that dorsal CA3c is only involved in pattern separation processes when the animal is required to detect a metric change in object location. This was observed during the metric change in object location task (with both decreased object exploration and rearing; but at a smaller magnitude than the DG effect). There was no apparent effect of a CA3c lesion during the environmental change task. The inability to react to the metric shift in object location was reflected in the negative value of the difference scores displayed in Figure 5. It must be noted, however, that dorsal CA3c lesions never caused effects as dramatic as those caused by dorsal DG lesions.

One interpretation of these data may be that the DG selectively recruits CA3c to assist in the metric detection and not the detection of the overall environmental change. This interpretation is in agreement with the suggestions of Scharfman (2007) and de Almeida et al. (2007), who suggest that CA3c cooperates with the DG to support efficient spatial memory formation and subsequent recall. Also, it is possible that if CA3c forms a circuit with the DG (Fig. 6) that participates in sparse encoding of entorhinal inputs, the feedback circuit would be more critical for detection of discrete changes, such as after metric shifts in object locations. Since the change in overall environmental geometry is a very large and prominent change, the feedback circuit may not be as relevant due to the size of the change (i.e., the DG can easily orthogonalize the two environments with or without CA3c feedback modulation). This difference is akin to the concept of discrete pattern separation (viz. metric shift on object locations task) and the pattern separation of charts (viz. change in environmental geometry) analyzed by Rolls and Treves (1998) and Rolls and Kesner (2006).

The implication of this theory is that for small-scale or subtle changes, the dorsal DG and dorsal CA3c cooperate to orthogonalize the incoming visuospatial data with a fair degree of resolution. The CA3c projection to the hilar mossy cells would act to inhibit the granule cells, facilitating even sparser encoding than the DG would normally provide (i.e., a more orthogonal representation). For larger changes, such as for an overall environmental change, CA3c is not needed because the requirement for sparse encoding is not present (low spatial resolution is more than sufficient to distinguish a circle from a square environment). That is to say, it is easier for the animal to identify orthogonal charts than to process metric relationships between stimuli (Rolls and Kesner, 2006).

OVERALL DISCUSSION

The data presented suggest that animals with lesions to the dorsal DG, dorsal CA3c, and dorsal CA3a,b subregions of the hippocampus react differentially to metric changes in object location as well as to changes in the environmental geometry. These data also suggest that there is functional heterogeneity of function within CA3.

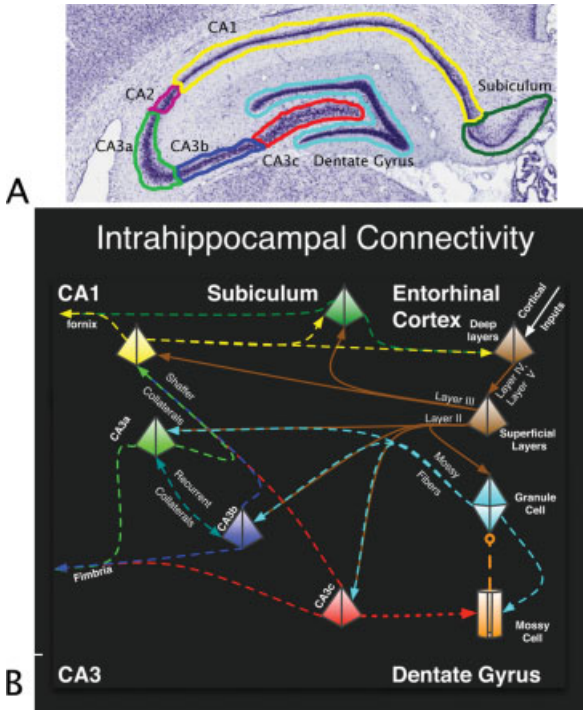

FIGURE 6. Anatomy of the CA3c system. (A) Nissl stained hippocampus with the DG, CA3c, CA3a,b, and CA1 outlined. (B) Diagram of DG-CA3 circuitry with CA3c connections emphasized after Scharfman (2007) and de Almeida et al. (2007). All other connections depicted are those modeled extensively by Rolls and Treves (1998). The colors in the circuit diagram correspond to the colors used to outline the different hippocampal subregions in the plate above. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Additionally, it is of interest that the rearing measure and object exploration measure appeared to be somewhat independent, or at least not interchangeable as measures of exploration. That is to say, an observed deficit in object exploration does not necessarily correspond to a deficit in rearing, and vice versa. We suggest this means a change in object exploration reflects detection of a change in the metric relationship between stimuli within the environment (e.g., the animals perceive that the distance between the objects has changed), whereas a change in rearing reflects the detection of overall environmental changes (i.e., changes in the environmental geometry; cf. Lever et al., 2006). As examples of this independence, during the metric change in object location task, animals with lesions to CA3a,b, showed no deficits for object exploration, but showed deficits for rearing. Dorsal DG and CA3c lesioned animals, however, showed deficits for both rearing and object exploration during both tasks. It may be possible that, as Lever et al. (2006) postulate, rearing is a measure of exploration of the overall environment (objects within it included), whereas directed object exploration is a measure of object recognition directly or else a

measure of the animal's detection of changes within small portions of the environment (Poucet, 1993; cf. Andersen et al., 2006). It also may be possible that rearing and object exploration are somewhat related, but that rearing is not as reliable a measure as object exploration in the present experiment.

CONCLUSIONS

The present experiment provides behavioral evidence that dorsal CA3c and the dorsal DG may interact for spatial information processing. This effect was only seen during the condition in which the animal is required to detect a discrete metric change in object location, a task that has been shown to be particularly sensitive to DG damage (Kesner, 2007a; Goodrich-Hunsaker et al., 2008). Also, the present experimental results provide further support for models that posit that the dorsal DG is more critical for detecting a subtle environmental change than CA3a,b and CA3c, because lesions to the dorsal DG resulted in deficits for spatial re-exploration, whereas lesions to dorsal CA3a,b had subtle effects at best.

These data support the premise of the assertions made by Lisman and coworkers that the feedback circuit between the DG and CA3c may play a role in spatial memory (specifically phase precession and spatial sequence learning; Lisman, 1999; Lisman et al., 2005). More specifically, it appears that dorsal CA3c may interact with the dorsal DG to support pattern separation processes by providing shunting inhibition, perhaps via excitatory connection with hilar mossy cells, which inhibit granule cells to facilitate sparse encoding by the dentate granule cells. Such a mechanism has been suggested by Scharfman (2007), and incorporated into the models described by Lisman and coworkers (Lisman, 1999; Lisman et al., 2005; de Almeida et al., 2007).

It is of note that dorsal CA3a,b, the subregion(s) lesioned in most CA3 lesion studies (Kesner et al., 2004, 2005; Lee et al., 2005a,b; Gold and Kesner, 2005; Jerman et al., 2005; Gilbert and Kesner, 2006; Kesner, 2007b; cf. Li and Chao, 2008 for an example of an electrolytic CA3 lesions that also failed to lesion CA3c) and inactivated in pharmacological manipulations (cf. Hunsaker et al., 2007a,b) had only a very limited effect during the present experiment. In fact, dorsal CA3a,b lesions only showed a small effect after a metric change in object location, and then only in behaviors associated with environmental exploration (e.g., rearing). The present experiment does not provide support for theories suggesting that the dorsal CA3a,b (viz. "CA3" as described by most computational models) is organized as a homogeneous associative network that participates in pattern separation processes (Morris and McNaughton, 1987; Levy, 1989; Rolls, 1996; Samsonovich and McNaughton, 1997; Rolls and Treves, 1998). It must be noted that the present experiment did not involve spatial memory per se, but emphasized detection of a spatial change in either the metric distance between objects or a change to the overall environment. Further work is required to evaluate the role of dorsal CA3c and dorsal CA3a,b for pattern completion processes as pattern completion was beyond the scope of this present experiment (cf. Gilbert and Kesner, 2006; cf. Kesner, 2007b).

Although the present behavioral data do not show any dramatic effects of dorsal CA3a,b lesions, the dorsal DG effect is clear. Additionally, the dorsal CA3c lesion data suggest that there is a circuit involving dorsal CA3c and the dorsal DG that is perhaps important for pre-processing spatial information prior to dorsal CA3a,b processing stages (i.e., for the computational processes modeled in Morris and McNaughton, 1987 among others for "CA3"). Further experiments are needed to better characterize the role of this circuit in sequential processing.

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