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The Role of Hippocampal Subregions in Detecting Spatial Novelty

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Previous literature suggests that the hippocampus subserves processes associated with the encoding of novel information. To investigate the role of different subregions of the hippocampus, the authors made neurotoxic lesions in different subregions of the dorsal hippocampus (i.e., CA1, dentate gyrus [DG], or CA3) of rats, followed by tests using a spontaneous object exploration paradigm. All lesion groups explored normally an object newly introduced in a familiar location. However, when some of the familiar objects were moved to novel locations, both DG and CA3 lesion groups were severely impaired in reexploring the displaced objects, whereas the CA1 lesion group was only mildly impaired in reexploration. The results suggest that the DG–CA3 network is essential in detecting novelty for spatial, but not for individual object, information.

Successful encoding and retrieval of memory demand a continuous comparison of the current environment to the internal representations of the environment to determine an appropriate mode of network operation (e.g., encoding novel information vs. retrieving old information). It has been strongly suggested that the hippocampus performs as a key neural substrate for such a match–mismatch process between the internal representation and the sensory information from the environment (Chun, 2000; Gothard, Skaggs, & McNaughton, 1996; Hampson, Simeral, & Deadwyler, 1999; Honey, Watt, & Good, 1998; Knierim, Kudrimoti, & McNaughton, 1998; Knight, 1996; Vann, Brown, Erichsen, & Aggleton, 2000; Wan, Aggleton, & Brown, 1999; Wiebe & Staubli, 1999).

The hippocampus is anatomically composed of three main subregions: dentate gyrus (DG), CA3, and CA1 (Ramon y Cajal, 1911; Lorente de Nó, 1934). What is the function of these hippocampal subregions in the match–mismatch process? Is a certain subregion more important than the others in the match–mismatch process? Theoretical models differ with respect to the locus of the match–mismatch process within the hippocampus. Most models focus on CA1 as the subregion in which a critical comparison is made between the sensory environment and the internal representation of the environment, mainly on the basis of its unique, anatomical position receiving the information simultaneously both from the DG–CA3 network and directly from the entorhinal cortex (Hasselmo, Fransen, Dickson, & Alonso, 2000; Hasselmo & Schnell, 1994; Lisman & Otmakhova, 2001; McClelland, McNaughton, & O'Reilly, 1995). Similarly, CA3 receives converging afferents originating from multiple brain areas (e.g., DG, entorhinal cortex, and medial septum). Therefore, some hippocampal models incorporate CA3 as a potential comparator (Mizumori, Ragozzino, Cooper, & Leutgeb, 1999; Vinogradova, 2001). With respect to the DG subregion, theoretical models have not emphasized a direct role for DG in the match–mismatch process. However, DG seems essential in pattern separation (McClelland et al., 1995; McNaughton & Morris, 1987; Morris, 2001; Treves & Rolls, 1994), which may play a key role in the match–mismatch process by orthogonalizing similar input patterns and amplifying the differences in those patterns. It is conceivable that this pattern separation process is essential in determining the intrahippocampal representation of the environment. The match–mismatch process is also likely to be affected by such a pattern separation process; that is, a more orthogonalized representation of the changed environment is likely to facilitate the detection of a mismatch.

In the present study, using an object exploration paradigm (Save, Poucet, Foreman, & Buhot, 1992), we tested the behavioral effects of lesions in different subregions of the dorsal hippocampus to gain insights into the differential roles of those subregions in the match–mismatch process. We predicted that CA3 would play a pivotal role in the match–mismatch process, because previous studies (Lee & Kesner, 2002, 2003; Nakazawa et al., 2003) have suggested that CA3 is important for rapid formation of spatial or contextual memory (especially in the acquisition of novel information). Without a rapid memory formation of the surrounding environment in CA3, it may be difficult to perform a match–mismatch process in the hippocampus, mainly because of a lack of a concrete representation of the most recently experienced environment to which the sensory information of the current environment can be compared. We also hypothesized that, for CA3 to form a representation of the environment rapidly, the role of DG as a pattern separator would be essential (Gilbert, Kesner, & Lee, 2001; Kesner, Gilbert, & Wallenstein, 2000). Without such "filtering" by DG of neocortical inputs to CA3, there would be too much interference in CA3, as a result of the highly recurrent connections within CA3. Therefore, we also predicted that DG lesions would disrupt the match–mismatch process in the hippocampus.

Natural exploratory behavior of an animal has served as an important behavioral paradigm to assess the match–mismatch process (Malleret et al., 2001; Roullet, Sargolini, Oliverio, & Mele,

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2001; Save et al., 1992). Normal animals tend to quickly notice and respond to changes in their environment (e.g., changes in the locations of objects), usually by increasing their exploratory behavior selectively for the changed stimuli, compared to unchanged ones. However, animals with dorsal hippocampal lesions fail to exhibit this natural tendency, suggesting a crucial role of the hippocampus in processing environmental changes (Save et al., 1992). Axon-sparing neurotoxic lesions were produced in different hippocampal subregions (i.e., DG, CA1, or CA3) in the current study. The results suggest that the DG–CA3 network plays a key role in processing novel spatial information, but not novel object information.

Method

Subjects

Forty-one male Long–Evans rats (300–400 g) were housed individually in standard rodent cages. They were maintained on a 12-hr light–dark cycle. All behavioral experiments were performed during the light phase of the light–dark cycle. Each rat was allowed access to water and food ad libitum during the 1-week pretesting period.

Behavioral Apparatus

The behavioral apparatus was an open, circular platform (i.e., a dry land version of the Morris water maze; Gilbert, Kesner, & DeCoteau, 1998). The platform was painted white and elevated 65 cm from the floor. The surface of the apparatus was 119.0 cm in diameter and 3.5 cm in thickness. One hundred seventy-seven food wells (each 2.5 cm in diameter and 1.5 cm in depth) were originally drilled into the surface of the platform in evenly spaced parallel rows and columns 2 cm apart. However, these holes were covered by a green, heavy-duty curtain draped over the platform to prevent the rats from being distracted by the holes. Rats were not able to feel the holes through the heavy-duty curtain on the surface of the platform. The platform was kept in a well-lit room with one door, a chair, a long shelf on one wall, and different posters of various sizes placed on the walls. Numerous visual cues (e.g., toys) were present on the shelf. A black start box (24 cm long × 15 cm wide x 17 cm high) was used to introduce the subjects to the platform. Rats were kept in the box during an intersession interval outside of the testing room. A video camera was attached to the ceiling of the room to capture the entire platform and was connected to both a VCR and a TV monitor in an adjacent room for recording behavior.

Six different toy objects were used for the object exploration paradigm: Object A, a green rubber frog (10 cm × 6 cm); Object B, a green toy figure (7 cm × 4 cm); Object C, a yellow rubber duck (9 cm × 5 cm); Object D, a yellow plastic vertical bar (11.0 cm × 1.5 cm); Object E, a white wooden bear (6 cm × 4 cm); and Object F, a sky-blue plastic cup (10 cm × 7 cm). Objects B and D were attached to circular metal bases (4 cm in diameter) to support the objects on the platform. A cork cap was attached to the bottom of each object and plugged into the hole of the circular platform to further ensure secure placement of the objects and prevent the rats from knocking them over during exploration. Five objects (i.e., Object A to Object E) were present on the platform during Sessions 2–6, and Object A was replaced by Object F in Session 7 (see Figure 1). The objects maintained a similar spatial configuration that had been used for a previous study (Figure 1 in Save et al., 1992).

Surgery

Rats were randomly assigned to receive neurotoxic lesions in different subregions of the dorsal hippocampus. Each subject was injected with atropine sulfate (0.2 mg/kg ip) and deeply anesthetized with sodium pentobarbital (Nembutal; 60 mg/kg ip). The rat was placed in a stereotaxic instrument (David Kopf Instruments, Tujunga, CA), and an incision was made along the midline of the scalp. The skull was exposed, and the instrument was adjusted to ensure a flat skull surface. Small burr holes were drilled on the skull for the following coordinates: DG lesion group—(a) 2.7 mm posterior to bregma, 2.1 mm lateral to midline, and 3.4 mm ventral from dura, and (b) 3.7 mm posterior to bregma, 2.3 mm lateral to midline, and 3.0 mm ventral from dura; CA1 lesion group—3.6 mm posterior to bregma, 1.0, 2.0, and 3.0 mm lateral to midline, and 1.9 mm ventral from dura; CA3 lesion group—(a) 2.5 mm posterior to bregma, 2.6 mm lateral to midline, and 3.2 mm ventral from dura, (b) 3.3 mm posterior to bregma, 3.3 mm lateral to midline, and 3.2 mm ventral from dura, and (c) 4.1 mm posterior to bregma, 4.2 mm lateral to midline, and 3.1 mm ventral from dura.

Neurotoxins were infused into different subregions of the dorsal hippocampus to produce axon-sparing, subregion-specific lesions. Colchicine (2.5 mg/ml, 0.6 μl/site, 0.2 μl/min; Sigma-Aldrich, St. Louis, MO) was used for bilateral DG lesions ($n = 5$) for its well-known specificity for DG lesion (Emerich & Walsh, 1989; Mundy & Tilson, 1990; Walsh, Schulz, Tilson, & Schmechel, 1986). Ibotenic acid was used for both CA3 and CA1 lesions ($n = 12$ for CA3 and $N = 9$ for CA1; Sigma-Aldrich). For CA1 lesions, ibotenic acid (6 mg/ml, 0.1–0.15 μl/site for three sites per hemisphere, 0.07 μl/min) was slowly infused into pyramidal cell layers of the CA1 region to have both septotemporal and mediolateral spread in the

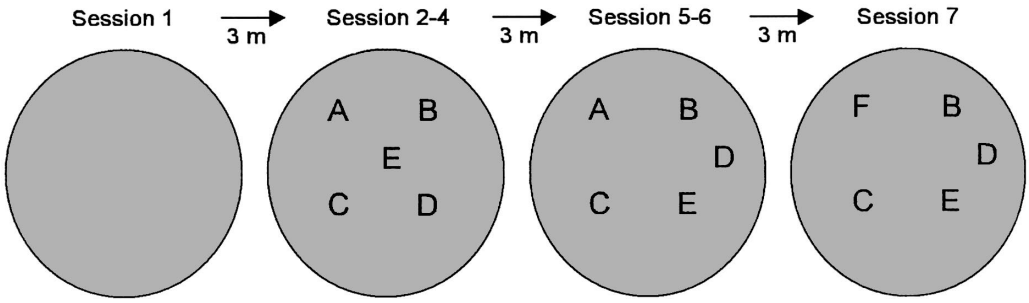

Figure 1. A schematic illustration of the configurations of objects in the object exploration paradigm. Letters A, B, C, D, E, and F represent the six different objects used throughout the experiment. Each 6-min session was followed by a 3-min (3 m) intersession interval (not shown between Sessions 2 and 4 and between Sessions 5 and 6). No object was available in Session 1. The configuration of the five objects introduced in Session 2 was maintained until two objects (Objects D and E) were moved to different locations in Session 5. A novel object (Object F) replaced Object A in Session 7.

dorsal CA1 region. For CA3 lesions, ibotenic acid (6 mg/ml) was injected into three different dorsal CA3 regions per hemisphere (0.1–0.2 μl/site for three sites per hemisphere, 0.07 μl/min). All injections were made with a 10-μl Hamilton (Reno, NV) syringe with a microinjection pump (Cole Parmer Instrument Company, Vernon Hill, IL). Vehicle solution (phosphate-buffered saline [PBS]) was injected into the corresponding subregions to produce control lesions ($n = 5$ for DG, $n = 5$ for CA1, $n = 5$ for CA3).

The rats were given 7 days for recovery after surgery. Some of the subjects included in the current study were previously tested for approximately 3 weeks in different behavioral paradigms (i.e., spatial working memory task in Lee & Kesner, 2003; Hebb-Williams navigation task in Lee & Kesner, 2004a). The data from those rats were not significantly different from the data from those that had not been exposed to other tasks. All animal care and experimental procedures conformed to National Institutes of Health and Society for Neuroscience guidelines for care and use of experimental animals.

Behavioral Procedure

The same behavioral procedure used for the previous study (Save et al., 1992) was adopted for the current study. Each rat was placed in the start box and was introduced to the platform to start the first session (Session 1). No object was present on the platform for Session 1. The start box was removed from the room by an experimenter as soon as the rat exited the box. Each session lasted for 6 min, and there was a 3-min intersession interval. The rat's behavior was recorded on videotape for offline analysis. The circular area of the monitor screen corresponding to the platform was divided into nine grid zones. The activity level of each rat was measured by counting the crossover between the grid zones during each session (i.e., crossing a boundary line between grid zones with both hind paws), and the crossover scores were averaged for all the sessions. The experimenter entered the room with the start box at the conclusion of a session and placed the rat in the box to start a 3-min intersession interval before the next session started. The box containing the rat was placed outside of the testing room during the intersession interval, during which the cover of the box prevented the rat from viewing the outside environment.

During the first 3-min intersession interval after Session 1, five objects (A-E) were arranged on the platform (Figure 1), following the same configuration adopted from the previous study (Save et al., 1992). Four objects (A-D) were arranged as a square-shaped configuration (50 × 50 cm), the approximate center of which was occupied by Object E. The rat was then introduced to the platform to start the exploration of the objects in Session 2. The amount of exploration for each object was measured by monitoring the contact duration of the rat's snout with the object. An observer who was blind to the lesion group information of the rats performed the scoring. The timer of the VCR displayed on the monitor was recorded with all behavioral sessions, which provided fairly accurate measurement of the object exploration amount during the offline analysis. One second was given as a minimal contact-time score if the rat exhibited contact time less than 1 s (determining the onsets and offsets at millisecond resolution was difficult using the current offline analysis method). The same procedure was used for Sessions 3–4 with the same configuration of the objects (Figure 1). For Sessions 5–6, however, Object E was moved to the position of Object D, and Object D was displaced to a new location outside the square-shape configuration to make a polygon-shaped configuration of the objects (Figure 1). For the last session (Session 7), the same polygon-shaped configuration was maintained, but a new object (i.e., Object F) replaced Object A (Figure 1).

Data Analysis

Locomotor activity was measured by the number of grid crossings for each lesion group across the seven sessions and the session scores were blocked on the basis of the experimental manipulations (i.e., Block 1 = Session 1; Block 2 = Sessions 2–4; Block 3 = Sessions 5–6; Block 4 = Session 7). To measure the amount of habituation, we calculated a difference score by subtracting the time spent for exploring each object in Session 4 from the time spent for the same object during Session 2. A habituation index was then the average of the difference scores for all five objects between Sessions 2 and 4. A spatial mismatch index was calculated to quantify the amount of exploration for the objects moved to different locations from their original locations; the sum of the exploration time for each of the displaced objects (i.e., Object D and Object E) during Sessions 3–4 was subtracted from the sum of the exploration time for those objects during Sessions 5–6 (higher score thus indicating more exploration of the displaced objects). The spatial mismatch index was also calculated similarly for the nondisplaced objects. We calculated an object mismatch index by subtracting the average exploration time of the other four objects that were not changed (Objects B-E) from the time spent exploring the newly introduced object during Session 7 (Object F).

A one-way analysis of variance (ANOVA) was used to compare different lesion effects on the habituation index or the object mismatch index. An ANOVA was performed for the spatial mismatch index, separately for the displaced or nondisplaced object, during Sessions 2–4 with lesion group as the between-subjects variable. The activity level was compared among the groups by an ANOVA with lesion group as a between-subjects variable and blocked session as a within-subject variable. A post hoc comparison (Newman-Keuls) was performed when necessary.

Histology

Each rat was deeply anesthetized with an intraperitoneal injection of 1.5 ml sodium pentobarbital (70 mg/kg), and perfused intracardially with normal saline followed by a 10% (wt/vol) formalin solution. The brain was removed from the skull and stored in a 10% formalin-30% (wt/vol) sucrose solution in a refrigerator (4 °C) for 72 hr to equalize shrinkage rate of tissues across brains. A tissue block containing the dorsal hippocampus was cut perpendicularly from each brain. The blocks were then frozen and cut at 40-μm sections, and every third section was mounted on glass slides. The sections were stained with cresyl violet for histological verification of the lesions. A 3-D volumetric analysis was performed as described previously (Lee & Kesner, 2003, 2004a, 2004b) to quantify the lesion amounts. Briefly, the sections in the dorsal hippocampus (bregma 2.0 to 4.2) were projected onto tracing paper by means of a microslide projector (final magnification 30×), and the boundaries of principal cell layers were traced in CA1, CA3, and DG by an experimenter who was blind to the lesion group. After the tracing, aligned serial sections were converted into two-dimensional digital images. The images were then imported into a 3-D reconstruction software package (Voxwin 1.2.2; Voxar, Edinburgh, U.K.) for 3-D reconstruction and volumetry. The percent damage to each subregion of the hippocampus was calculated by comparing the average number of voxels composing each subregion of the control group with the average number of voxels in the corresponding subregion of each lesion group in the dorsal hippocampus.

Results

Lesions

Colchicine-induced lesions in DG of the hippocampus produce severe damage in the granule cells of DG, while minimally affecting pyramidal cells in CA1 and CA3 (Emerich & Walsh, 1989; Mundy & Tilson, 1990; Walsh et al., 1986; Xavier, Oliveira-Filho, & Santos, 1999). The DG lesion protocol used for the current study has been shown to be effective in eliminating granule cells in DG (Lee & Kesner, 2003, 2004a, 2004b). Microscopic verifications of Nissl-stained sections confirmed the previous observation. Granule

cells in the dorsal DG area were almost completely eliminated (97% reduction in volume compared with the control group; see Figure 2b). In addition, the cells in the hilar region were also damaged by the colchicine injections, presumably as a consequence of the granule cell loss. There was a little damage in CA3 pyramidal cells (18%), and there was a thinning of pyramidal cell layers in CA1 (19%). It is unknown whether this thinning of CA1 pyramidal cell layers was produced by the action of colchicine on CA1 as well as on DG, or was a by-product of cell loss in DG/hilar areas. Previous literature has shown that the colchicine-induced DG lesion spares the direct perforant path input into CA1 and CA3 (Xavier et al., 1999). On the contrary, DG lesions eliminate the mossy fiber connections between DG and CA3 almost completely (Xavier et al., 1999).

Ibotenic acid was used to produce lesions in pyramidal cells in CA1 or CA3. Ibotenic acid has been well known for its axon-sparing excitotoxicity (Jarrard, 1989). Because neither CA1- or CA3-selective neurotoxin is currently available, we have developed injection parameters suitable to induce subregion-specific lesions with ibotenic acid, which has produced fairly selective damage to either CA1 or CA3 (Lee & Kesner, 2003, 2004b). As shown in Figure 2c, the ibotenic acid injections into CA1 produced almost complete removal of the pyramidal cells in CA1 (84%), while saving most of the pyramidal cells in CA3 and granule cells in DG (90–95% intact). In some sections, there was still some damage of granule cells from downward spread of ibotenic acid while it was injected into CA1. Some cells in CA2 were also affected in some sections. Ibotenic acid injected into CA3 eliminated most pyramidal cells in CA3 (81%; Figure 2d). Some damage in CA1 pyramidal cells close to CA2 was observed in some sections. The lesioned pyramidal cell layers were heavily infiltrated by glial cells, thus still allowing the identification of pyramidal cell layers in most sections. On the basis of microscopic observation on Nissl-stained sections, both ibotenic acid and colchicine lesions produced very little damage in other extrahippocampal areas of the brain, including the entorhinal cortex and the subiculum.

Although the volumetric analysis was performed only within a certain region of the hippocampus (i.e., dorsal hippocampus), there was some damage outside of the analyzed zone. Specifically, ~15% of the granule cells were damaged in the DG lesion group beyond the zone of analysis, with minimal damage in the pyramidal cells in CA1 and CA3 (~5%). In the CA3 lesion group, ~30% of pyramidal cells in CA3, ~25% of CA1 pyramidal cells, and ~15% of DG granule cells were also damaged. The CA1 lesion group exhibited ~10% damage in CA1 pyramidal cells outside the zone of analysis, with little additional damage in other cell layers in CA3 and DG (~2%). However, the number of lesions outside the main zone of volumetric analysis was relatively more variable among the rats. It has been repeatedly shown that the hippocampus closer to the septal pole is more important for spatial cognition (Bannerman et al., 2004; Moser & Moser, 1998). Therefore, it is less likely that the additional damage in subregions toward the temporal pole of the hippocampus contributed significantly to the main results of the current study.

Session 1: Locomotor Activity

Figure 3a shows locomotor activity (measured by grid crossings) for each lesion group across sessions throughout the experiment. The different control lesion groups were combined into one control group, because there were no significant differences among the groups ($p > .10$). The rats generally decreased their level of activity across sessions, demonstrating habituation to the environment as they were exposed to the same or similar environment repeatedly. A regression analysis showed that the slope for each lesion group was significantly negative ($p < .0001$ for the control, CA1, and CA3 lesion groups; $p < .001$ for the DG lesion group), and there were no significant differences among the groups with respect to the slope of the regression lines ($p > .50$; analysis of covariance). The results also indicate that subregional lesions produced increased locomotor activity on the platform (Figure 3a). The increased locomotor activity was especially observed during the earlier sessions, and the lesions produced in CA1 or DG especially resulted in hyperactivity in earlier sessions. An ANOVA for a repeated measures design showed that there was a highly significant effect of blocks, $F(3, 105) = 131.9$, $p < .0001$, and the interaction between groups and blocks, $F(9, 105) = 2.7$, $p =$

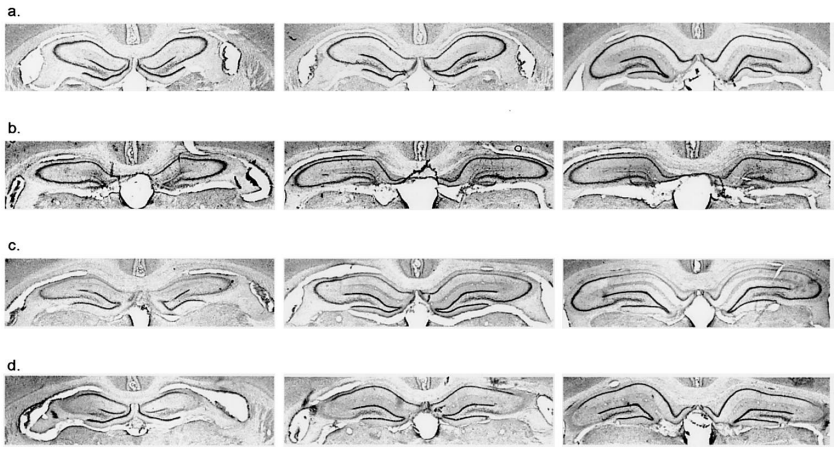

Figure 2. Subregional lesions in the dorsal hippocampus. Three coronal sections along the septotemporal axis from the control (a), dentate gyrus (b), CA1 (c), and CA3 (d) lesion groups are shown.

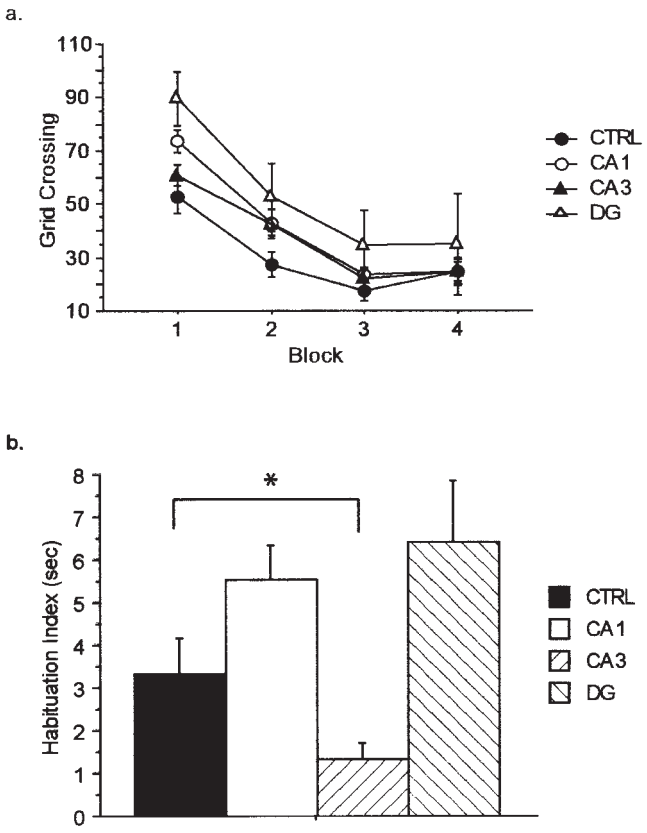

Figure 3. a: Habituation of different subregional lesion groups, measured by the decrease in locomotor activity across blocks (Block 1: Session 1; Block 2: Sessions 2, 3, and 4; Block 3: Sessions 5 and 6; and Block 4: Session 7). Note the similarly decreasing activity curves among the groups. b: Habituation measured by the decrease in the amount of exploration of the same objects between Sessions 2 and 4. Error bars represent standard error. CTRL = control; DG = dentate gyrus. *$p < .01$

.0074. The effect of lesion groups failed to reach significance, $F(3, 35) = 2.3$, $p = .099$. A Newman-Keuls post hoc test revealed that the DG- and CA1-lesioned rats were significantly more active than controls for Blocks 1 and 2 ($p < .001$). The CA1-lesioned rats were not significantly different from controls for Blocks 3 and 4. The DG-lesioned rats were more active than controls for Block 3 ($p < .001$), but were not significantly different from controls for Block 4.

Sessions 2–4: Habituation to Objects

Although the activity level measured by grid crossing reflects general habituation of the rats to the environment, it does not show that the habituation specifically occurred to the objects in the environment. The habituation index was calculated by comparing the time spent for exploring the objects when they were first introduced in Session 2 with the time spent in reexploring the same objects in Session 4 after multiple exposures to the objects (Figure 3b). The larger the habituation index, the greater the habituation. A one-sample t test showed that all the lesion groups and controls significantly increased habituation to the same objects between Sessions 2 and 4 ($p < .001$). A one-way ANOVA for the habituation index showed significant differences among the lesion groups in the habituation index, $F(3, 201) = 11.6$, $p < .0001$. However, a post hoc test (Newman-Keuls) showed that only the DG lesion group was significantly different from the control group by increasing habituation to the same objects in the environment ($p < .01$).

Sessions 5–6: Detection of Spatial Novelty

In Session 5, two of the objects (i.e., Objects D and E) were removed from their original locations and were placed in different locations on the platform (Figure 1). Those displaced objects were maintained in the changed locations in Sessions 6 and 7. Such spatial manipulation did not affect the amount of reexploration for nondisplaced objects (i.e., Objects A, B, and C) in most lesion groups (see Figure 4a); a one-sample t test for each group showed that only the rats with CA3 lesions significantly decreased the amount of reexploration for nondisplaced objects ($p < .001$), compared to rats with other subregional lesions and controls ($p > .1$). However, although the CA3 lesion group showed a significant decrease in reexploring the nondisplaced objects, an ANOVA showed no significant differences among the groups, $F(3, 119) = 2.5$, $p > .06$.

In contrast to the minimal effects of the subregional lesions on reexploration of the nondisplaced objects described above, there were striking effects of different subregional lesions on reexploration of the displaced objects (Figure 4b). A one-sample t test showed that the control group demonstrated a significantly increased reexploration for the displaced objects ($p < .01$), whereas the CA3 and DG lesion groups exhibited significantly reduced reexploration for those objects ($p < .01$, CA3; $p < .05$, DG). The CA1 lesion group, however, showed no change in reexploring the displaced objects ($p > .4$), with the spatial mismatch index at around zero. A one-way ANOVA revealed highly significant differences among different groups in reexploring the displaced objects, $F(3, 78) = 8.7$, $p < .0001$. A post hoc test (Newman-Keuls) produced highly significant differences between the CA3 lesion group and controls ($p < .001$), and between the DG lesion group and controls ($p < .001$). The CA1 lesion group appeared to exhibit a more reduced amount of reexploration for the displaced objects compared with controls, and the Newman-Keuls post hoc test showed a significant difference between the CA1 lesion group and the control group ($p < .05$; see Discussion). In sum, rats with subregional lesions in the hippocampus (especially DG and CA3 lesions) showed a pronounced deficit, compared with controls, in reexploring the familiar objects moved to novel locations in the environment.

On the basis of the spatial mismatch index separately calculated for each of the displaced objects (i.e., Objects D and E; Figure 4c), the significance of the differences among the lesion groups largely originated from reexploring Object D (the object that was moved to a completely novel location that had never been occupied by any object; Figure 1). The control group showed a significant increase in reexploring Object D ($p < .01$). An ANOVA with lesion group as the between-subjects variable and the two objects (Object D and E) as the within-subject variable showed significant effects of lesion group, $F(3, 37) = 5.8$, $p = .002$, and the Lesion $\times$ Object interaction, $F(3, 37) = 4.7$, $p = .007$. A Newman-Keuls post hoc test for Object D revealed that the DG and CA3 lesion groups did

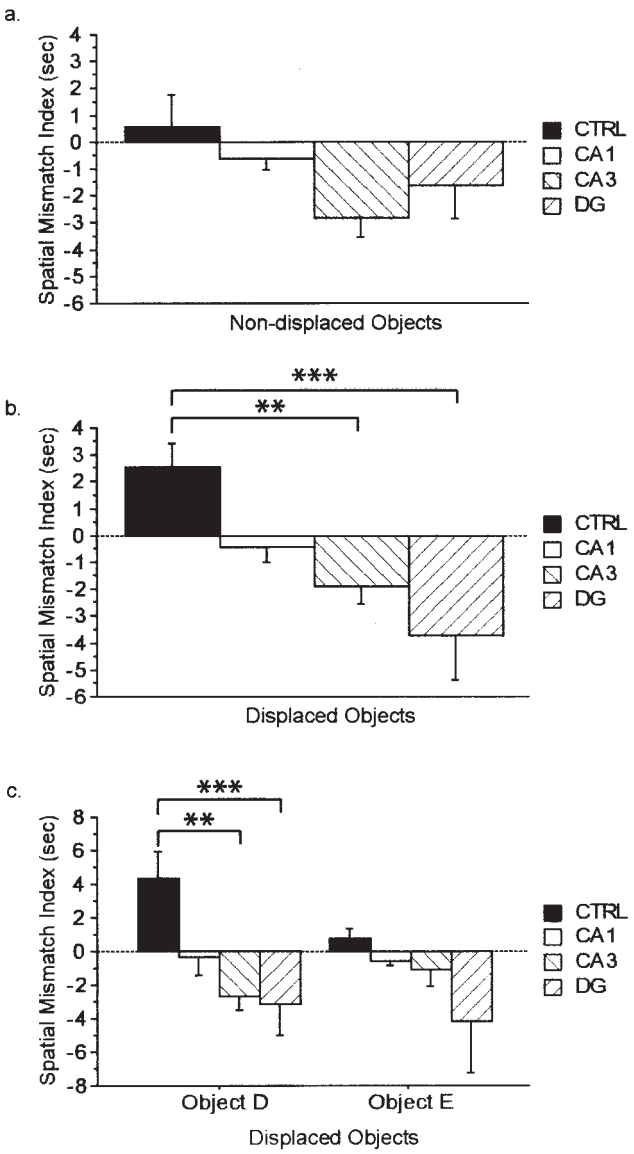

Figure 4. Spatial novelty detection. a: Spatial mismatch index for the nondisplaced objects. b and c: Spatial mismatch index for the displaced objects. Object D was moved to a new location previously unoccupied by any object, and Object E was moved to the previous location of Object D in Sessions 5 and 6. Error bars represent standard error. CTRL = control; DG = dentate gyrus. $^{**}p < .002$ (CTRL vs. CA3); $^{**}p < .001$ (CTRL vs. DG).

not show a significant increase in exploratory behavior relative to controls ($p < .05$), whereas CA1 was comparable to the control group. In contrast, a one-sample t test showed no significant change in reexploring Object E in any group, including controls ($p > .1$).

Session 7: Detection of Object Change

Figure 5 shows the object mismatch index calculated by subtracting the average time spent for reexploring the four objects that were not changed in the same session from the time spent for exploring a new Object F during Session 7. The results indicated that none of the lesion groups showed a deficit in exploring the novel object introduced to a familiar location during Session 7. A one-way ANOVA showed no significant group effect, $F(3, 36) = 0.6$, $p > .50$. The result suggests that none of the hippocampal subregions included in our study is involved in recognizing a novel object when only the identity information of the individual item is changed, with no alteration in the spatial information associated with that item.

Discussion

The current study investigated the role of the hippocampus in a possible match–mismatch process for spatial and object information, based on a hippocampal subregional analysis. The main results are as follows: The overall habituation rates to the familiar environment were similar among the lesion groups. The pattern of exploratory behavior, however, was different among the lesion groups when spatial information associated with some objects in the environment was changed; the lesions in DG or CA3 produced a significant impairment in detecting spatial novelty, whereas the effect of CA1 lesions was modest. In contrast, there was no significant deficit in reexploring the objects that maintained their original locations. Lesions in any of the three hippocampal subregions did not affect the detection of a novel object placed in a familiar location.

The dissociation of the DG and CA3 subregions from CA1 is noteworthy with respect to their roles in detecting spatial changes in the environment. Some hippocampal models suggest CA3 as a potential comparator for detecting novelty (Mizumori, Ragozzino, Cooper, & Leutgeb, 1999; Vinogradova, 2001). These models focus on a possible match–mismatch process associated with CA3, based either on its capability of monitoring information both from DG and from the entorhinal cortex via the perforant path (Mizumori et al., 1999) or on its unique anatomical position of receiving both cortical and subcortical signals (Vinogradova, 2001). Those models could explain the deficit of the CA3 lesion group in reexploring displaced objects in our study. Because the rats with DG lesions were also severely impaired in reexploring the displaced objects, DG may support a mechanism through which

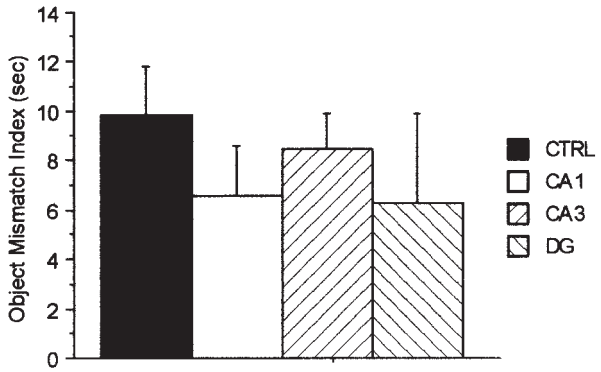

Figure 5. Object novelty detection measured by the reexploration amount for a new Object F that replaced the Object A in Session 7. Note the similar amounts of exploration of the Object F by the different lesion groups. Error bars represent standard error. CTRL = control; DG = dentate gyrus.

sensory inputs can be represented in CA3 without interfering with the memory representations already established in the CA3 network. This could be accomplished by a pattern separation process in DG, which would attenuate the interference in CA3 whenever new sensory inputs arrive. This may result in the slightly facilitated habituation displayed in the DG lesion group in our study (Figure 3b), as the pattern completion process may override the pattern separation process in the CA3 network in the absence of DG, thus increasing the possibility of recognizing different environments as similar. In sum, both DG and CA3 seem essential in monitoring and responding to spatial changes in the environment.

Computational models have also emphasized CA1 as a critical hippocampal subregion where the match–mismatch computation may occur (Hasselmo, Fransen, Dickson, & Alonso, 2000; Lisman & Otmakhova, 2001; McClelland, McNaughton, & O'Reilly, 1995). According to those models, a comparison occurs in CA1 between the direct inputs from the entorhinal cortex and the representations coming from CA3. As a result of this comparative function, CA1 may detect mismatch between the internal representation and the external environment whenever there is a significant change in the environment. However, our data do not provide an unconditional support for such a hypothesis. Instead, CA3 and DG seem more important in detecting novel spatial information, as described above. The spatial mismatch index of the CA1 lesion group remained at around zero for the displaced objects, which suggests that a match–mismatch computation possibly occurred in the hippocampal system (possibly in the DG–CA3 network). In the CA1 lesion group, when the rats encountered the displaced objects, it is possible that the match–mismatch system functioned just enough to release the system from the habituation mode (thus allowing the similar amount of exploration shown during the previous session). However, the mismatch signal might not be expressed fully (possibly via CA1) to activate an increased reexploration of the environment in the CA1 lesion group, as CA1 lesions block cortical outputs of the hippocampus and leave only subcortical outputs from CA3. Fyhn, Molden, Hollup, Moser, and Moser (2002) showed that principal cells in CA1 changed their firing rates when a novel platform became available in an annular water maze task. It is difficult to know from their study, however, whether the firing rate changes in CA1 reflected a mismatch signal from an upstream network (i.e., DG–CA3) or whether mismatch signals were actually generated in CA1. If the signals were generated in CA1, it is possible that a more demanding memory paradigm such as the water maze task may activate the match–mismatch process in CA1, versus a natural exploration paradigm used in the current study. Testing animals with CA1 lesions in more demanding memory tasks that require mismatch detection seems necessary to address the issue.

It is possible that comparing a sensory environment with "recently" acquired representations (e.g., minutes old) may depend on the DG–CA3 network, whereas CA1 is more involved in comparing the current environment with the representations that have been subject to long-term consolidation (e.g., for hours or days); the behavioral paradigm used in the present study allowed only 3-min intersession intervals. Supporting such a possibility, previous studies reported a selectively increased level of a *c-fos*-related protein, that is, Fos, in CA1 as a result of the presentation of familiar items arranged in novel configurations (Jenkins, Amin, Pearce, Brown, & Aggleton, 2004; Wan, Aggleton, & Brown, 1999). It is important to note that, in those studies, the subjects had long-term resting periods before they were sacrificed after the behavioral experiment. Furthermore, in Jenkins and colleagues' study (2004), rats were overtrained with familiar stimuli, and long-term intersession intervals (2 hr) were imposed between sessions, during which a consolidation might occur in CA1. Those conditions might have produced a situation wherein a comparison between a novel spatial configuration of stimuli and a long-term representation of the environment occurred (however, a control experiment with a shorter survival time is necessary to verify such subregional dynamics). Furthermore, previous literature (Lee & Kesner, 2002; Shimizu, Tang, Rampon, & Tsien, 2000) suggests that the NMDA receptors in CA1 may play an important role in long-term consolidation of acquired information. At the physiological level, the firing patterns of principal cells in CA1 in a spatial environment are reactivated during sleep, presumably reflecting the consolidation process (Louie & Wilson, 2001; Wilson & McNaughton, 1994). In sum, CA1 may still function as a match–mismatch processor. However, the previous experimental literature described above suggests that CA1 is critical in comparing the current environment with relatively "older" memories and that the DG–CA3 network is more critical in performing the match–mismatch process at a shorter time scale (see also Lee, Rao, & Knierim, 2004).

If the DG–CA3 network is essential in detecting spatial novelty, CA1 lesions should lead to similar deficits, because CA1 has been considered to receive CA3 outputs and send the major hippocampal efferents to the cortex (Andersen, Blackstad, & Lomo, 1966). However, a dissociation was observed in our data between the CA1 and CA3 lesion groups in detecting spatial novelty. Such a dissociation between CA1 and CA3 has been reported repeatedly in previous lesion studies using other behavioral paradigms (Lee & Kesner, 2002, 2003, 2004b). It may reflect merely insufficient lesions or inactivation in the CA1 subregion; that is, the intact CA1 cells after the lesions in CA1 might still convey the CA3 outputs to cortical areas. However, the dissociation between CA1 and CA3 is also observed at the physiological level in normal animals (Lee et al., 2004), which suggests that CA1 does not passively convey the information represented in CA3 to the neocortex. Therefore, a simplistic view of CA1 as a relay station for CA3 needs to be reconsidered. Instead, CA3 and CA1 may be able to function independently, and the communication between those two subregions may be determined by certain conditions, possibly controlled by dynamic modulatory signals (e.g., cholinergic signals from the septum; Hasselmo, 1999; Vinogradova, 2001). Most researchers have neglected the CA3 efferents to the brain stem circuits via the lateral septum, and only its cortical outputs via CA1 have been emphasized when discussing the function of the hippocampal subregions (but see Vinogradova, 2001). The CA3 may convey novelty information in certain conditions to control the motivation level of the animals to explore the environment, independent of the hippocampal outputs to the cortex via CA1 (Vinogradova, 2001).

The present results demonstrate that the dorsal hippocampal subregions (i.e., DG and CA3 in the current experiment) are more specialized for detecting spatial changes than changes in objects in the environment, consistent with the result from the previous study with dorsal hippocampal lesions (Save, Poucet, Foreman, & Buhot, 1992). That is, no subregion lesion group (i.e., CA1, CA3, or DG) showed a deficit in detecting the novel object (i.e., Object F)

that replaced one of the familiar objects (i.e., Object A). This result disproves the possibility that the deficit in detecting the displaced objects in the DG and CA3 lesion groups might be due to a decrease in the natural tendency to explore novelty, because the rats were normal in exploring the novel object introduced to the environment.

Although the DG and CA3 lesion groups were impaired in detecting the changes in locations of familiar objects, they were normal in habituating to the repeated spatial environment throughout the experiment. Considering both the impaired detection of spatial changes and the generally intact habituation, we suggest that the habituation shown in the subregion lesion groups may be due to a mechanism that is not dependent on the hippocampal function in representing the stimuli in an environment (e.g., elemental coding scheme compared to a configural or relational coding strategy; Fanselow, 2000; Marr, 1971; O'Reilly & McClelland, 1994); that is, hippocampal lesions may drive the system to process the items in the environment individually rather than to process configural relationships among stimuli.

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