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# The CA3 Subregion of the Hippocampus Is Critical for Episodic Memory Processing by Means of Relational Encoding in Rats

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This experiment tested the theory that the CA3 subregion of the hippocampus mediates episodic learning of arbitrary associations. The authors developed 2 tasks based on the episodic flavor-place paired-associate task described by M. Day, R. Langston, and R. G. Morris (2003): an object-cued spatial location recall task and a spatial location–cued object recall task. After rats were trained to a criterion of 80% correct on 1 of the 2 tasks, they received either a dorsal CA3 lesion or a vehicle control lesion. Control animals continued performing well on both tasks. Rats with lesions to dorsal CA3 were impaired on both tasks and performed at chance but were able to perform a nonepisodic version of the task as a control. These data suggest that CA3 mediates episodic learning of arbitrary associations as tested in the 1-trial object-cued spatial location recall and spatial location–cued object recall tasks.

*Keywords:* CA3, hippocampus, arbitrary association, paired-associate learning, episodic memory

The major aim of this study was to investigate whether the CA3 subregion of the hippocampus is important for the mediation of arbitrary associations in an episodic memory task based on rapid (one-trial) acquisition of novel information. David Marr (1971) proposed that the hippocampus should be capable of rapid formation of simple associations because of modifiable synaptic recurrent collateral connections among its neurons. In recent years, it has been proposed that the CA3 recurrent collateral system operates as an attractor network that may function to rapidly form such arbitrary associations (McNaughton & Morris, 1987; Rolls, 1989).

It has been suggested that the hippocampus and its subregions support the formation of arbitrary associations during paired-associate learning (Eichenbaum & Cohen, 2001). Computational models have suggested that the CA3 autoassociative network enables arbitrary associations to be formed and stored in the CA3 network. Subsequently, the extensive recurrent collateral connectivity in CA3 then allows for the efficient retrieval of the whole representation after exposure to a partial (or degraded) retrieval cue (Hasselmo & Wyble, 1997; McNaughton & Morris, 1987; Rolls, 1996; Rolls, in press; Rolls & Kesner, 2006). In this case, arbitrary associations refer to associations between items being formed regardless of type or class (e.g., space or object). Computationally, arbitrary associations are important in that they allow efficient and rapid recall of an association on the basis of cues from any of the elements of the association, not preferentially one over the other. For example, information from the parietal cortex regarding the location of an object may be associated with information from temporal cortex regarding the identity of an object (cf. Rolls, 1996). These two kinds of information may be projected to the CA3 region of the hippocampus via the medial and lateral perforant path projections from the entorhinal cortex to enable the organism to remember a particular location and an object, respectively (Breindl, Derrick, Rodriguez, & Martinez, 1994). Support for this idea comes from the observation that under the influence of direct infusions of D,L-2-2-amino-5-phosphonovalerate (APV; a *N*-methyl-D-aspartate [NMDA] antagonist) into dorsal CA3, which alters medial perforant path plasticity, or naloxone (a μ opiate antagonist), which alters lateral perforant path plasticity, there was a disruption of both novelty detection for a spatial location and novelty detection for objects (Hunsaker, Mooy, Swift, & Kesner, 2007).

In a previous study designed to directly test the involvement of the CA3 subregion of the dorsal hippocampus in spatial paired-associate learning over multiple trials, rats were trained on a successive discrimination task to examine object–place paired-associate learning. In this task, two paired associations were reinforced, consisting of one particular object in one particular location and a different object in a different location. Rats learned that if an object was presented in its paired location, then they should displace the object to receive a reward. However, rats should withhold from displacing the object if it is not in its paired location. Rats with CA3 lesions were impaired in learning object–place paired associations relative to controls as well as to animals with dentate gyrus or CA1 lesions (Gilbert & Kesner, 2003).

Even though the study mentioned above measured the acquisition of paired-associate information, it failed to address whether the learning is based on arbitrary associations, in part because the stimuli in the object–place task are integral and cannot be separated from each other. Furthermore, because the tasks are biconditional discrimination tasks, they are, according to Morris (2007) and O'Reilly and Rudy (2001), likely based on the learning of conjunctions of the cues that make up each association, implying that the cues cannot be separated from each other. In an ingenious

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experiment, Day et al. (2003) trained rats to learn an association between two flavors of food and two spatial locations during a study phase in a single trial. During a recall test phase, they were presented with a flavor that served as a cue for the selection of the correct location. They found that injections of an NMDA receptor antagonist (APV) or alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonist (6-cyano-7-nitroquinoxaline-2,3-dione, or CNQX) to the dorsal hippocampus before the study phase impaired encoding, but injections of APV before the test phase did not impair place recall, whereas injections of CNQX did. The interpretation is that hippocampal NMDA receptors are necessary for forming one-trial flavor–place associations and that recall can be performed without further involvement of NMDA receptors. Cuing the location to recall the flavor has not been tested, so one cannot be sure whether the hippocampus supports arbitrary associations on the basis of this set of experiments.

To examine the role of the CA3 subregion in subserving arbitrary associations in the context of episodic memory, we developed a pair of tasks based on the Day et al. (2003) experiment, namely an object-cued spatial location recall task and a spatial location–cued object recall task. Animals with lesions to dorsal CA3 showed similar deficits for both the object-cued spatial location recall task and the spatial location–cued object recall task, but were able to learn the same object–spatial location associations when presented repeatedly. These data provide the first direct evidence that the CA3 subregion of the hippocampus mediates episodic learning of arbitrary associations.

## Method

### *Subjects*

Eighteen male Long-Evans rats were used as subjects. Each rat was initially food deprived to 85% of its free-feeding weight and supplied with continuous access to water. Each rat was housed separately in a plastic cage and randomly assigned to either the CA3 lesion group ($n = 9$) or the control group ($n = 9$). Four rats in each group were used in the first task, and 5 rats in each group were used in the second.

### *Apparatus*

Each rat was trained and tested on a white cheeseboard maze (Gilbert & Kesner, 2003). The cheeseboard apparatus was 119 cm in diameter and 3.5 cm in thickness and stood 65 cm above the floor. There were 177 food wells drilled into the board in 15 parallel rows and columns evenly spaced 2 cm apart. Each hole was 2.5 cm in diameter and 1.5 cm deep. The training took place in a fully lit room with shelves, one chair for the experimenter, and posters on the wall, all of which served as distal cues for spatial location.

The start box used in the study was 38 cm long, 22 cm wide, and 17 cm high. The box was divided into two chambers by a guillotine door, which was raised and lowered manually, as was the opening door to the box. The front chamber of the start box was 13 cm long with a hole drilled into the bottom. This hole was then lined up with a food well on the cheeseboard. The front chamber was used only in keeping the cue object separated from the rat immediately preceding the test phase of each trial in the object–place cued-recall task.

The start box was positioned on the edge of the cheeseboard. Two fields of possible object placement were selected for the task, one in the left field and one in the right. Forty-eight wells, 24 on each side, made up these two fields and were separated from each other by three holes. Fifty possible objects were chosen for the project. Each object was between 4 to 12 cm tall and 2 to 6.5 cm wide. Each object was distinct and varied by width, texture, shape, and color. Each object was attached to a flat metal washer that was 4.5 cm in diameter and served as a base to completely cover the food well. Two identical items were used for the neutral objects in the test phase of each trial for the object-cued spatial location recall version, and one item was used in the spatial location–cued object recall version. The neutral objects were both cylinders 10 cm in height and 3.5 cm in diameter, were attached to washers in the same manner as the other objects, and were spray-painted black.

The task was designed such that no single object was seen more than once per day and was paired in such a way that no object–spatial location set would be repeated within 1 month during training. The locations were also set up to ensure that no single location was repeated within 10 trials, each location appeared with roughly equal frequency within a 1-month period, and the right and left positions were counterbalanced. During each trial, one object was located in the left field and one in the right.

### *Preoperative Training—Shaping*

An experimenter handled rats for 15 min daily for 2 weeks before experimentation to habituate the rat to human contact. Rats were trained to displace neutral objects for cereal (Froot Loop) reward. During this time, the rats were food deprived to 85% of their free-feeding weight. First, the object was placed behind the well; the rat simply had to approach the object, and the reward was available. As the rat moved more quickly to the object and consumed the reward, the object was slowly moved to increasingly cover the rewarded food well. Once the rat rapidly displaced the objects, the rat began the tasks.

### *Object-Cued Spatial Location Recall Task*

On the basis of the Day et al. (2003) experiment, we developed an object-cued spatial location recall task using the cheeseboard. In this task's study phase, rats were placed in the start box, and when the door in front of the start box was opened, the rats were allowed to displace 1 object in one location to consume a cereal reward. Then 10 s after returning to the start box, the door was opened again, and the rats were allowed to displace a 2nd object in another location to consume a reward (cf. Figure 1A). Fifty possible objects and 48 spatial locations were used. After a 15-s delay before the test phase, the rats were shown 1 object (first or second randomized) in the start box as a cue, and then after a 10-s delay, the door was opened and they were presented with 2 identical cylindrical objects that occupied the locations that had been occupied by the 2 objects experienced during the sample phases. Rats were rewarded for displacing the cylinder that occupied the location of the object that was presented as the retrieval cue, but not for displacing the other cylinder.

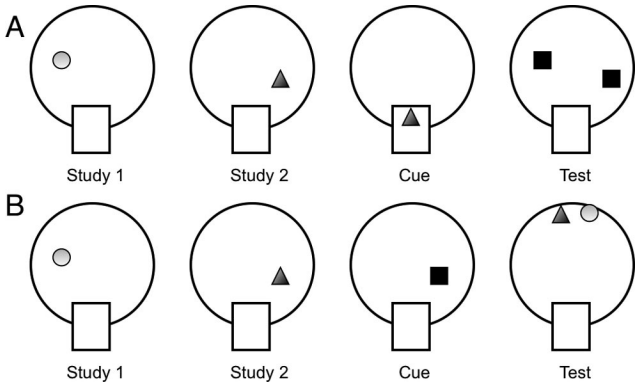


*Figure 1.* A: Object-cued spatial location recall. Each shape represents a different object, and the black squares represent spatial locations covered by neutral blocks. Each trial consisted of two study phases followed 15 s later by an object cue and 10 s later by a test between two previously experienced spatial locations. B: Spatial location–cued object recall. Each shape represents a different object. The black square represents the spatial location covered by a neutral block given as a cue. Each trial consisted of two study phases followed 15 s later by a spatial location cue and 10 s later by a test between two previously experienced objects.

Each subject was given a total of 10 trials per day with an intertrial interval of 60 s. Different objects and locations were used for the 10 trials within any given day (also randomized across days). All rats learned the task with 75% or better accuracy in about 200 trials (range = 160–240).

*Surgery.* After criterion was reached, each animal was randomly assigned to either receive a dorsal CA3 lesion ($n = 4$) or be in the control group ($n = 4$). Previous research has shown that selective lesions to the CA3 subregion can be successfully made (Gilbert & Kesner, 2003). Half of the animals were anesthetized with pentobarbital (Nembutal; 60 mg/kg ip), and the other half were anesthetized with isoflurane (2%–4% in 1–2 L/min medical air) because our lab converted from injected to inhaled anesthesia during the experiment. Each animal was then given atropine sulfate (0.2 mg/kg ip) and placed in a stereotaxic device (Kopf Instruments, Tujunga, CA). At this point, an incision was made following the midline of the skull, appropriate coordinates were taken, and the skull overlying the injection points was removed using a dental burr.

Each dorsal CA3 lesion consisted of six lesion points, three in each hemisphere. Ibotenic acid (6 mg/ml) was infused at a rate of 6 μl/h into each site via an injection cannula connected to a Hamilton syringe (Reno, NV) placed in a microinjection pump. The locations were (a) 2.8 mm posterior to bregma, 3.2 mm lateral to the midline, and 3.2 mm ventral from the dura mater (0.05 μl volume infused); (b) 3.3 mm posterior to bregma, 3.4 mm lateral to the midline, and 3.2 mm ventral from the dura mater (0.08 μl volume infused); and (c) 4.1 mm posterior to bregma, 4.2 mm lateral to midline, and 3.3 mm ventral from the dura mater (0.15 μl volume infused). The injection cannulas were left in place for at least 60 s after injections to allow for diffusion and spread of the drug before retraction. The control rats received a phosphate-buffered saline injection using the same volumes as with the ibotenic acid and at the same sites mentioned above. After surgery, rats were given a 1.5-mL injection of saline in each thigh subcutaneously to rehydrate them and Children's Tylenol (2 ml/50 ml water) as an analgesic.

*Postoperative testing.* After surgery, each rat was given a 2-week recovery period before testing was resumed. Each rat was then tested for 6 days, 10 trials per day, for a total of 60 trials. These trials were grouped into two sets of 3 consecutive days of training with 1 day of no training between sets. The postoperative testing was identical in nature to the preoperative training.

*Fixed object–cued spatial location recall task.* After completion of the postoperative trials, each rat was tested for 6 days on a fixed association task. The fixed task consisted of the same study and test phases as the regular task, but the object–location pairs were held constant across all 60 trials (i.e., the exact same two object–place associations were presented during the study phase of every trial). The nonepisodic fixed task was presented to verify that the animals could form object–spatial location associations over repeated trials.

## *Spatial Location–Cued Object Recall Task*

*Preoperative training—shaping.* The shaping procedure was identical to that described for the object-cued spatial location recall task.

*Spatial location–cued object recall.* To determine whether the CA3 region subserves arbitrary associations, another set of naïve rats were tested in a newly developed spatial location–cued object recall task. In this task, the study phase was the same as the previous task, with two object–location pairings presented in turn separated by 10 s. Again, 50 possible objects and 48 spatial locations were used. However, in this case after a 15-s delay during the test phase (cf. Figure 1B), when the door was opened the rat was allowed to displace a neutral object in one spatial location (first or second randomized) on the maze as a location cue, return to the start box, and after a 10-s delay, the door was opened and the rats had to select the correct object (choosing and displacing one of two objects that were located at the end of the cheeseboard perpendicular to the start box). The rats received a Froot Loop reward for selecting the correct object associated with the location cue. Each rat was given a total of 10 trials per day with an intertrial interval of 60 s and learned the task with 75% or better accuracy in about 200 trials (range = 150–270).

*Surgery.* After criterion was reached, each animal was randomly assigned to either receive a dorsal CA3 lesion ($n = 5$) or be in the control group ($n = 5$). All the animals were anesthetized with isoflurane (2–4% in 1–2 L/min medical air). The same surgical procedures were used as described for the object-cued spatial location recall task.

*Postoperative testing.* After surgery, each rat was given a 2-week recovery period before testing was resumed. Each subject was then tested for 6 days, 10 trials per day, for a total of 60 trials. These trials were grouped into two sets of 3 consecutive days of training with 1 day of no training between sets. The postoperative testing was identical in nature to the preoperative training.

*Fixed spatial location–cued object recall task.* After completion of the postoperative trials, each rat was tested for 6 days on a fixed association task. The fixed task consisted of the same study and test phases of the regular task, but in this task the object–location pairs were held constant across all 60 trials (i.e., the exact same two object–place associations were presented during the

study phases of every trial). The nonepisodic fixed task was presented to verify that the animals could form spatial location–object associations over repeated trials.

### *Histology*

After the testing was completed, every animal was deeply anesthetized using a 1.5-ml intraperitoneal injection of sodium pentobarbital (mixed at 60 mg/ml). At this point, the animal was perfused intracardially using a 10% formalin solution. The brain was then removed from the skull and stored in a 10% formalin/30% sucrose solution. The brain of each animal was frozen and cut into 24-μm sections from bregma to the posterior region of the hippocampus. Every third slice was taken and mounted on a glass slide. These sections were then stained with Cresyl violet and a histological examination of the lesion placement was made.

All sections were photographed and imported into ImageJ (version 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. The lesion region of interest (CA3) was 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) was calculated and recorded. The ratio of remaining stained pixels to the total area was calculated and used to compute the percentage of tissue unlesioned in the region of interest. This value was then used to ascertain percentage of damage (i.e., 100% − % stained tissue remaining = % tissue ablated). We calculated percentage of damage this way rather than by measuring unstained pixels to be as conservative as possible. We also measured damage to the other hippocampal subregions (CA1 and dentate gyrus) to verify that we did not obtain lesions from unintended spread of the neurotoxin. We also limited the analyses to the dorsal half of the hippocampus (cf. Bannerman et al., 2002; Moser & Moser, 1998). The percentage of damage for each animal was calculated and then averaged across animals.

## Results

### *Histology*

Schematic drawings of the largest (gray) and the smallest (black) of dorsal CA3 lesioned hippocampi are shown in Figure 2A, and photomicrographs of a representative lesion of dorsal CA3 are shown in Figure 2B. A quantitative analysis revealed that lesions to dorsal CA3 were approximately 90% complete, with sparing in dorsal CA3c and no cortical damage (>95% damage to dorsal CA3a and CA3b and approximately 55% damage to dorsal CA3c). There was only damage to the medial blade of the dentate gyrus in one case (5% damage to the dentate gyrus). There was no observed damage to dorsal CA1, but CA2 was usually about 50% ablated. There was no damage to the overlying cortex. In all lesions, there was some sparing mostly at the septal pole of the hippocampus, but the lesion could be verified from 2.3 to 4.3 mm posterior to bregma. There was no observed damage to the ventral 50% of the hippocampus, nor was there damage to postrhinal, perirhinal, entorhinal, or posterior parietal cortices. Also, it has previously been demonstrated that excitotoxic lesions to dorsal CA3 do not result in entorhinal cortex degeneration, but do result in reliable CA3 damage (Jerman, Kesner, Lee, & Berman, 2005).

### *Object-Cued Spatial Location Recall Task*

The results indicate that CA3-lesioned rats performed more poorly than controls and their own preoperative performance for the object-cued spatial location recall task (see Figure 3). Data for the object-cued recall for spatial location task were grouped for analysis into blocks of 30 trials. We performed a two-way analysis of variance using a repeated measures design on the data, with lesion (CA3 or control) serving as the between-groups factor and block (presurgery, postsurgery 1, or postsurgery 2) as the within-group factor. The analysis revealed a significant effect of lesion groups, $F(1, 6) = 77.1$, $p < .0001$, and blocks, $F(2, 12) = 25.5$, $p < .0001$. The analysis also showed a significant interaction effect of Lesion × Blocks, $F(2, 12) = 10.7$, $p < .002$. A Newman–Keuls post

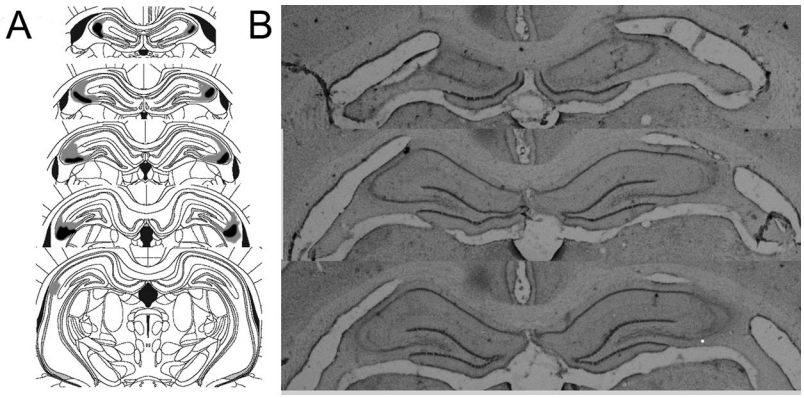


*Figure 2.* Serial sections were taken along the septotemporal axis from top to bottom. A: Schematic drawings of the largest (gray) and the smallest (black) of CA3 lesioned rat brains. Adapted from *The Rat Brain in Stereotaxic Coordinates* (5th ed.), G. Paxinos and C. Watson, 2005. Copyright 2005, with permission from Elsevier. B: Photomicrographs (12.5×) of a CA3 lesion. Note the almost complete degeneration of the pyramidal cells in CA3.

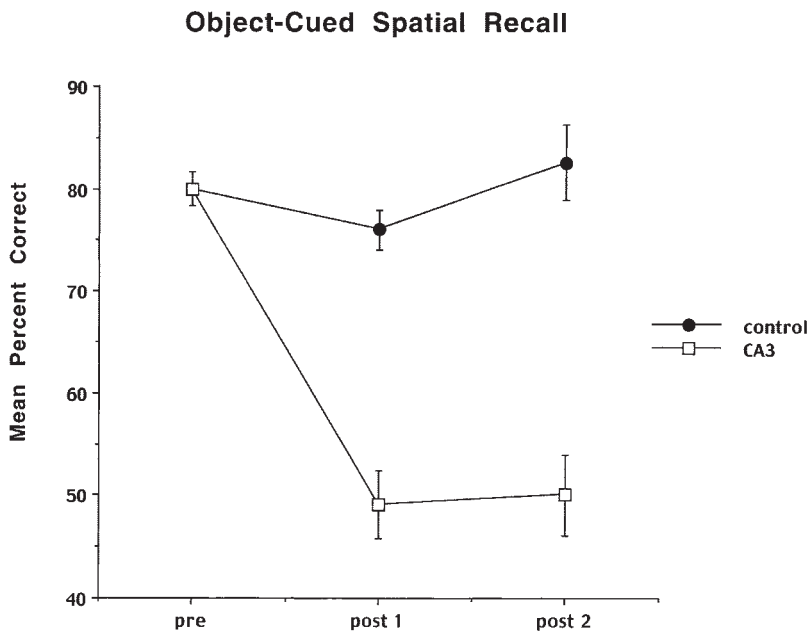


*Figure 3.* Mean percentage of correct performance for the control and CA3-lesioned rats on the object-cued spatial location recall task before (pre) and after (post 1 and post 2) surgery. Note the profound CA3 lesion effect.

hoc analysis performed on blocks revealed a significant difference ($p < .05$) for mean percentage of correct performance between the presurgery and both postsurgery 1 and postsurgery 2 blocks of trials, yet no significant difference was found between the postsurgery 1 and postsurgery 2 blocks. Results from a Newman–Keuls comparison test of the Lesion × Block interaction revealed no significant difference in performance on the presurgery block of trials, indicating that both groups learned the task equally well. The analysis did show a significant difference ($p < .05$) between the control and CA3 groups on both postsurgery 1 and postsurgery 2 blocks. Finally, no significant differences were found in the control group's performance on the presurgery, postsurgery 1, and postsurgery 2 blocks of trials. There was a significant difference ($p < .05$) found in the CA3 lesion group between the presurgery block and both postsurgery blocks, but not between the postsurgery 1 and the postsurgery 2 blocks.

### *Fixed Object–Cued Spatial Location Recall Task*

Figure 4 shows the mean percentage of correct performance on the fixed object–cued spatial location recall task for control and CA3-lesioned rats. It appears that control rats initially had a difficult time learning the changed procedure associated with the fixed task, but eventually they performed back to 80% correct performance, and the CA3-lesioned rats were able to learn the fixed version of the task so that by the sixth block they also performed at 80% correct performance. Data for the fixed task were grouped into six blocks of 10 trials for analysis. A repeated measures two-way analysis of variance was performed with lesion (CA3 or control) as the between-group variable and blocks (1–6) as the within-group variable. The analysis revealed a significant effect only for blocks, $F(5, 30) = 4.09$, $p = .006$. The results indicated that the CA3-lesioned rats could learn the associations over multiple trials. This result suggests that CA3-lesioned rats had no motivational or response selection problems.

### *Spatial Location–Cued Object Recall Task*

The results indicate that the CA3-lesioned rats performed more poorly than controls and their own preoperative performance for the spatial location–cued recall for object task (see Figure 5). Data for the spatial location–cued object recall task were grouped for analysis into blocks of 30 trials. A two-way analysis of variance using a repeated measures design was performed on the data, with lesion (CA3 or control) serving as the between-group and blocks (presurgery, postsurgery 1, and postsurgery 2) as the within-group factor. The analysis revealed a significant effect of lesion group, $F(1, 8) = 47.4$, $p < .0001$, and blocks, $F(2, 16) = 30.4$, $p < .0001$. The analysis also showed a significant interaction effect of Lesion × Blocks, $F(2, 16) = 27.3$, $p < .0001$. A Newman–Keuls post hoc analysis performed on blocks revealed a significant difference ($p < .05$) for mean percentage of correct performance between the presurgery and both the postsurgery 1 and the postsurgery 2 blocks of trials, yet no significant difference was found between the postsurgery 1 and postsurgery 2 blocks. Results from a Newman–Keuls comparison test of the Lesion × Block interaction revealed no significant difference in performance on the presurgery block of trials, indicating that both groups learned the task equally well. The analysis did show a significant difference ($p < .05$) between the control and CA3 groups on both the postsurgery 1 and the postsurgery 2 blocks. Finally, no significant differences were found in the control group's performance on the presurgery, postsurgery 1, and postsurgery 2 blocks of trials. There was a significant difference ($p < .05$) found in the CA3-lesioned group between the presurgery block and both postsurgery blocks, but not between the postsurgery 1 and the postsurgery 2 blocks.

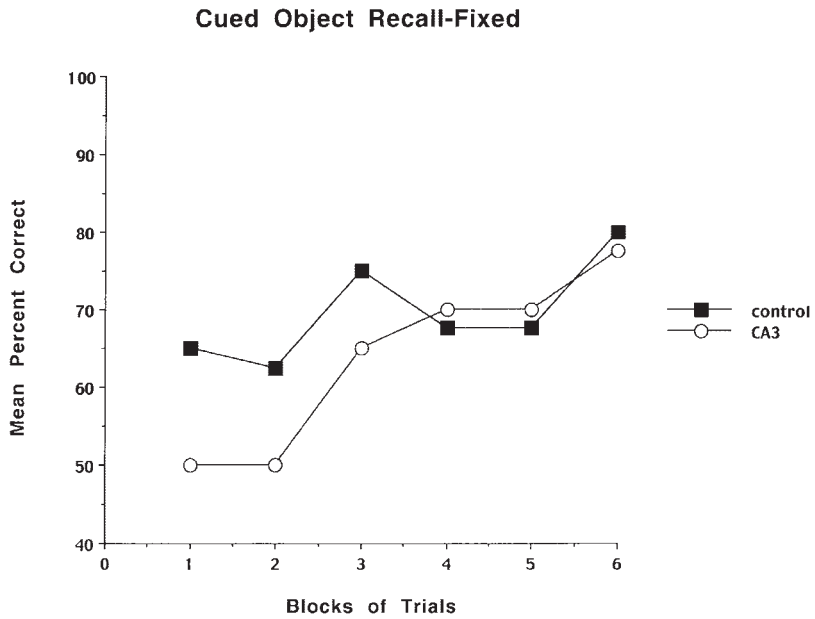


*Figure 4.* Mean percentage of correct performance for the control and CA3-lesioned rats on the object-cued spatial location recall task for the fixed condition postsurgery. Note recovery in performance of the CA3-lesioned group.

### *Fixed Spatial Location–Cued Object Recall Task*

Figure 6 shows the mean percentage of correct performance on the fixed spatial location–cued object recall task for control and CA3-lesioned rats. It appears that control rats maintained steady performance across the six blocks of trials and the CA3-lesioned rats were able to learn the fixed version of the task so that by the sixth block they performed at 80% correct performance. Data for the fixed task were grouped into six blocks of 10 trials for analysis. A repeated measures two-way analysis of variance was performed with lesion (CA3 or control) as the between-group variable and blocks (1–6) as the within-group variable. The analysis revealed a significant effect for lesion, $F(1, 8) = 85.6$, $p = .0001$; a significant effect for blocks, $F(5, 40) = 44.9$, $p = .0001$; and a significant effect for the interaction between lesion and blocks, $F(5, 40) = 15.1$, $p = .0001$. A Newman–Keuls post hoc test of the interaction between lesion and blocks indicated a significant difference ($p < .05$) between the two groups for the first three blocks, but no significant difference for the last three blocks. Analysis furthermore showed no significant difference in performance for the control group. The results indicated that the CA3-lesioned rats could learn the associations over multiple trials. This result suggests that CA3-lesioned rats had no motivational or response selection problems.

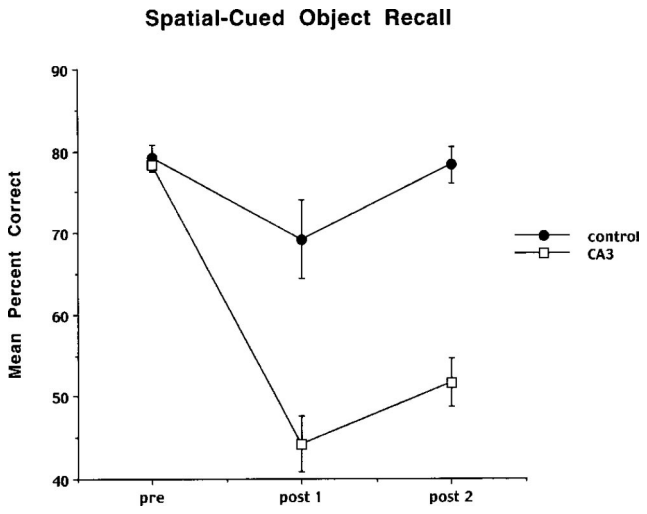


*Figure 5.* Mean percentage of correct performance for the control and CA3-lesioned rats on the spatial location–cued object recall task before (pre) and after (post 1 and post 2) surgery. Note the profound CA3 lesion effect.

## Discussion

The present data demonstrate that CA3 lesions disrupt performance of both the one-trial object-cued spatial location recall task and the spatial location–cued object recall task. Additional support comes from the finding that a subset of primate hippocampal (especially CA3) neurons respond with increased activity in the correct spatial location during and after the object recall cue in a similar one-trial object–place learning task followed by recall of the spatial position in which to respond when shown the object (Rolls, Xiang, & Franco, 2005). Thus, some hippocampal neurons appear to reflect spatial recall given an object recall cue. These data are consistent with the prediction of computational models that emphasize the importance of CA3 in mediating the rapid formation of arbitrary associations (Rolls & Kesner, 2006).

The present data are also consistent with those of other studies in animals and humans demonstrating a role for the hippocampus (Holdstock et al., 2002; Kesner & Hopkins, 2006; Malkova &

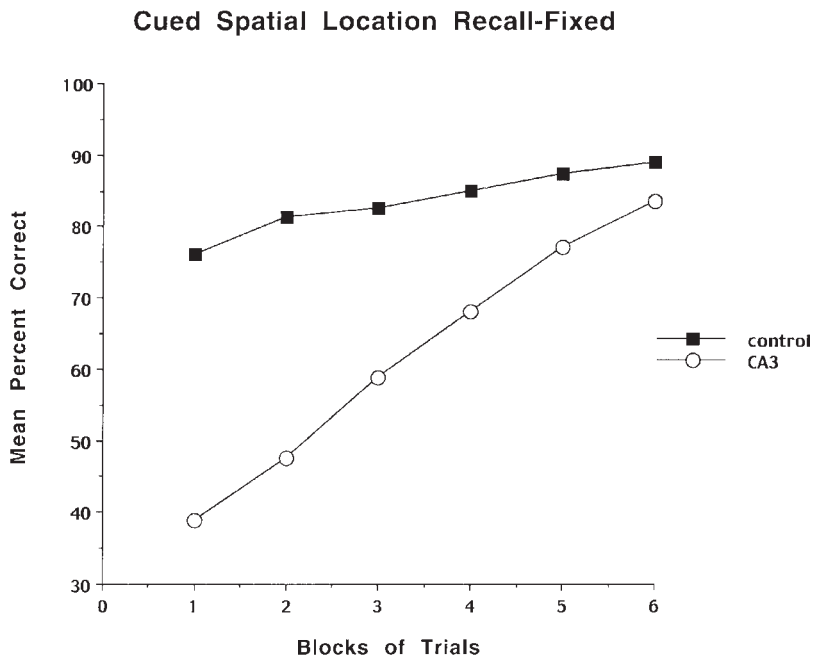


*Figure 6.* Mean percentage of correct performance for the control and CA3-lesioned rats on the spatial location–cued object recall task for the fixed condition postsurgery. Note recovery in performance of the CA3-lesioned group.

Mishkin, 2003; Owen, Sahakian, Semple, Polkey, & Robbins, 1995; Parkinson, Murray, & Mishkin, 1988; Smith & Milner, 1981; Stepankova, Fenton, Pastalkova, Kalina, & Bohbot, 2004) and especially the CA3 region (Gilbert & Kesner, 2003) in subserving object–place associations. Even though the studies above measure the acquisition of paired-associate information, they fail to address whether the learning is based on configural or conjunctive associations or on arbitrary associations because the stimuli in the object–place task are integral and cannot be separated from each other during recall. Furthermore, because the tasks are biconditional discrimination tasks, they are likely based on the learning of conjunctions of the cues that make up a given association, implying that that the cues cannot be separated from each other (cf. Morris, 2007; O'Reilly & Rudy, 2001). Thus, the present study is one of the first to demonstrate that the CA3 region of the hippocampus supports arbitrary associations in support of computational models of CA3 function and other theoretical views of hippocampal function. This is not to exclude the possibility that in some other tasks the hippocampus processes conjunctive associations as the task demands (O'Reilly & Rudy, 2001). One possible mechanism for the cued recall deficits could be an impairment in pattern completion in that the recall cue provides access to a previously experienced set of cues. Furthermore, research has shown that the CA3 region is involved in pattern completion using visual and spatial cues (Gold & Kesner, 2005).

The present data also support a role of CA3 in subserving episodic memory. Tulving (1983) has suggested that episodic memory in humans is a form of memory that "receives and stores information about temporally dated episodes or events and temporal-spatial relations between them" (p. 385). This has been translated into the idea that animal episodic-like memory depends on concurrent recall of what, where, and when an event happened. Thus, the present task is similar to cued recall tasks that measure episodic memory in humans. In this experiment, correct performance of this task required that the rat be able to discriminate between two behavioral episodes (each object–spatial location pairing in this case was a distinct event or behavioral episode) based on the retrieval cue provided. Other investigators have also suggested that these cued recall tasks represent good examples of the need to process information within episodic memory (Eacott & Easton, 2007; Gaffan, 1994; Hunsaker & Kesner, in press; Morris, 2007).

Effective performance on the present reciprocal cued recall tasks requires relational processing of the visual objects and their spatial locations present during the episode. Even though recalling a correct pairing is all that is necessary for correct performance, only a single element present during the episode is provided to cue recall of the rest of the episode. This supports assertions that animals rapidly learn relationships between visual objects and spatial locations while concurrently maintaining the stimuli represented independently to guide effective and flexible recall (cf. Eichenbaum & Cohen, 2001). Animals are required to recall the specific episodes to match the retrieval cue with the corresponding portion of the episode to be rewarded, so no temporal ordering mechanism or logical reasoning could be used to solve the task via a nonepisodic mechanism (cf. Morris, 2007). Thus, on cued recall, the animal recalls all of the objects present during the behavioral episode and the spatial relationships between those stimuli (and thus the episode itself), not just an abstract rule to guide behavior or a previously learned conjunctive representation. Because CA3-lesioned rats were able to relearn the task when the same stimuli were repeated across trials, but not when each association was trial

unique (i.e., episodic), this experiment provides additional support for a critical role for CA3 in episodic memory. The fixed task did not provide new information on each trial and thus cannot be considered to be an episodic task (cf. Hunsaker & Kesner, in press; Morris, 2007), suggesting that the CA3 neural network may not be necessary for nonepisodic associative learning tasks.

Alternative interpretations of the results of this experiment would include a potential CA3-lesion–induced deficit in motivational or response selection processes or a deficit in working memory for object or spatial location information. The purpose of testing the rats in the fixed task version of the object–place association experiments was to test these possible interpretations. The results showed that CA3-lesioned rats can learn the task when the same objects and locations are used for the study phase on repeated trials, ruling out a problem with motivation or response selection. Also, because the same delay (15 s) was used in the variable and fixed versions of the tasks, it would be more difficult to suggest that the CA3-lesioned rats were impaired in the variable task situation because of the inability to remember object–place information across a 15-s delay. Furthermore, it has been shown that at short delays, working memory for objects is not disrupted by hippocampus (including CA3) lesions in a continuous recognition and delayed nonmatching-to-sample task (Jackson-Smith, Chiba, & Kesner, 1993; Kesner, Bolland, & Dakis, 1993). Also, CA3-lesioned rats are not impaired in an object–trace odor paired-associate task (Kesner, Hunsaker, & Gilbert, 2005). With respect to spatial location information, the delay period between the study and test phase may be a critical variable in that CA3-lesioned rats can perform well in the context of short delays. Lee and Kesner (2003a) showed that CA3-lesioned rats are initially impaired in learning a one-trial nonmatching-to-place task on an eight-arm maze using a 10-s delay, but they did learn the task in about 80 trials. When a 5-min delay was introduced, the rats performed poorly at the 5-min delay, but continued to perform well for the 10-s delay trials, suggesting that the time between the study and the test phase may be an important variable. Also, when rats are trained first on a 10-s delay before receiving hippocampal lesions, then rats with dorsal hippocampus (including dorsal CA3) lesions are only transiently impaired in performing at the 10-s delay. They recover very quickly, but they do have a deficit at 5-min delays (Lee & Kesner, 2003b). In a different study, CA3-lesioned rats were impaired with a delay of 30 s between the study and the test phase, but this task was unique because test phase performance was based on judgment of spatial distance and not associative memory (Gilbert & Kesner, 2006). In this experiment, the delay between the study and the test phases was 15 s, which should not have produced a working memory deficit for spatial location information, suggesting that the CA3 lesion deficit may be more likely a result of the lesion of CA3 acting to disrupt the ability of the animal to support arbitrary associations.

The results of this study suggest that CA3 is important for the relational encoding of object–spatial location associations that allow for rapid and efficient recall based on a partial retrieval cue. The results also suggest that rats show episodic-like learning for associations and that CA3 is important for this process. Further studies involving concurrent training on both the object-cued spatial location recall and spatial location–cued object recall tasks are required to conclusively determine the role of the rodent hippocampus and CA3 for truly reciprocal cued recall tasks, when either cue may be presented to recall the other element.

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