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The Role of CA3 and CA1 in the Acquisition of an Object–Trace–Place Paired-Associate Task

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The hippocampus mediates associative learning involving spatial and temporal information. Specifically, paired associations in which a trace interval separates the elements appear to be associated within CA1. In contrast, CA3 appears to be involved in associations containing spatial elements. This suggests that CA3, but not CA1, is involved as long as the spatial association does not contain temporal elements; conversely, CA1 is involved when a temporal element is included, regardless of whether there are spatial elements present. In the present study, rats were run on an object–trace–place paired-associate learning paradigm. Rats with CA3 as well as rats with CA1 lesions showed deficits in the acquisition of this task. These results suggest that CA1 is involved in making arbitrary associations involving a temporal (trace) element, whereas CA3 is involved in making associations that involve spatial elements; furthermore, CA1 and CA3 interact in the presence of both spatial and temporal information.

Keywords: temporal association, paired-associate learning, CA1, CA3, hippocampus

Many mnemonic processes that contribute to learning and memory have been attributed to the hippocampus, including novelty detection, pattern separation, encoding, pattern association, pattern completion, retrieval, short-term memory, and intermediate-term memory (Kesner, Lee, & Gilbert, 2004). O'Reilly and McClelland (1994), Rolls (1996), Rolls and Kesner (2006), and Rolls and Treves (1998) have suggested that the hippocampus mediates the formation of arbitrary associations. For example, information concerning the location of an object may become associated with information regarding the identity of the object (Gilbert & Kesner, 2002).

Although paired-associate learning has been attributed to the hippocampus, the types of information that the hippocampus associates have not been specified in the models. Kesner, Gilbert, and Wallenstein (2000) have suggested that the hippocampus does not mediate all associative learning but is involved in paired-associate learning when one stimulus is associated with a spatial location. Rats that have hippocampal damage do not show deficits in working memory for olfactory information, visual objects, or egocentric responses (Cho & Kesner, 1995; Gilbert & Kesner, 2006; Wood, Agster, & Eichenbaum, 2004). It has been suggested that although the hippocampus processes various types of sensory information, it does so in search of spatial and temporal information to associate (Rolls & Kesner, 2006). The hippocampus may serve the purpose of enabling organisms to learn and remember where and when an event took place.

Humans, nonhuman primates, and rats with hippocampal damage have shown significant impairments in object–place paired-associate learning (Gaffan, 1994; Gilbert & Kesner, 2002, 2003), whereas learning nonspatial paired associations involving odor–odor, object–object, visual response, and auditory–visual associations have not been affected (Bunsey & Eichenbaum, 1996; Cho & Kesner, 1995; Jarrard & Davidson, 1990; Li, Matsumoto, & Watanabe, 1999; Murray, Gaffan, & Mishkin, 1993; Wood et al., 2004). Research has also shown that hippocampal-lesioned rats are able to discriminate between locations, odors, and objects used for paired-associate tasks as well as controls, and they do not show deficits for response inhibition. Therefore, data indicate that learning stimulus–place associations can occur in the hippocampus.

Rats with neurotoxin-induced lesions to DG, CA3, or CA1 were tested on tasks requiring either object–place or odor–place paired associations: tasks affected by hippocampal damage (Gilbert & Kesner, 2002). The results have revealed that rats with lesions to either dorsal DG or CA1 learned the object–place and the odor–place tasks as quickly and efficiently as control rats. In contrast, rats with lesions to CA3 were impaired for these tasks (Gilbert & Kesner, 2003). This suggests that the CA3 subregion mediates paired-associate learning involving spatial information (i.e., "place").

Rats with lesions to either dorsal CA1 or CA3 were tested on an object–trace–odor task (Kesner, Hunsaker, & Gilbert, 2005). This object–trace–odor task involved a 10-s time delay interposed between the presentation of an object and an odor. Rats with lesions to dorsal CA3 learned the task as readily as controls. However, rats with dorsal CA1 lesions were impaired and unable to perform the task. This research suggests that CA1 mediates paired-associate learning if a time component is involved (i.e., "trace") even when CA3 does not appear to play a role. These data suggest that the hippocampus is involved in arbitrary associations

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that do not involve spatial components as long as they contain a temporal component.

Although research has identified specific mnemonic functions for CA3 and CA1, their roles for paired-associate learning have not been studied with association tasks that involve both spatial and temporal components. It has been shown that the CA3 subregion subserves learning spatial associations and that the CA1 subregion mediates temporal associations (cf. Kesner et al., 2004; Rolls & Kesner, 2006). In the present study, rats with control lesions or lesions to either dorsal CA1 or CA3 were tested on a task that had both spatial and temporal components: an object–trace–place task. We hypothesized that rats with either a CA1 or a CA3 lesion should be impaired in learning the task because it requires that both spatial and temporal information be processed concurrently.

Method

Subjects

Twenty male Long–Evans rats (300–450 g) were used in this experiment and maintained on a 12-hr light–dark cycle. All experimentation was carried out during the light phase of the cycle. The rats were maintained at 300–450 g and handled approximately 10–15 min a day for 7 days prior to surgery. After surgery, the rats were fed once a day throughout the experiment and had ad libitum access to water. All surgical and experimental procedures conformed to the guidelines of the University of Utah Institute of Animal Care and Use Committee.

Apparatus

A cheeseboard maze served as the test apparatus for all testing. The surface of the apparatus stood 65 cm above the floor, was 119 cm in diameter, and was 3.5 cm in thickness. A total of 177 food wells (2.5 cm in diameter and 1.5 cm in depth) had been drilled into the surface of the maze in evenly spaced rows and columns (rows were 7.5 cm apart [center to center]). A start box (24 cm long, 15 cm wide, and 17 cm high) was placed on the maze surface, with the long axis of the box centered perpendicular to the rows of food wells, and the back of the box placed along the edge of the apparatus. This box was equipped with a hinged top and two guillotine doors that were raised and lowered manually by the experimenter. Two small toys (a soft fabric Winnie the Pooh and a solid plastic Garfield toy; each approximately 7 cm tall) were used for the task. The objects were attached to a door that could be inserted and removed from the start box. A gray wood block (2 cm wide and 5 cm tall) was used to mark potentially rewarded spatial locations that were 68 cm apart and approximately 70 cm in front of the start box. The apparatus was kept in a well-lit room with a cabinet, one door, and various pictures on the wall that served as distal, spatial cues. Froot Loops were placed in the food wells covered by the gray block during rewarded trials.

Surgery

The rats were randomly assigned to three groups: vehicle-injected controls ($n = 6$), dorsal CA3 ($n = 7$), and dorsal CA1 ($n = 7$) ibotenic acid lesions. The subregional lesion procedures were identical to those used by Kesner et al. (2005). Rats were anesthetized with isoflurane (2%–4% in 1–2 L per min flow rate adjusted for maximal anesthesia) and given atropine sulfate (0.54 mg/kg ip). Rats were then placed on an isothermal heating pad to maintain their body temperature at 37 °C. Small burr holes were drilled in the skull at the following coordinates: (a) CA1-lesioned group: 3.6 mm posterior to bregma; 1.0, 2.0, and 3.0 mm lateral to midline; and 2.1, 1.9, and 2.4 mm ventral from dura; and (b) CA3-lesioned group: 2.5 mm posterior to bregma, 2.6 mm lateral to midline, and 3.2 mm ventral from dura; 3.3 mm posterior to bregma, 3.3 mm lateral to midline, and 3.2 mm ventral from dura; and 4.1 mm posterior to bregma, 4.2 mm lateral to midline, and 3.1 mm ventral from dura. Neurotoxins were infused into CA1 and CA3 to produce axon-sparing, subregion-specific lesions (cf. Jerman, Lee, Kesner, & Berman, 2005). Ibotenic acid (6 mg/mL; 0.10–0.15 μL per site at 0.07 μL per min) was infused into pyramidal cell layers for CA1 and CA3 lesions. We made all injections using a 10-μL syringe with a microinjection pump, and we left the needle in place for at least 1 min after the injection to allow for proper diffusion of the acid. Phosphate-buffered saline was injected into the corresponding coordinates to produce control lesions ($n = 3$ for CA1; $n = 3$ for CA3). For 3 days after surgery, rats received Children's Tylenol (1–2 mg/mL) as an analgesic in their drinking water. Rats were given 2 weeks to recover from surgery prior to experimentation.

Behavioral Procedure

After recovery from surgery, the rats were shaped to retrieve a Froot Loops reward from a food well covered by a gray wooden block over a 7-day period. After shaping, each rat was then trained on a successive discrimination go/no-go task to examine object–trace–place paired-associate learning. The task consisted of pairing an object (A or B) with a place (1 or 2) separated by a 10-s trace interval (see Figure 1). Correct pairings were A1+ (Garfield—Location 1 rewarded) or B2+ (Winnie the Pooh—Location 2 rewarded) and were rewarded. Incorrect pairings were A2− (Garfield—Location 2 not rewarded) or B1− (Winnie the Pooh—Location 1 not rewarded) and were not rewarded.

During the task, the rat was placed in the start box and was presented with one of the two objects (A or B). The rat was given 3 s to explore the object, after which the object was removed. After the object was removed, there was a 10-s trace period during which the rat was forced to stay in the start box with no stimuli present. After the 10-s trace period, the door to the start box was opened. The rat left the box and ran to the spatial location (either 1 or 2) marked by a gray wooden object. The spatial locations were 68 cm apart to reduce interference between spatial locations (cf. Gilbert, Kesner, & Lee, 2001). Latency was measured from the opening of the door of the start box until the rat knocked over the wooden block. A latency of greater than 10 s was set as the criterion for a no-go. If the rat did not knock over the object within 10 s, the rat was placed in the start box. The intertrial interval was 30 s. There were 24 trials per day, consisting of 12 go trials (A1+ and B2+) and 12 no-go trials (A2− and B1−). The reward consisted of half a piece of Froot Loops cereal located inside a food well covered by the gray block. Go and no-go trials were intermixed identically to the study by Kesner et al. (2005) to facilitate comparison of observed effects. Rats were tested 24 trials per day, 5 days per week, for 4 weeks, resulting in a total of 480 trials per rat.

Data Analysis

The dependent measure for the object–trace–place task was the latency difference between go and no-go trials. Two-way repeated measures analyses of variance (ANOVAs) with lesion group as the between variable and blocks of 60 trials as the within variable were performed on the data. All statistical analyses were done with SPSS 12 base statistical analysis software package (SPSS Inc., Chicago, IL), and Tukey's honestly significant difference (HSD) post hoc pairwise comparisons were performed on all significant main effects. Alpha was set at $p > .05$ for all analyses.

Histology

After experimentation, all rats were sacrificed with 1 mL sodium pentobarbital (70 mg/mL ip) and intracardially perfused with 0.9% phosphate buffered saline (pH 6.0) for 2 min followed by 10% buffered formalin (pH 7.0) for another 5 min. The brains were stored for 72 hr at 4 °C in 30%

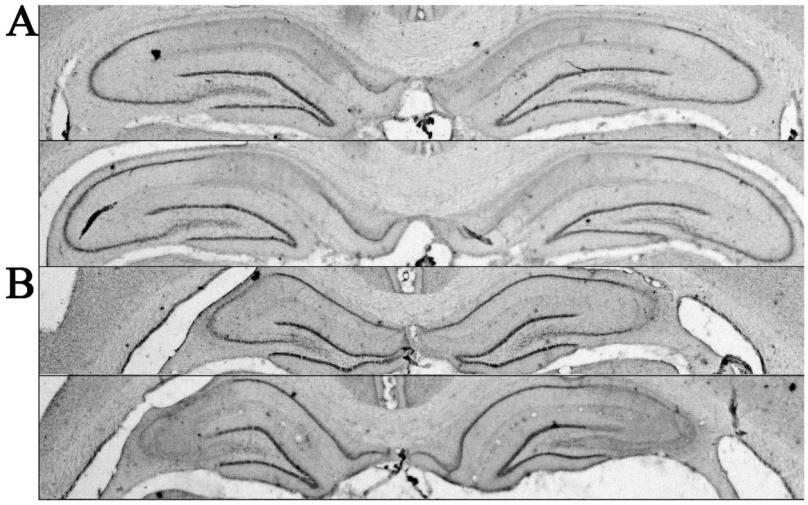

Figure 1. Photomicrographs (10 ×) of representative anterior and posterior sections of (A) CA1- and (B) CA3-lesioned rats.

sucrose/formalin. Each brain was frozen and cut into 40 μm sections beginning at bregma −1.8 mm and ending at bregma −4.80 mm. We stained every third section with cresyl violet, and we examined for microscopic verification of the lesion placement as well as quantitative volumetric analysis using ImageJ software Version 1.33 (National Institutes of Health, Bethesda, MD).

Results

Histological Analysis

Because neither a CA1- nor CA3-unique neurotoxin is available, we have developed injection parameters suitable to induce subregion-specific lesions with ibotenic acid, which produce selective damage to either CA1 (Gilbert et al., 2001) or CA3 (Gilbert & Kesner, 2003; Lee & Kesner, 2004; cf. Jerman et al., 2005) pyramidal cells. Although it is difficult to define the boundary between the dorsal and ventral portions of the hippocampus, the dorsal region was defined as the anterior 50% of the hippocampus (Moser & Moser, 1998). Data from two rats had to be excluded from analysis because their lesions appeared to be bilateral hippocampectomies ($n = 1$ CA3 rat, and $n = 1$ CA1 rat). A quantitative analysis revealed that CA1 lesions resulted in 85% ± 3% damage to the dorsal CA1 pyramidal cell layer with 4% ± 1% damage to CA3 pyramids and 6% ± 1% damage to DG granule cells, whereas CA3 lesions resulted in 90% ± 3% damage to CA3 with 3% ± 1% damage to CA1 and 4% ± 1% damage to DG. As shown in Figure 1A, ibotenic acid injections into CA1 produced almost complete degeneration of the pyramidal cells in CA1; Figure 1B shows the result of a representative CA3 lesion. There was no observed extrahippocampal cortical damage in any of the rats.

Behavioral Analysis

The results of the experiment are shown in Figure 2. The graphed results show that rats with CA3 lesions as well as CA1-lesioned rats did not learn this task within 480 trials, and there was no marked improvement. A two-way repeated measures ANOVA

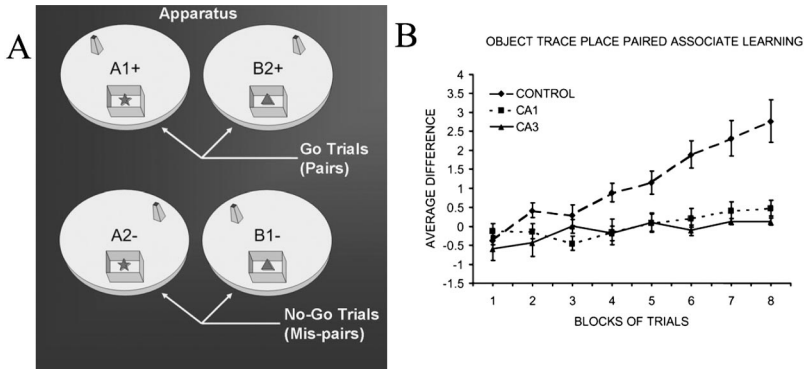

Figure 2. A: Behavioral apparatus for the object–trace–place paired-associate task. B: Mean latency differences (latency on mispaired trials − latency on paired trials) as a function of blocks of trials (60 trials per block) for control-, CA1-, and CA3-lesioned rats on acquisition of the object–trace–place paired-associate task. Note that CA1- and CA3-lesioned rats did not acquire the task, whereas control-lesioned rats did.

with groups as the between variable and blocks of trials as the within variable revealed a significant effect for groups, $F(2, 12) = 14.56$, $p = .001$; a significant blocks of trials effect, $F(5, 60) = 26.48$, $p < .0001$; and a significant interaction between groups and blocks of trials, $F(10, 60) = 6.92$, $p = .01$. Subsequent Tukey's HSD post hoc paired comparison tests on the interaction effect revealed that the CA1-lesioned group was significantly different ($p < .01$) from controls, as was CA3 ($p < .01$), but there were no significant differences between CA3 and CA1 ($p > .05$). Control rats improved their performance across blocks as determined by a Tukey's HSD paired comparisons test ($p < .01$), whereas CA1- and CA3-lesioned rats did not improve as a function of blocks ($p > .05$ in both cases).

The mean latency for the go trials was 3.29 s ± 0.67 s for the control group, 3.35 s ± 0.55 s for the CA1 group, and 3.21 s ± 0.70 s for the CA3 group. To test whether the latency differences in go trials contributed to the results, we ran a one-way ANOVA on the mean latency for go trials between groups. The results indicate that there was not a significant effect for groups, $F(2, 14) = 0.26$, $p = .78$. The mean latency for the no-go trials was 6.28 s ± 0.77 s for the control group, 3.41 s ± 0.85 s for the CA1 group, and 3.38 s ± 0.72 s for the CA3 group. To test whether the latency differences in no-go trials contributed to the results, we ran a one-way ANOVA on the mean latency for no-go trials between groups. The results indicate that there was a significant effect for groups, $F(2, 14) = 12.65$, $p = .001$. Tukey's HSD post hoc pairwise comparisons indicated that CA3- and CA1-lesioned rats did not significantly differ from each other ($p = .945$) but did significantly differ from controls ($p = .003$ for CA3 group, and $p = .002$ for CA1 group). These results suggest that the lack of an increase in latency for the CA1 and CA3 groups compared with the control group was due to the failure to inhibit responding on no-go trials.

Discussion

The present experimental data provide evidence supporting both the assertion that CA3 is critical for the performance of spatial paired-associate tasks (Gilbert & Kesner, 2003) as well as that CA1 is critical for making associations across time (Kesner et al., 2005). When rats are required to perform a task requiring both spatial and temporal processing (e.g., trace–place), both CA1 and CA3 are involved.

CA1 and CA3 can be dissociated based across mnemonic roles: traditionally time and space. CA3 is critical when a spatial location is to be learned. At short delays, CA3, but not CA1, is critical for delay-nonmatch-to-place acquisition (Lee & Kesner, 2003). Furthermore, CA3, but not CA1, is involved in spatial paired-associate learning in which the spatial location and the other element of the association are spatially and temporally contiguous (Gilbert & Kesner, 2003). CA1, however, is involved when information must be remembered over a temporal interval. CA1, but not CA3, is involved in paired-associate learning when the elements of the association are temporally noncontiguous (Kesner et al., 2005). Other differences between CA3 and CA1 involve processing of information during encoding within short-term memory and retrieval of information from intermediate memory. Support for this idea comes from the observation that CA3, but not CA1, is involved in encoding during the acquisition of a Hebb–Williams maze measured within 1 day on the basis of short-term memory (Jerman, Kesner, & Hunsaker, 2006; Vago & Kesner, 2005). In contrast the CA1, but not CA3, is involved in retrieval during retention of the Hebb–Williams maze measured between days on the basis of intermediate-term memory (Jerman et al., 2006; Lee & Kesner, 2004; Vago & Kesner, 2005). Furthermore, the CA3, but not CA1, mediates acquisition of delay fear conditioning (Lee & Kesner, 2004). However, the CA1, and not the CA3 region, mediates retention of fear conditioning. Also, CA1, but not CA3, is critically involved in the retention of trace fear conditioning (Rogers, Hunsaker, & Kesner, 2006).

The present data are also in line with previous reports that have shown that there are tasks that are dependent on both CA1 and CA3. For example, both CA3 and CA1 are required for performance of a delay-nonmatch-to-place task at intermediate delays (5 min; Lee & Kesner, 2003). Both CA3 and CA1 are necessary for memory of a sequence of spatial locations (Lee, Jerman, & Kesner, 2005). CA1 and CA3 are also both involved in forming representations of space and detecting spatial novelty (Lee et al., 2005; Lee, Rao, & Knierim, 2004; Lee, Yoganarashimha, Rao, & Knierim, 2004).

It is proposed that for tasks involving spatial information that must be associated with other stimuli across an interposed temporal gap, both CA3 and CA1 are necessary. This takes advantage of the role of CA3 for rapidly encoding information and the ability of CA1 to hold or maintain information over a trace interval (cf. McEchron, Tseng, & Disterhoft, 2003).

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