Learning Associations Between Places and Visual Cues Without Learning to Navigate: Neither Fornix Nor Entorhinal Cortex Is Required

E.A. Gaffan,1* D.M. Bannerman,2 and A.N. Healey1

1School of Psychology, University of Reading, Reading, United Kingdom
2Department of Experimental Psychology, Oxford University, United Kingdom

ABSTRACT: Rats with fornix transection, or with cytotoxic retrohippocampal lesions that removed entorhinal cortex plus ventral subiculum, performed a task that permits incidental learning about either allocentric (Allo) or egocentric (Ego) spatial cues without the need to navigate by them. Rats learned eight visual discriminations among computer-displayed scenes in a Y-maze, using the constant-negative paradigm. Every discrimination problem included two familiar scenes (constants) and many less familiar scenes (variables). On each trial, the rats chose between a constant and a variable scene, with the choice of the variable rewarded. In six problems, the two constant scenes had correlated spatial properties, either Allo (each constant appeared always in the same maze arm) or Ego (each constant always appeared in a fixed direction from the start arm) or both (Allo + Ego). In two No-Cue (NC) problems, the two constants appeared in randomly determined arms and directions. Intact rats learn problems with an added Allo or Ego cue faster than NC problems; this facilitation provides indirect evidence that they learn the associations between scenes and spatial cues, even though that is not required for problem solution. Fornix and retrohippocampal-lesioned groups learned NC problems at a similar rate to sham-operated controls and showed as much facilitation of learning by added spatial cues as did the controls; therefore, both lesion groups must have encoded the spatial cues and have incidentally learned their associations with particular constant scenes. Similar facilitation was seen in subgroups that had short or long prior experience with the apparatus and task. Therefore, neither major hippocampal input-output system is crucial for learning about allocentric or egocentric cues in this paradigm, which does not require rats to control their choices or navigation directly by spatial cues. 


KEY WORDS: retrohippocampal region; subiculum; allocentric; egocentric; incidental learning; rats

INTRODUCTION

Gaffan et al. (2000a) described a test of spatial learning that does not involve navigation. In this task, rats had the opportunity to learn new associations involving spatial cues, without using those cues to control their movements. In essence, the task was a visual discrimination, using the constant-negative paradigm in an automated Y-maze (Gaffan and Woolmore, 1996). The stimuli were large abstract displays called “scenes.” Rats learned a series of constant-negative problems. In each problem, they learned to avoid a small number of scenes with which they became familiar (constants) and associate them with particular spatial cues, even though that is not required for problem solution. Fornix and retrohippocampal-lesioned groups learned NC problems at a similar rate to sham-operated controls and showed as much facilitation of learning by added spatial cues as did the controls; therefore, both lesion groups must have encoded the spatial cues and have incidentally learned their associations with particular constant scenes. Similar facilitation was seen in subgroups that had short or long prior experience with the apparatus and task. Therefore, neither major hippocampal input-output system is crucial for learning about allocentric or egocentric cues in this paradigm, which does not require rats to control their choices or navigation directly by spatial cues. 

In some problems, spatial cues varied randomly: each constant scene could appear in different arms of the maze (i.e., its allocentric position varied across trials) and in different directions (left or right) relative to the start arm (i.e., its egocentric position varied). These were control problems, with no added spatial cue. In other problems, each constant scene had a spatial cue additionally associated with it—either an allocentric place, or an egocentric direction, or both. 

An allocentric (Allo) cue was added by having each constant (on trials when it occurred) always appear in a particular arm of the maze; e.g., if there were two constant scenes—A and B—A might always appear in the north arm and B always in the southwest arm. In our maze, the arms might be distinguished either by extramaze cues, such as visual and auditory landmarks in the room surrounding the maze, or by intramaze features within the three nominally identical arms, such as their appearance, smell, or texture. We refer to all arm-specific cues as allocentric; we consider whether the extramaze—intramaze distinction is important, in the Discussion section. An egocentric (Ego) cue was added by having a given constant always appear in a fixed direction from the start arm; e.g., constant A might always appear to the left of the start arm, constant B always to the right. In a fourth

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*Correspondence to: E.A. Gaffan, School of Psychology, University of Reading, Reading RG6 6AL, UK. E-mail: e.a.gaffan@reading.ac.uk
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rats; either Allo or Ego added cues resulted in faster learning, while combined Allo + Ego cues yielded the fastest learning of all (Gaffan et al., 2000a). This enhanced learning shows that normal rats encode allocentric and egocentric spatial cues and learn new associations between specific visual cues (constant scenes) and either type of spatial cue, even when the task does not require them to do so. We refer to this learning about the correlated spatial cues as incidental (cf. Good et al., 1998). We did not test incidental spatial learning directly, by requiring the rats to choose between spatial alternatives or navigate by means of the cues—rather, we tested it indirectly (Richardson-Klavehn and Bjork, 1988). The enhancement of visual learning that we observed served as an indirect test of spatial learning.

Gaffan et al. (2000a) studied rats with hippocampal lesions in the same paradigm. Lesions that damage the hippocampus or closely connected structures disrupt the learning of navigational tasks in which rats must approach particular allocentrically defined locations to gain reward (O’Keefe et al., 1975; Sutherland and Rodriguez, 1989; Aggleton et al., 1992; Jarrard, 1995) and leave learning that is based on egocentric cues unaffected (Rasmussen et al., 1989; Aggleton et al., 1996; Neave et al., 1997). But would hippocampal-lesioned rats be impaired in the indirectly tested learning about allocentric or egocentric cues; i.e., would they show less enhancement of constant-negative learning by added Allo or Ego cues than did normal rats? Because their learning of standard egocentric tasks is normal, we expected that hippocampal-lesioned rats would readily learn about egocentric cues, and therefore show clear enhancement of constant-negative learning by an added Ego cue. That is what we found (Gaffan et al. 2000a, experiment 1). In the case of allocentric cues, the prediction depends on how one explains the impairment of normal allocentric learning. If the impairment reflects difficulty in encoding the conjunctions of cues that define places, as assumed in some theories (e.g., O’Keefe, 1991; Eichenbaum, 1996; O’Reilly and Rudy, 2001), incidental learning (for which the cues must be encoded) should also be impaired, and there should be no enhancement of visual learning by added Allo cues. However, if hippocampal rats can encode allocentric cues and learn where stimuli are in allocentric space, but have difficulty in controlling or registering their own movements through space (McNaughton et al., 1996; Redish and Touretzky, 1997; Whishaw, 1998), they should show enhancement by added Allo cues. The results obtained by Gaffan et al. (2000a) were more consistent with the second type of account. In two experiments, when we compared learning of problems with an added Allo cue to problems with no added cue, the hippocampal-lesioned group showed as much benefit from the Allo cue as did sham-lesioned rats. In the first experiment, there were signs of a mild impairment because (unlike sham controls) hippocampal-lesioned rats showed no benefit of adding an Allo to an Ego cue—the Allo cue appeared to be overshadowed when an Ego cue was also available. These results suggest that rats without a hippocampus did spontaneously encode the added Allo cue in our paradigm, though it may have been slightly less salient than it was for normal rats. We concluded that our task was able to reveal such encoding precisely because it tests allocentric spatial encoding, and associative learning, indirectly—dependent of navigation.

**FIGURE 1.** Schematic illustration of the four problem types. Each row represents the positions of start arm, rewarded and nonrewarded scenes in four possible examples of trials. Each session comprised 80 trials analogous to those shown. In the Allo + Ego added condition, each constant scene appears in a fixed arm and a fixed direction from the start arm. In the Ego added condition, each constant appears in a fixed arm (but not in a fixed direction from the start arm). In the Allo added condition, each constant appears in a fixed direction from the start arm (but not in a fixed arm). In the No-Cue added condition, each constant can appear randomly in any arm and in any direction from the start arm. S, start arm; A,B: Constant scenes. C–F: Variable scenes; +, arm that must be chosen for reward, i.e., arm containing a variable scene.

Rats are not required to learn the associated spatial properties of the constants to solve the visual discrimination; any problem can be mastered simply by learning to avoid the constant visual scenes A and B and to approach the variable scenes. Moreover, it is the constants that have fixed spatial properties, not the variables, but it is the variables that must be approached to gain food reward; therefore, the rats’ approach behavior need not come under the control of any spatial cue, and the task does not entail spatial navigation. However, we hypothesized that if rats nonetheless learned about the added spatial cues, they might solve such problems faster than control problems that had no added spatial cue, because the correlated spatial cues might increase the discriminability of the constants from the variables, an “acquired distinctiveness” effect (Hall, 1991). We found this to be true of normal condition, each constant had both Allo and Ego properties added. See Figure 1 for illustrations and further details.

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The present study followed up that of Gaffan et al. (2000a) with two further lesion groups. The new groups received (1) fornix transection (FX group), which disconnects the hippocampus from its major diencephalic output targets and also from its septal inputs; and (2) a neurotoxic retrohippocampal lesion (group RH), which removed the cells of the entorhinal cortex along with much of the ventral part of the subiculum and subicular cortices.

Fornix transection does not have completely equivalent effects to selective hippocampal ablation. For example, rats with fornix transection learn at a normal rate in two nonspatial configural tasks (McDonald et al., 1997) and discriminate better than controls in a conditioned place preference test (Ferbinteanu and McDonald, 2001), all of which are impaired by hippocampectomy. However, fornix transection produces qualitatively similar effects to hippocampectomy (although sometimes quantitatively smaller or larger) in a wide variety of paradigms, including locomotor activity, circadian patterns, object–place association, and, most relevant to the present study, allocentric spatial navigation (Sutherland and Rodriguez, 1989; Aggleton et al., 1992; Whishaw and Jarrard, 1995; McDonald et al., 1997; Cassel et al., 1998; Sziklas et al., 1998). Thus, the FX group constitutes a possible replication of the pattern of results reported by Gaffan et al. (2000a), namely that the lesioned animals do learn incidentally about both allocentric and egocentric cues, when tested with our indirect, non-navigational procedure.

The lesion sustained by group RH has different effects. Selective neurotoxic lesions of entorhinal cortex produce little or no impairment in many allocentric spatial navigation tests (Hagan et al., 1992; Pouzet et al., 1999; Bannerman et al., 2001b)—surprisingly, given that so many cortical inputs to the hippocampus, and return projections from hippocampus to the cortical regions of origin, are mediated by entorhinal cortex (Witter, 1993). (It should be noted, however, that more extensive spatial impairments have been observed after cytotoxic retrohippocampal lesions, which, like ours, encroached upon subicular areas (Good and Honey, 1997; Oswald and Good, 2000).) Another example of dissociation is that cytotoxic retrohippocampal or entorhinal lesions do not affect fear conditioning to contextual cues, whereas hippocampal or fornix lesions do (Phillips and LeDoux, 1995; Good and Honey, 1997; Bannerman et al., 2001a). We have also found the same RH lesion to have no effect on performance in a scene processing task which is altered after transection of the fornix or other diencephalic projections from hippocampus (Gaffan et al., 2001).

Conversely, entorhinal cortical ablation does disrupt at least one process that may be unaffected by selective hippocampal lesions: latent inhibition (Yee et al., 1995; Coutureau et al., 1999; Shohamy et al., 2000). It has also been found to abolish the difference between intra- and extra-dimensional shifts in a discrimination paradigm (Oswald et al., 2001). These can be broadly described as transfer effects, where past experience with stimuli influences subsequent learning about the same or similar stimuli; theoretical interpretations of these transfer effects are mentioned in the Discussion. Our paradigm requires rats to learn a series of problems in which the role of spatial cues changes between tasks, resulting in potential negative or positive transfer. There are several reasons, then, why we might observe different patterns of results in groups FX and RH.

In addition, we examined the effect of varying the amount of prior experience. A possible limitation to the conclusions of Gaffan et al. (2000a) was that their rats had extensive practice with the constant-negative procedure in the same apparatus (using different constant stimuli, of course) before the main experiment began. Thus, the allocentric stimuli—the three maze arms and their associated intra- and extramaze cues—were highly familiar to the rats; it could be argued that even the hippocampal-lesioned group had had ample opportunity to encode the three places, and the task of associating the places with new constant scenes was therefore not a very demanding one. Therefore, we split our subjects into two subgroups, one of which (Early) completed the experiment after minimal pre-training in the maze, whereas the other (Late) completed it later, after prolonged experience with the constant-negative task, similar to that given to rats in the previous study. According to the above argument, if there were Allo-cue enhancement in either lesion group, it should be more evident in the Late subgroups than the Early ones.

In other respects, the study was similar to experiment 1 of Gaffan et al. (2000a); other minor changes are mentioned under Materials and Methods. Each rat learned eight constant-negative problems, two of each of the four types—Allo (Allo cue added), Ego (Ego cue added), Allo + Ego (both Allo and Ego cues added), and NC (No-Cue, i.e., no added spatial cues). In each problem, there were two constant scenes (a different pair in each of the eight problems) and many possible variable scenes. There were three groups of rats: FX (fornix-transected), RH (which received retrohippocampal lesions), and SH (sham-operated controls). One-half of each group (Early subgroup) took part in the experiment after very brief pre-training in the maze, while the remaining Late subgroup took part several months later, after extensive experience with the constant-negative task in the maze, where the stimuli were dissimilar to those used in the present experiment.

Materials and Methods

Animals

Thirty-six male rats of the Dark Agouti strain, supplied by Harlan UK (Bicester, Oxon, England) took part in the study. Their age was approximately 4 months at surgery. Surgery and postsurgical activity testing took place at Oxford University, and during this time the rats received ad libitum food and water. They were transferred to Reading University for behavioral testing in the main experiment. They were caged in pairs on a 12:12-h light cycle and testing took place during the light phase. They had free access to water and were maintained at 85–90% of free-feeding weight by controlled feeding after testing sessions.

Surgery

Thirteen rats were assigned to the RH group (retrohippocampal lesions) and 14 to the FX group (fornix transection), and 9 received sham surgery (SH group). All rats were anesthetized with tribro-
moethanol (Avertin, 290 mg/kg, i.p.) and were placed in the stereotactic frame (Stoelting, Wood Dale, IL) with the head level between bregma and lambda. For the retrohippocampal lesion, an incision of the scalp was made along the midline and the appropriate portion of the bone overlying the neocortex was removed. Injections were made with a 5-μl syringe needle which was mounted on the stereotactic frame. N-Methyl-D-aspartate (NMDA) was dissolved in phosphate-buffered saline (PBS) (pH 7.4) at a concentration of 10 mg/ml. Injections of NMDA (0.025–0.10 μl) were made over 30–60 s at each of 16 injection sites. For the stereotactic coordinates of the injection sites and the corresponding volumes of NMDA injected, see Table 1.

For fornix transection, the right temporal muscle was retracted and a hole drilled through the side of the skull. The fimbria-fornix was cut mechanically with an adapted pair of specially ground fine watchmakers’ forceps and was held horizontally on the stereotactic manipulator such that the tips of the forceps were exactly 1.5 mm apart (measured using a pair of digital calipers). The forceps were inserted at a point 1.2 mm posterior to bregma, with the lower tip a distance of 5.6 mm below the skull surface as measured at bregma. Using a screw drive, the forceps were inserted to a point in the contralateral hemisphere at a distance of 4.0 mm beyond the midline as defined by bregma. Once in position, the forceps were clamped by tightening a screw, held shut for 2 min, and then opened and retracted.

With the sham-operated rats, the skull opening was made in an identical manner to the lesioned groups (four rats as for the retrohippocampal lesion, five as for the fornix transection), but the injection needle or forceps was not inserted into the brain.

On completion of surgery, all animals were sutured and a topical antibiotic powder (PEP 2% powder; Intervet Laboratories, UK) sprinkled over the wound. The animals also received a subcutaneous injection of antibiotic (Baytril 2.5%; Bayer Ltd, Ireland) and were allowed to recover in a temperature-controlled recovery chamber (30°C). All rats were allowed at least 2 weeks to recover before undergoing any behavioral testing.

### Apparatus

The postsurgical tests of locomotor activity used a 39 × 25-cm floor activity cage having two photocell beams along its long axis, 1.5 cm above the floor and 13 cm apart. For the main experiment, two computerized Y-mazes were used (Gaffan and Eacott, 1995). At the far end of each of the three arms of the maze there were two computer monitors, side by side, on which monochromatic displays could be presented on 23 × 18-cm screens, and a food tray between the screens into which 45-mg diet pellets (Noyes Type P) could be dispensed. The screens were 46 cm from the maze center; interruption of infrared beams, 23 cm from the screens, indicated when a rat entered a particular arm. The mazes were roofed in plexiglass so that extramaze cues (e.g., a doorway, computers, a rack of cages) were available to the rat; however, the room lighting was dim compared with the stimuli displayed within the maze. Presentation of stimuli and reward pellets, and monitoring of the rat’s location and collection of pellets from the food tray, were under computer control. For more details, see Gaffan et al. (2000b).

### Stimuli

The constant and variable stimuli for all constant-negative problems were drawn from a pool of 120 different complex scenes. Scenes were monochromatic displays drawn in low-resolution Borland Turbo Pascal graphics using gray levels 4–12 from a possible 16 (for more information, see Gaffan and Eacott, 1995; for illustrations, see Gaffan et al., 2000a). Each scene extended across the two screens within a maze arm, the left and right screens showing mirror images. A scene comprised a number of abstract shapes (e.g., ellipses, polygons) scattered across a contrasting diffuse background. One-half of the scenes had figures in lighter gray against a background of darker gray; the remaining scenes had the converse.

The absolute brightness of scenes was varied quasi-randomly, to counteract a potential artifact in the Allo-enhancement effect. A constant scene that always appears in the same maze arm (added Allo condition) is always displayed on the same pair of monitors, whereas in the control and added-Ego conditions, the same constant scene is displayed in different arms across trials and therefore on different monitors. Monitor screens that are nominally identical inevitably differ both between and within screens in local and global brightness and in the precise mapping of logical gray levels to actual luminance (Harris et al., 1987). Perhaps, then, learning about the constant scenes was improved by the added Allo cue, not because each scene stayed in the same place, but because its visual appearance was more consistent across trials. If the benefit of the Allo cue is independent of its spatial nature, it is not surprising that hippocampal rats showed the benefit. Therefore, in this experiment, all scenes were made to vary randomly in absolute brightness. The pattern of relative brightness and spatial layout of parts within a given scene stayed the same so that the scene retained its identity across trials. This was achieved by having three different palettes, i.e., mappings of logical grays to absolute luminance. In the brightest palette, the luminance of gray level 12 was about 7 candelas per square meter (cd/m²) and that of gray level 4 was 0.06 cd/m². In the dimmest, corresponding luminance was 3 and 0.025 cd/m².

### Table 1

<table>
<thead>
<tr>
<th>A-P</th>
<th>M-L (from brain surface)</th>
<th>D-V</th>
<th>Vol (μl)</th>
</tr>
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<tbody>
<tr>
<td>−6.3</td>
<td>±5.8</td>
<td>−6.9</td>
<td>0.05</td>
</tr>
<tr>
<td>−6.7</td>
<td>±4.7</td>
<td>−7.4</td>
<td>0.025</td>
</tr>
<tr>
<td>−6.7</td>
<td>±6.0</td>
<td>−5.8</td>
<td>0.075</td>
</tr>
<tr>
<td>−7.2</td>
<td>±4.5</td>
<td>−6.6</td>
<td>0.05</td>
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<td>−7.2</td>
<td>±5.8</td>
<td>−5.8</td>
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<tr>
<td>−8.7</td>
<td>±4.8</td>
<td>−3.6</td>
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NMDA, A-P, anteroposterior; M-L, mediolateral; D-V, dorsoventral.
cd/m², the other palette being intermediate. A given scene always used the same set of logical grays, but each half-scene was displayed in a palette chosen at random from the three; thus, absolute brightness of a given scene could vary more than twofold across trials. This degree of variation exceeded that normally found between and within monitors and therefore compensated for the fact that any constant scene which had an added Allo cue always appeared on the same pair of monitors.

**Procedure**

**Activity testing**

This took place about 2 weeks after surgery and was intended as a preliminary check on adequacy of lesions (rats in the FX group were expected to show increased locomotion). A single 2-h test of locomotor activity in the wire cage was carried out in the dark, with the rats not food-deprived. To ensure that beam interruptions reflected ambulation, the two beams had to be broken in succession (a crossover) to make one count. The total number of crossovers in 2 h was the measure of activity.

**Maze pre-training**

All rats were first trained to collect food pellets from any of the three dimly lit food trays and to approach any maze arm in which a bright horizontal line was displayed; the horizontal line was used as a trial start signal during later problems. Food tray and start signal training required 6 sessions of 5–15 min.

Each lesion group was randomly divided into an Early subgroup (7 from group RH, 7 from the FX group and 5 SH controls) and a Late subgroup (6 RH, 7 FX, and 4 SH). The Early subgroups received minimal pre-training with the constant-negative paradigm, comprising two constant-negative problems each with one constant scene; this pre-training lasted around nine 80-trial sessions on average. The two constant scenes for the two problems were chosen randomly from the 120 available; variable scenes were randomly chosen from the 119 scenes other than the constant. The pre-training of the Late subgroups consisted of a separate experiment during which they learned 11 different constant-negative problems. The first two were similar to the Early subgroups' pre-training problems; the rest used different kinds of constant and variable stimuli from the scenes to be used in the present study (see Gaffan et al., 2001, for details). This series of problems occupied more than 100 sessions, spread over 5–8 months. After completing them, the rats took part in the present study. The Early subgroups were 7–8 months old when they started the main experiment, the Late subgroups were 12–15 months old.

The procedure for each constant-negative pre-training problem was as follows. A problem consisted of a series of 80-trial sessions in which the same constant scene appeared on every choice trial, accompanied by a variable scene that was different on every trial of a session. In the first trial, one arm was randomly designated the start arm, signaled by a white horizontal stripe. When the rat entered this arm, the constant and variable scenes were displayed, one in each of the other two arms, the constant being in the arm to the left or right of the start arm at random. Choice of the variable was correct. If the rat chose the arm containing the variable, two 45-mg food pellets were dispensed, this arm was designated the start arm for the following trial, and the next trial began 2 s after the rat had collected the reward. If the rat made an error by choosing the constant, both scenes immediately disappeared. Again, the arm chosen became the start arm for the following trial, but there was an additional 3-s delay before the next trial could begin. (If the rat had left the new start arm during this time, the white stripe was displayed there to call him back.) There was no correction procedure after errors.

At the beginning of each session, before the block of choice trials, there were two “errorless reminder” trials in which the rat could choose between the constant and a brightness-matched blank screen, but approaching the blank screen had no effect. On the first of these trials, approaching the constant resulted in delivery of one food pellet, on the second, approaching the constant terminated it without reward. Thus the errorless trials followed the normal rule that the constant was rewarded when relatively unfamiliar (having not been seen for more than a day) and nonrewarded throughout the rest of the session when it was familiar. The errorless trials were not included in data analysis.

Each problem continued until a criterion of 80% correct choices in two successive sessions was attained, or eight sessions had been completed. The next problem with a new constant scene began in the following session. Note that the constant-negative paradigm is a completely general procedure that can use any set of visual stimuli. Each pre-training problem required the rat to learn a fresh constant stimulus.

There was no systematic basis for positive or negative transfer between the pre-training problems and the problems to be learned in the main experiment. The major difference between pre-training and the main experiment (apart from the different stimuli) was that spatial cues varied irrelevantly throughout pre-training. Only during the main experiment were spatial cues added in some problems.

**Main experiment**

The rats learned a sequence of eight constant-negative problems in which each problem had two constant scenes, rather than one as in pre-training problems. Across all trials of a problem, the two constants appeared in quasi-random alternation. Each appeared together with a variable scene that was different on every trial, with a reward given for avoiding either constant scene and approaching the variable, as usual. The start arm for each trial was predefined and was signaled by the horizontal-striped stimulus; when the rat entered the start arm, constant and variable scenes were presented in the other arms. A correct choice was rewarded with three pellets, and the interval until the next start signal appeared was about 2 s. After an error, the interval was prolonged by 4 s. The series of choice trials was preceded by four errorless reminder trials, as described in the section on Pre-training, where each constant appeared twice, first with a single-pellet reward then with no reward.

Scenes were drawn from the set of 120 described in the section on Stimuli. Any scenes that had been used as constants in the early pre-training problems were not reused as constants. Variable
scenes were randomly sampled from all 118 scenes that were not constants for the current problem. As before, no scene appeared more than once per session as a variable scene.

Each block of four problems comprised one each of four types—Allo + Ego, Ego, Allo, and No-Cue (NC), illustrated schematically in Figure 1. In problems with an added Allo cue (type Allo, or type Allo + Ego), each constant always appeared in a particular arm, in other problem types, the arm in which it appeared varied across trials. In problems with an added Ego cue (Ego, or Allo + Ego), a given constant always appeared in the same direction (left or right) from the start arm; in other problem types, its direction varied. NC problems had no added spatial cue.

Problems of type Allo + Ego had to have the same start arm on every trial (cf. Fig. 1), while in types Allo and Ego, the start arm necessarily varied across trials. NC problems could have employed either a fixed or a variable start arm. So that Allo + Ego would not be unique in this respect, a fixed start arm was used for NC problems as well. Where a fixed start arm was employed, the particular arm used varied between rats and across problems within rats. Similarly, the particular pair of arms where the constants appeared in Allo problems varied between and within rats; the same pair of arms was never repeated in consecutive problems.

The four problem types were given in different orders to different rats, using an approximate $4 \times 4$ Latin square design. It could not be perfect, as the group sizes were not multiples of four. The order of types in the second block, Problems 5–8, was different from that in the first block. Eight pairs of scenes were selected randomly from those available, the only constraint being that one member of each pair had a dark gray background and one a light gray background. The pairs were allocated as constants to the eight problems learned by each rat, in an effort to counterbalance assignment of particular constant-scene pairs to problem types, using independent Latin squares. No attempt was made to control the similarity of the scenes used as constants in different problems, so positive and negative transfer across problems, based on accidental similarities among scenes, was a possibility. To minimize the degree of transfer, the assignment of dark- and light-background constants to left- and right-sided directions in problems with an Ego cue, or to particular arms in problems with an added Allo cue, was counterbalanced within rats.

Each problem comprised a series of 80-trial sessions, and was run for a minimum of four sessions, to allow four-session learning curves to be drawn. If a criterion of learning had not been reached at that point, further sessions were run until it was attained. The criterion was that the rat should score 80% correct on each constant scene separately, across the last 50 trials of two successive sessions. This was slightly more stringent than the criterion set by Gaffan et al. (2000a), to reduce the variability of the errors-to-criterion measure. The reason for basing criterion only on the last 50 trials was that rats commonly show a “warm-up” effect, making more errors early in a session, so omitting the early trials from the criterion sample produced more reliable estimates. However, all trials were included when measuring performance during the first four sessions.

RESULTS

Histology

Retrohippocampal lesions

All rats in the RH group displayed substantial bilateral cell loss in both the medial and lateral entorhinal areas (Fig. 2). Generally, the lesion extended from the level of the temporal pole of the hippocampus to the level of the hippocampal flexure. The extent of the lesion was greatest in ventral areas where entorhinal cell loss was complete, or virtually complete, in all 13 animals. There was extensive cell loss in both the deep (IV–VI) and superficial layers (I–III), although the damage extended farther dorsally in the superficial layers. As the lesion extended dorsally, the cell loss became progressively more restricted to the superficial layers. All subjects also showed a minimal amount of cell loss in ventral areas of the perirhinal cortex.

In addition, there substantial cell loss in the extrasubicular cortices (pre- and parasubiculum) and in the subiculum proper. Again, the amount of subicular and extrasubicular cortical damage was much greater in ventral areas and was invariably complete at these levels. The dorsal subiculum was generally spared in all subjects. There was also subicular sparing at the ventralmost extent of the lesion (at the temporal pole of the hippocampus), with cell loss restricted to entorhinal areas at this level.

Therefore, the RH lesions comprised substantial entorhinal and subicular damage, especially in more ventral areas where the lesions were complete. Most lesions were similar in terms of size and location to those reported by Good and Honey (1997), Yee et al. (1995), and Pouzet et al. (1999), although there may have been slightly more subicular cell loss in the current study than in the latter two. All cases were considered acceptable in terms of the extent of retrohippocampal damage; the right column of Figure 2 shows the smallest lesion.

Two animals had no hippocampal damage at all. In six cases (Fig. 2, center column), the lesion extended to the very edge of the hippocampus, but the resulting damage was minimal and was restricted to the caudalmost part of the dentate gyrus and CA1 subfield in the ventral hippocampus. In five cases (Fig. 2, left column), the hippocampal damage was more substantial, encompassing a significant portion from the caudal end of DG and some damage to CA1. Again, the cell loss was limited to the ventral hippocampus, with no damage to the dorsal hippocampus whatsoever. Although such small hippocampal lesions would be expected to have a negligible effect on their own, we thought it important to focus on selective retrohippocampal lesions for purpose of comparison with the hippocampal lesion group of Gaffan et al. (2000a). Therefore, the latter five animals, three in the Early and two in the Late subgroup, were omitted from analysis. The resultant Early and Late subgroups of the RH group each contained four rats.
FIGURE 2. Reconstructions of the retrohippocampal lesions. Maximal (left column), representative (center column), and minimal (right column) extent of the lesions seen in horizontal sections between −3.10 mm from the brain surface (top row) and −8.82 mm from the brain surface (bottom row) (plates 116 and 93, respectively, of Paxinos and Watson, 1998).
Fornix transection

All 14 rats in the FX group displayed substantial damage to the fimbria fornix (Fig. 3). In addition, there was consistent damage to the lateral septum, and also a small amount of unilateral damage to the cortex and to the caudate putamen along the route of entry of the forceps. In four of the animals in this group, the point of entry of the forceps was slightly more caudal; consequently, there was a small amount of damage to the dorsalmost portions of the thalamus (Fig. 3, right column). This included bilateral damage to the stria terminalis. In addition, there was damage to the dorsal half of the rostral regions of both the anterodorsal and anteroventral thalamic nuclei. However, in the remaining 10 rats, the lesion was located slightly more rostrally, and there was no evidence of any thalamic damage (Fig. 3, left column).

Three of the four rats that sustained thalamic damage were in the Late subgroup, and one was in the Early subgroup. The three in the Late subgroup showed evidence of severely impaired learning during pre-training, failing to reach criterion in the final problem which meant that they were excluded from the separate study of which the pre-training problems formed a part (Gaffan et al., 2001). All four rats, whether tested Early or Late, did prove able to complete the present experiment. However, we excluded them from analysis because the results from the Late subgroup’s pre-training suggested that the extrafornical damage was influential. The resultant Early and Late subgroups of the FX group contained six rats and four rats, respectively.

Behavioral Testing

One rat in the Early subgroup of the FX group became ill and completed only seven of eight problems, so the final number of rats in that subgroup was five.

Postsurgical activity test

The mean activity scores (crossovers during 2 h) were 397.7 for the RH group, 680.9 for the FX group and 235.4 for the SH group, $F(2,23) = 8.05, P < 0.01$. By a Newman-Keuls post-hoc test, only the rats in the FX group were more active ($P < 0.05$) than the SH controls.

Constant-negative pre-training

Early subgroups. The mean total numbers of sessions to learn both pre-training problems (including criteria sessions) were 7.5 (RH group) 9.8 (FX group) and 7.8 (SH group). The lesion groups did not differ significantly, $F(2,11) = 1.09$.

Late subgroups. Details of performance of these subgroups in the preceding study, which constituted the pre-training, were reported by Gaffan et al. (2001). Briefly, the FX group were slightly but not significantly slower at learning the 11 constant-negative problems than the RH and SH groups, which learned at a similar rate. The total numbers of sessions taken up by the whole preceding study were on average 111 (RH group), 129 (FX group), and 106 (SH group), which did not differ significantly ($F = 1$). The mean time elapsed from the start of training in the maze to the beginning of the present experiment (after completion of pre-training) was 6.7 months for the RH group, 6.2 months for the FX group, and 5.6 months for the SH group, which again did not differ ($F < 1$).

Main experiment

Two types of learning measure were collected for each problem—errors to criterion (discounting the two criteria sessions), and percentage correct performance in the first four sessions, as each problem was run for a minimum of four sessions. Errors rather than trials to criterion are reported, to be comparable with Gaffan et al. (2000a).

Errors to criterion: Early and Late subgroups. The errors-to-criterion data are illustrated in Figure 4, which shows results from the Early and Late subgroups separately and combined (All). (Scores are averaged from problem blocks 1 and 2, i.e., the first and second replication of the four cue types, because there were no significant interactions with Block, see below.)
The data for the sham control rats (Fig. 4, top) show the same pattern of cue effects observed by Gaffan et al. (2000a) in their sham control group. These rats learned faster when either a pure Allo or a pure Ego cue was added (Allo vs NC, Ego vs NC) and faster still when an Allo was added to an Ego cue (Allo + Ego vs Ego). Moreover, the pattern of cue effects was very similar in Early and Late subgroups, although the Late subgroup (which had lengthy prior experience with the constant-negative task) learned faster overall.

Both lesion groups clearly benefited from pure Allo and pure Ego added cues, just as the sham groups did, and this was true in Early and Late subgroups, as suggested in Figure 4 (middle, bottom). The main hint of a difference between lesioned and sham groups appears in the Allo + Ego versus Ego comparison. When tested Early, neither retrohippocampal- nor fornix-lesioned rats benefited from the addition of an Allo to an Ego cue. When tested Late, the lesioned groups did appear to benefit, although not so much as the sham controls. However, these apparent group differences in cue effects proved not to be statistically reliable, as reported below. It may also be noted from Figure 4 that the Early and Late subgroups learned at similar rates within both lesioned groups (unlike the controls, where the Late subgroup learned faster) and that both lesion groups, particularly fornix, learned slightly slower overall than the controls; however, these differences were small, so the cue effects can be assessed against similar baselines in all three groups.

Analysis focused on the effects of different types of added cue and on whether these were affected by the lesions. We also wished to know whether there were overall differences between the lesion groups, whether Early versus Late training affected the groups differently, and whether time of training modified the effects of the added cues.

Omnibus tests on the Cue effect are of limited interest because they do not give separate information about the Allo and Ego cues. So Cue effects were also broken down into three designed contrasts: pure Allo (Allo vs NC), pure Ego (Ego vs NC) and Allo added to Ego (Allo + Ego vs Ego). An alternative approach would be to treat the four conditions as a 2 × 2 factorial (Allo present/absent × Ego present/absent). Such factorial analyses were reported in our previous study. They have the disadvantage, however, that main effects are difficult to interpret; for example, the main effect of the Allo cue is the average of two distinct effects, adding an Allo cue alone (Allo vs NC) and adding Allo to Ego (Allo + Ego vs Ego). It is more informative to test separately the pure Allo, pure Ego, and combined effects. We chose to test the combined effect of Allo added to Ego (Allo + Ego vs Ego) rather than Ego added to Allo (Allo + Ego vs Allo) because rats with hippocampal system lesions are impaired in allocentric but not egocentric navigation (see Introduction), therefore Allo-cue effects observed after lesions are more interesting than Ego-cue effects.

Our objective was to examine the effects of each lesion in detail, so we compared each lesion group separately with the sham group. This approach incurs some redundancy, but has the advantage that specific effects of each lesion are clearly brought out. Therefore, ANOVAs were run to compare first the retrohippocampal group with sham controls, then the fornix group with controls. The between-subject factors were lesion (RH vs SH or FX vs SH) and time of training (Early/Late). Within-subject factors were the type of added Cue (4 types—Allo + Ego/Allo/Ego/No-Cue), and problem Block, i.e., the first versus the second replication of the four cue
The primary purpose of the first set of analyses was to examine whether Early versus Late testing moderated lesion or Cue effects. Therefore, we mention only those effects that are relevant to that question. Others are reported below, from the analyses that combined both subgroups.

The comparison of the retrohippocampal and sham groups yielded no main effect of Time, \( F(1,13) < 1 \), but a significant Lesion \( \times \) Time interaction, \( F(1,13) = 4.02, P < 0.05 \). This arose because the sham control rats learned faster when tested Late (after much pre-training) than when tested Early, but the retrohippocampal-lesioned rats did not benefit from pre-training. There was also a significant Cue effect, \( F(3,39) = 13.89, P < 0.001 \), but Cue had no interactions involving Lesion or Time, \( F(1,13) < 1 \). Figure 4 suggests that, if there was any difference between the lesion groups, it was confined to one cue manipulation (addition of an Allo to an Ego cue). However, the analysis of separate contrasts did not confirm this. All three contrasts (Allo vs NC, Ego vs NC, and Allo + Ego vs Ego) were significant, \( P < 0.05 \), but none was involved in any significant interaction with either Lesion or Time, \( F(1,13) \leq 1.06 \). These effects are described more fully below in analyses of combined subgroups. Therefore, the retrohippocampal and sham groups both benefited to a similar extent from the addition of Allo and Ego cues. The ostensible weakening of the benefit of Allo added to Ego cues in group RH when tested Early was not great enough to reach statistical significance, perhaps because of the small numbers in each subgroup.

Broadly similar conclusions emerged from the comparison between fornix-transected and sham control groups. The fornix group (unlike the retrohippocampal) showed slightly impaired learning overall – Lesion \( F(1,14) = 5.78, P < 0.05 \). The effect of Cue was significant, \( F(3,42) = 12.98, P < 0.001 \), but there were no significant interactions involving Cue, Lesion or Time, \( F(1,14) < 1 \). Tests on the three separate Cue contrasts showed that the Allo versus NC and Ego versus NC contrasts were significant, \( P < 0.01 \), and the Allo + Ego versus Ego contrast was not, but importantly none of these interacted with either Lesion or Time, \( F(1,14) \leq 1.25 \). Fuller details from the combined subgroups will be presented below. Again, the apparent difference between Early and Late subgroups in the fornix group, relative to controls, was not confirmed statistically.

**Errors to criterion: combined subgroups.** Because of the lack of statistical support for a difference between Early and Late subgroups, subsequent analyses combined the subgroups to form single groups RH (\( n = 8 \)) FX (\( n = 9 \)) and SH (\( n = 9 \)). The errors-to-criterion scores of these groups are labeled “All” in Figure 4. Figure 4 suggests that each combined lesion group showed as much benefit as controls did from a pure added Allo or Ego cue (Allo vs NC, Ego vs NC) but did not benefit as much as the shams when an Allo was added to an Ego cue (Allo + Ego vs Ego). This pattern resembled that shown by the hippocampal-lesioned group in Gaffan et al. (2000a). We analyzed the data from the combined groups by carrying out two Lesion \( \times \) Cue \( \times \) Block ANOVAs, the first comparing groups RH and SH, the second groups FX and SH. These yielded effects which were similar in most respects to the Lesion \( \times \) Time analyses reported above, but the main purpose was to gain greater power to detect whether any of the cue effects, particularly that of an Allo added to an Ego cue, was reliably weakened by either the retrohippocampal or the fornix lesion.

The comparison of groups RH and SH yielded a nonsignificant Lesion effect, \( F(1,15) = 1.83, \) an effect of Block, \( F(1,15) = 11.48, P < 0.01 \) (because both groups showed a practice effect, learning faster in the second block of four problems than in the first) and a Cue effect, \( F(3,45) = 15.75, P < 0.001 \). The Cue effect did not interact with Lesion, \( F(1,15) < 1 \), and there were no interactions involving Block, largest \( F(3,45) = 2.02 \).

To discover whether there were group differences on any of the specific cue effects, the contrasts Allo versus NC, Ego versus NC and Allo + Ego versus Ego (and their interactions with Lesion) were tested separately. The results are summarized in Table 2, section 1, left columns.

In Table 2, tests on simple effects of cues are directional, because added cues could not plausibly impair learning, they could only enhance it, and all differences were in the expected direction. Therefore, one-tailed significance levels are reported. However, because the impact of lesions on cue enhancement is unknown, two-tailed significance levels are reported for Cue \( \times \) Lesion interactions. Table 2 shows significant main effects \( [F(1,15) \geq 8.77] \) for all three comparisons, but none of these interacted with Lesion (\( F(1,15) < 1 \)). Thus, the small differences in pattern of cue effects between the RH and SH groups, suggested in Figure 4, were again found to be unreliable; the RH group showed comparable benefits of Allo and Ego cues as did the SH group.

Comparison of the FX and SH groups showed that there was a significant Lesion effect, \( F(1,15) = 4.50, P < 0.05 \), because (as noted previously) learning in fornix-transected rats was slightly retarded on average. There was a Block effect, \( F(1,16) = 35.57, P < 0.001 \), and a Lesion \( \times \) Block interaction, \( F(1,16) = 5.07, P < 0.05 \), because the FX group improved more from block 1 to block 2 than did the controls; but Block did not interact with any of the Cue effects. The Cue effect was significant, \( F(3,48) = 14.59, P < 0.001 \), but did not interact with Lesion, \( F(1,16) < 1 \).

We decomposed the Cue effect as before, and its interaction with Lesion, into three specific contrasts (see Table 2, under 2. Fornix vs sham, left columns). Once again, there were strong main effects \( [F(1,16) \geq 13.19] \) of the Allo and Ego cues in isolation, whereas the effect of Allo added to Ego was weaker and not significant, \( F(1,16) = 1.78 \). The main finding was that none of the Cue effects interacted with Lesion, \( F(1,16) < 1.57 \). As with group RH, we conclude that the FX group showed enhancement by Allo and Ego cues to a similar extent as did the SH group, especially when the cues were added in isolation; the apparent group differences were not reliable.

**Performance in sessions 1–4: combined Early and Late subgroups.** Figure 5 illustrates the alternative measure of learning: performance during early sessions of new problems. Mean scores during the first four sessions of problems of all types are shown, with data averaged from both problem blocks. The data are also pooled from the Early and Late subgroups, because preliminary
analyses not reported here showed (in a similar way to the first analyses on the errors-to-criterion scores) that cue and lesion effects were not reliably moderated by the time of testing.

Figure 5 presents a similar impression to that shown in Figure 4. The sham controls performed better when a pure Allo or pure Ego cue was added (Allo vs NC, Ego vs NC) and better still when both cues were added (Allo + Ego). The RH and FX groups’ performance was enhanced by pure Allo and pure Ego added cues, but there was less obvious benefit of Allo added to Ego cues.

These data were analyzed similarly to the errors, with Sessions as an additional within-subject factor. Two Lesion (2) × Cue (4) × Block (2) × Session (4) ANOVAs were carried out, one comparing the RH and SH groups and one comparing the FX and SH groups. Each was followed up by decomposing the Cue and Cue × Lesion effect into three contrasts: pure Allo, pure Ego, and Allo + Ego versus Ego. All analyses yielded highly significant effects of Sessions, F > 50.0, reflecting the steep upward slope of all graphs in Figure 5.

### TABLE 2.

**Designed Contrasts on Cue Effects, Group Comparisons: Pure Allo, Pure Ego, Allo Added to Ego**

<table>
<thead>
<tr>
<th>Group Comparisons</th>
<th>Percentage correct, sessions 1–4a</th>
<th>Errors to criteriona</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F(Cue)</td>
<td>F(Cue × Lesion)</td>
</tr>
<tr>
<td>1. Retrohippocampal vs sham df 1,15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allo vs NC</td>
<td>20.98***</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ego vs NC</td>
<td>10.00**</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Allo+Ego vs Ego</td>
<td>4.87*</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2. Fornix vs sham df 1,16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allo vs NC</td>
<td>18.66***</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ego vs NC</td>
<td>13.19**</td>
<td>1.06</td>
</tr>
<tr>
<td>Allo+Ego vs Ego</td>
<td>1.78</td>
<td>1.57</td>
</tr>
<tr>
<td>3. Hippocampal vs sham (Gaffan et al., 2000a) df 1,14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allo vs NC</td>
<td>3.04</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ego vs NC</td>
<td>4.99*</td>
<td>1.67</td>
</tr>
<tr>
<td>Allo+Ego vs Ego</td>
<td>&lt;1</td>
<td>3.37†</td>
</tr>
</tbody>
</table>

df, degrees of freedom; Allo, allocentric; Ego, egocentric; NC, no cue; NC, no cue problems; Allo vs NC, pure Allo cue effect; Ego vs NC, pure Ego cue effect; Allo+Ego vs Ego, Allo cue added to Ego cue.

Significance tests on simple cue effects are one-tailed, interaction tests are two-tailed; *P < 0.05; **P < 0.01; ***P < 0.001; †P < 0.10.

**FIGURE 5.** Percentage of correct choices in the first four sessions of the four problem types by the sham controls and two lesion groups. Vertical bars represent 2 × standard error of difference between problem types, for that group.
Comparison of the RH versus SH groups showed no overall Lesion effect, F < 1, but significant effects of Block, F(1,15) = 46.03, P < 0.001, and Cue, F(3,45) = 18.22, P < 0.001. There was a Block × Session interaction, F(3,45) = 2.89, P < 0.05, which arose because rats learned faster in block 2 so there was a steeper slope across sessions, but Block did not interact with any other factors and has been ignored in Figure 5. The Cue effect did not interact with Lesion, F(3,45) = 1.14 or with any other combination of factors, F (9,135) ≈ 1.56.

The results of breaking down the Cue and Cue × Lesion effects into three designed contrasts are shown in Table 2 (under 1. Retrocortical vs sham, right columns). The pattern is closely similar to that seen with errors to criterion. Both pure Allo and pure Ego added cues produced strong benefits [F(1,15) ≈ 8.37] that did not differ between groups. The benefit of adding Allo to Ego was also significant, and showed more sign of differing between Lesion groups, interaction F(1,15) = 2.87, but not significantly.

Comparison of the FX versus SH groups also yielded similar results to the analysis of errors to criterion. In the four-way analysis, the FX group performed at a lower level overall than the SH group, although not significantly in this case, F(1,16) = 2.27. There were significant effects of Block [F(1,16) = 65.91, P < 0.001] Block × Lesion [F(1,16) = 6.20, P < 0.05] Block × Session [F(3,48) = 4.30, P < 0.05] and Block × Session × Lesion [F(3,48) = 3.11, P < 0.05], reflecting the fact that both groups performed better in block 2, and therefore showed steeper learning curves in block 2, but both practice effects were more pronounced in the FX group. However, Block did not interact with Cue or any combination involving it, F(9,144) ≈ 1.64, so the block differences are irrelevant to the major effects of interest. Cue was significant overall, F(3,48) = 17.37, P < 0.001, but did not interact with Lesion, F < 1.

Table 2 (under 2. Fornix vs sham, right columns) shows the results of breaking the Cue and Cue × Lesion effect into three contrasts. As with the RH group, this analysis gives similar results to the errors measure. The pure Allo and Ego effects were significant, F(1,16) ≈ 15.41, and did not differ between Lesion groups, F < 1; the benefit of adding Allo to Ego was weaker but significant, F(1,16) = 4.28, but the difference between Lesion groups in this respect was nonsignificant, interaction F(1,16) = 1.59.

In sum, these analyses confirm that both lesion groups, RH and FX, showed a strong benefit of both Allo and Ego cues when these were added in isolation, to a similar extent as did sham controls. Figures 4 and 5 give the impression that adding an Allo cue to an Ego cue was of greater benefit to sham controls than to the lesioned rats, but we did not confirm that the groups differed reliably in this respect.

**Comparison with effects of hippocampal lesions.** As mentioned above, the effects of added Allo and Ego cues on the RH and SH groups appeared similar to those found after hippocampal lesions by Gaffan et al. (2000a) (experiment 1). However, Gaffan and colleagues analyzed the cue effects in a slightly different way, so their statistical findings cannot be directly compared with ours. To permit comparison, we reanalyzed the results of the earlier study, splitting the Cue effect into the same three contrasts as in the present study: Allo versus NC, Ego versus NC, Allo + Ego versus Ego. The results are shown in Table 2 (under 3. Hippocampal vs sham; errors on the left, percentage correct in early sessions on the right). The tests are shown the hippocampal-lesioned group of Gaffan et al. (2000a) with the sham controls in that study. Gaffan et al. (2000a) used a less stringent criterion of learning than here and reported percentage correct only in the first three not four sessions, so the tests are not quite equivalent to ours. Furthermore, their hippocampal group were severely impaired in learning overall, so the effects of Allo and Ego cues were being assessed against different baselines. Even so, several conclusions can be drawn from Table 2. Like the RH and FX groups, the hippocampal group benefited from pure Allo and pure Ego added cues as much as did controls (significant Allo and Ego effects, which did not interact with Lesion). However, there was evidence of a Lesion difference in the benefit of Allo added to Ego [errors F(1,14) = 3.37, P < 0.10; sessions 1–3 F(1,14) = 7.32, P < 0.05]. The interaction took the form that the sham controls did benefit from adding Allo to Ego, the hippocampal group did not. This is the same pattern as can be seen in the data from groups RH and FX (Figs. 4 and 5), although the corresponding interactions did not reach significance in the present study.

**DISCUSSION**

We asked whether rats with retrohippocampal lesions or fornix transection were capable of incidental learning about associations between visual stimuli (scenes) and two types of spatial cue, allocentric (arms of a Y-maze) and egocentric (turn directions relative to the body axis). Our indirect test for the presence of this incidental spatial learning was that rats should learn faster to discriminate two familiar scenes (constants) from unfamiliar scenes (variables) when the constants were consistently associated with allocentric or egocentric cues (or both) than when the constants varied randomly in their allocentric or egocentric cue properties. We found that both groups of lesioned rats clearly showed incidental spatial learning. In the simple case in which only one cue was added, either Allo alone or Ego alone, the spatial learning was as clear in the lesion groups as it was in the sham control rats. Although both lesion groups showed apparently weaker learning about an allocentric cue than controls did when it was accompanied by an egocentric cue, especially the Early subgroups which had more limited experience with the maze, that overshadowing was not statistically reliable. The pattern of findings is broadly similar to that observed by Gaffan et al. (2000a) in rats with hippocampal lesions.

This study reinforces our earlier findings in other ways. First, the two lesion groups learned at a normal rate (retrohippocampal), or close to normal (fornix), whereas the previous hippocampal-lesioned group had been severely impaired in learning overall. In the present study we can be sure that the similar absolute size of the cue-enhancement effects in lesioned and control rats is not a consequence of different baseline levels. Second, the fact that Allo-cue enhancement emerged despite random manipulation of the
brightness of the scenes implies that the enhancement did reflect the spatial nature of the added allocentric cue and was not an artifact of particular pairs of monitors having idiosyncratic brightness characteristics (cf. Materials and Methods section).

The study also extends our previous findings. It shows, first, that neither input–output route from the hippocampus—the fornix or the entorhinal cortex (which was completely or near-totally removed by the retrohippocampal lesions)—is, on its own, crucial for mediating incidental learning about either allocentric or egocentric cues. Second, learning about the spatial cues when they were added in isolation (pure Allo or Ego enhancement) displayed by the lesioned rats was equally strong, whether they had brief or very long prior experience with the maze. Therefore, extended exposure to the maze and the constant-negative task, such as the hippocampal group had undergone in Gaffan et al. (2000a), is not a necessary condition for lesioned rats to show incidental spatial learning. There was some indication that learning about an added Allo cue might be weakened when an Ego cue was also present and the rats had only brief prior experience, but this finding was statistically ambivalent.

The focus of this discussion is on allocentric place cues, because the surprising feature of these experiments is that rats with lesions (especially hippocampus or fornix) that have a devastating effect on allocentric place learning or navigation, show normal or near-normal incidental learning about allocentric cues in our paradigm. Although we did not show directly that the fornix-transected group in this study were impaired in allocentric navigation as such, we know that fornix transection severely disrupts allocentric spatial working memory in our apparatus (Gaffan and Eccott, 1997). The present fornix-transected rats were hyperactive as expected, and differed from controls to the same extent as groups with comparable lesions (e.g., anterior thalamus) in a separate scene-learning experiment (Gaffan et al., 2001), implying that the lesions were behaviorally effective.

How might one explain the dissociation between navigating by spatial cues and incidental learning about spatial cues, as required here? To demonstrate Allo-cue enhancement in our paradigm, rats must encode the cues that define the places; concurrently learn two associations between specific visual scenes and places; and relearn different associations in eight successive problems, switching between problems for which place cues are relevant and problems for which they are irrelevant. What they need not do is learn to approach specific places. One account might be given in terms of theories that place heavy emphasis on the navigational role of the hippocampal system, i.e., “getting there,” as opposed to “knowing where” (Whishaw et al., 1995). Whishaw and colleagues have argued that hippocampal or fornix lesions disrupt the ability of rats to navigate by allocentric cues, specifically because they interfere with a path-integration or dead-reckoning process, but leave intact their encoding of or associative learning about allocentric cues, provided that the task does not call for path integration (Whishaw and Jarrard, 1996; Whishaw and Tomie, 1997; Whishaw and Maaswinkel, 1998; Whishaw and Gorny, 1999). The proposals made by Whishaw and colleagues have been disputed (White and Ouellet, 1997; Alyan and McNaughton, 1999; McDonald and Hong, 2000) and our results, although consistent with their theory, are far from providing direct evidence for it.

A second possibility is that the lesioned rats are indeed impaired in non-navigational aspects of place learning, i.e., in encoding and/or associative learning about places, but that the encoding required in our task is so simple that it does not tax their abilities. There are only three places (maze arms), and rats have the opportunity to learn the characteristics of those places thoroughly, even within the few sessions provided to the Early subgroups in this experiment. However, we do not consider the small number of places, or duration of exposure, crucial. Hippocampal- or fornix-transected rats are stably impaired in learning to approach a single place in Morris’s water pool, or one of two places such as the arms of a T-maze in spatial reversal or alternation tasks, despite extensive training. Furthermore, even though only a few places are available here, the specific visual-place associations change in every problem and, as noted above, the rats learn sometimes to ignore place cues (in Ego or No-Cue problems) and then use them again in Allo or Allo + Ego problems. Thus, the learning required is far from simple.

A third possibility is that learning about arms of the Y-maze does not satisfy the criteria of O’Keefe and Nadel (1978), who distinguished “locale” or place learning from “taxon” learning, namely that place learning does not “require the presence of local cues” (Morris, 1981). The three maze arms have different proximal or intramaze features (e.g., their walls, floors, and monitor surroundings), and rats could rely on those, rather than the distal or extramaze cues that are normally thought to define allocentric places. This point deserves consideration because, even though the three arms are very similar in terms of these intramaze features, some intramaze differences, such as odors, might be salient to rats. Our results raise the question of whether the lesioned rats encode the places in a more impoverished way than the sham controls. If only intramaze cues are available to lesioned rats, whereas normal rats can use both intramaze and extramaze (true place) cues, one would expect the lesioned rats to show less Allo-cue enhancement than controls (but comparable Ego-cue enhancement). Inspection of Figure 4 suggests that although pure Allo and pure Ego enhancement are (coincidentally) equal in controls, the Allo-cue benefit is numerically slightly smaller in both lesion groups, particularly fornix-transected. But there is no sign that the pure Allo enhancement was statistically weaker in either lesioned group. These observations suggest that the lesioned groups did not differ grossly from controls in terms of the wealth of cues available. However, the evidence is not conclusive either way. It would be desirable to confirm these findings in a setting where the place cues were unequivocally distal.

In short, our results are consistent with Whishaw and Jarrard’s contention that navigating to places and knowing what stimuli occur in those places have different neural bases, and it is only the former ability that is sensitive to hippocampal damage. However, they are open to alternative interpretations.

Finally, we consider the fact that retrohippocampal- and fornix-lesioned groups behaved very similarly. Both fornix transection and hippocampal lesions impair allocentric navigation (and fail to impair egocentric navigation), as reviewed in the Introduction; so
the results from the FX group are best viewed as a quasi-replication of the surprising absence of a hippocampal lesion effect on allocentric learning reported by Gaffan et al. (2000a). However, as described in the Introduction, entorhinal and retrohippocampal lesions typically have different effects from hippocampal or fornix lesions both in conventional spatial paradigms, and other forms of spatial and nonspatial learning. Can any conclusions be drawn from the pattern observed in this experiment?

Before answering this question, we should consider the possibility that the failure of retrohippocampal lesions to produce behavioral impairments reflects partial recovery of entorhinal function. Our main experiment started two or more months after surgery, and there is some degree of reinnervation of the dentate gyrus from surviving entorhinal cells within 2 weeks after a lesion (Reeves and Smith, 1987). This matter was considered in detail by Bannerman et al. (2001b), who argued that, not only could such a process not explain the pattern of spared and impaired function in their experiments, but it is unlikely to have much impact in rats with bilateral lesions because the reinnervation is transmitted via crossed fibers from the cortex contralateral to the lesion, and for this reason has been observed mainly after unilateral entorhinal lesions. We will assume, therefore, that the entorhinal cortex was in essence non-functional during behavioral testing.

It was possible that we might observe an effect of retrohippocampal lesions on our incidental spatial learning task, despite the fact that such lesions have little effect on conventional allocentric navigation. Our task requires rats to encode, and to learn new associations between, visual and spatial cues. It is known that lesions of cortical regions that project heavily into the hippocampus via entorhinal cortex (perirhinal and postrhinal cortex, in the rat) have effects on visual and spatial learning that are partly dissociated from those of hippocampal or fornix lesions. Neurotoxic lesions of perirhinal or postrhinal cortex (like entorhinal cortex) are reported to have either no effect (Bussey et al., 1999, 2000) or relatively mild effects (Liu and Bilkey, 1999, 2001) on allocentric navigation tasks that are severely disrupted by hippocampal or fornix lesions. Conversely, perirhinal lesions, separately or combined with damage to adjacent cortical areas, have been found to affect rats’ and monkeys’ performance in tasks such as object recognition (Save et al., 1992; Mumby et al., 1995; Bussey et al., 2000) and visual configurual or paired-associate learning (Murray et al., 1993; Buckley and Gaffan, 1998; Eacott et al., 2001) differently from fornix transection or hippocampal ablation. The effects of selective entorhinal lesions on such tasks have not been reported in rats; however there is evidence from monkeys that conventional entorhinal lesions mildly impair object recognition in a manner distinct from either perirhinal or hippocampal lesions (Meunier et al., 1993, 1996; Leonard et al., 1995). Also relevant to our study are tasks that require rats to remember object-place associations. There is evidence that both perirhinal/postrhinal cortex and the hippocampus and fornix are involved (Save et al., 1992; Bussey et al., 2000), but the role of entorhinal cortex has not been investigated. In view of the complex and incompletely studied pattern of dissociations between the various cortical areas and the hippocampus itself, one could not rule out an involvement of entorhinal cortex in our incidental spatial learning paradigm. But, having established in this experiment that entorhinal cortex is not necessary, we will in a future study examine the effects of perirhinal and postrhinal lesions.

Could any current theories account for our findings? One theory that ascribes a distinctive role to entorhinal cortex is that of Myers et al. (1995). These investigators propose that both entorhinal cortex and the hippocampus proper achieve “representational compression” whereby the neural representations of events that co-occur become more similar, thus influencing subsequent learning about those events. They hypothesize that entorhinal cortex is specialized purely for “stimulus-stimulus compression,” the convergence of representations of stimuli that co-occur, whereas the hippocampus subserves different forms of compression (e.g., “stimulus outcome”) and yet other representational changes such as differentiation. The theory can account for the fact that entorhinal, but not hippocampal, lesions abolish latent inhibition, which Myers et al. explain in terms of compression of the representation of a conditioned stimulus (CS) with that of the context in which it occurs (Shobany et al., 2000). That description bears some resemblance to our Allo-cue procedure where rats have the opportunity to learn that a visual cue (constant scene) always co-occurs with a particular place (maze arm), so the model reported by Myers et al. might seem to predict abnormal learning after entorhinal lesions. However, that is not a clear-cut prediction, because our task presumably also involves differentiation (whereby the representations of constant scenes that occur in different places become more distinctive) and that, according to Myers and colleagues is a hippocampal, and not an entorhinal, function. They set out to model only classical conditioning, not instrumental learning and novelty discrimination as entailed in our paradigm. Therefore the lack of effect of the retrohippocampal (or indeed hippocampal and fornix) lesions is inconclusive.

Another attempt to characterize the role of entorhinal cortex proposes that it plays a role in “attentional modulation” (Oswald et al., 2001), of which latent inhibition might be regarded as a special case. Broadly, attentional modulation refers to the effects of past experience with a discrimination in which certain stimuli are relevant or irrelevant on the later learning of discriminations in which the relevance of those stimuli changes or stays the same, i.e., extradimensional or intradimensional shifts (cf. Oswald et al., 2001). As previously noted, our procedure requires rats to learn a series of problems in which the relevance of spatial cues changes from problem to problem, so one might expect the retrohippocampal-lesioned group to show abnormal learning to some extent, according to this account. However, the pattern of changing relevance of different types of spatial cues—Allo, Ego, or the combination—is so complex across the eight problems, and is so variable between subjects because of counterbalancing, that a systematic analysis is not feasible. The present experiment was not designed to show such transfer effects, but this paradigm could well be adapted for future investigation of the attentional modulation theory.

In conclusion, the outcome of this study is consistent with the surprising finding of Gaffan et al. (2000a) that hippocampal lesions had little or no effect on incidental learning about allocentric cues in our paradigm, as we obtained a very similar pattern of results after fornix transection. The lack of effect of retrohip-
pocampal lesions adds to the evidence that entorhinal cortex on its own is not critical for learning about spatial cues—again surprisingly, in view of its apparently crucial position in the reciprocal interactions between hippocampus and cortex. Resolution of this paradox calls for further study of the role of other hippocampal-cortical interfaces, such as the perirhinal/postrhinal cortex and subicular region, and for more detailed analysis of what cues rats use to learn our task, as well as how they progress between one problem and the next.

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