

BRIEF REPORT

Hippocampal Formation Lesions Impair Performance in an Odor–Odor Association Task Independently of Spatial Context

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The rodent hippocampal system is known to play an important role in memory. Evidence that this role is not limited to spatial memory has come from studies using a variety of non-spatial memory tasks. One example is the social transmission of food preference paradigm, a task in which rats learn an odor–odor association with no explicit spatial memory component. However, because training and testing in this task typically take place in the same environment, it is possible that memory for the spatial context in which odors are experienced during training is critical to subsequent retention performance. If this is the case, it might be expected that lesions of the hippocampal system would impair memory performance by disrupting the establishment of a representation of the training environment. We addressed this issue by training rats in one spatial context and then testing them either in the same or a different spatial context. Normal control rats performed equally well when tested in an environment that was the same or different from that used during training, and the retention impairment exhibited by rats with hippocampus plus subiculum lesions was equivalent in the two test environments. These results support the view that the hippocampal system is necessary for the flexible expression of nonspatial memories even when the spatial context in which the memory is acquired is not critical to retrieval. © 2002 Elsevier Science (USA)

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The hippocampus and anatomically related, adjacent structures (subiculum, entorhinal, perirhinal, and postrhinal/parahippocampal cortex) are known to be important for memory

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in humans, monkeys, and rodents (e.g., Squire, 1992). One influential hypothesis (O'Keefe & Nadel, 1978) states that, in rodents, the role of the hippocampus is limited to spatial memory. However, a number of studies have now shown that damage to the hippocampus can produce deficits in tasks that have no explicit spatial memory component (Sutherland & Rudy, 1989; Alvarez, Zola-Morgan, & Squire, 1995; Bunsey & Eichenbaum, 1995, 1996; Dusek & Eichenbaum, 1997, 1998). One such task is the social transmission of food preference (Galef & Wigmore, 1983; Strupp & Levitsky, 1984), a paradigm in which animals learn that a particular odorous food is safe to eat (and therefore preferable) following exposure to the odor on a conspecific's breath. Several studies have now shown that damage to hippocampus, particularly if the damage also includes the subiculum, produces impairments in retention performance on this nonspatial task (Winocur, 1990; Bunsey & Eichenbaum, 1995; Alvarez, Lipton, Melrose, & Eichenbaum, 2001; Clark, Broadbent, Zola, & Squire, 2002). Importantly, this task differs from more commonly used visual recognition memory tasks such as delayed nonmatching to sample in that it requires a "flexible" response, that is, a response different from merely avoiding or approaching a stimulus presented in the same circumstances as the original exposure. In the social transmission task, the training situation involves a social interaction (smelling odors on the breath of another rat) outside the context of feeding, whereas the retention test involves feeding behavior without a social context. We have proposed that it is this requirement for expressing the learning "flexibly" that makes task performance dependent on the hippocampus and subiculum (Bunsey & Eichenbaum, 1995; Eichenbaum, 2000).

However, it has been argued that some overtly nonspatial tasks have a hidden but critical spatial memory component and that impairment following hippocampal damage may be due to a deficit in the hidden, spatial aspect of the learning (Gaffan & Harrison, 1989; Nadel, 1991). In the case of the social transmission of food preferences task, memory testing typically takes place in the same location as original training. Thus, the location of learning could be a necessary part of the memory such that loss of the capacity for spatial representation following hippocampal damage might prevent the expression of the nonspatial (odor) memory. To address this issue, we tested sham-lesioned controls and animals with damage to hippocampus plus subiculum on memory for the social transmission of food preference task in two different conditions, either in the same environment in which they were trained or in a different room in a different building. If memory for this odor-odor association was independent of spatial context, testing in different environments should have no effect on the performance of either normal rats or rats with hippocampal damage.

Subjects. Thirteen male, 350 to 400-g Long Evans rats were singly housed and maintained on a diet of Purina rat chow 5001 and a 12-/12-h light/dark cycle. They were placed on a food deprivation schedule of four pellets per day and had ad libitum access to water. Subjects underwent surgery 2 months prior to beginning training and had previously interacted with juvenile rats as part of a separate study. Six rats underwent hippocampus plus subiculum lesions (group HS), and seven rats underwent sham lesions (group N).

Nine male Long Evans rats weighing from 250 to 300 g were used as demonstrators. Demonstrators were housed in groups of three, maintained on a 12-/12-h light/dark cycle, and received a diet of Purina rat chow 5001. They were placed on a food deprivation schedule of three pellets per day and had ad libitum access to water.

HS and sham surgeries. Animals were anesthetized with halothane in a 75%/25% N₂O/O₂ mixture. After placement in the stereotaxic apparatus, the skull was exposed and bregma and lambda were made level. Skull flaps were removed bilaterally over the lesion sites. For sham-operated animals, small punctures were made in the dura with a 25-gauge needle at each bilateral lesion site. For HS animals, a Hamilton Syringe was lowered to the stereotaxic coordinates shown on Table 1, and ibotenic acid (10 $\mu\text{g}/\mu\text{l}$) was injected. The height of the dura used as origin for the dorsoventral coordinates was measured at 4.8 posterior to bregma and 4.1 lateral to midline ipsilateral to the injection site. The syringe was allowed to remain at each site for at least 1 min after the injection was completed to ensure that the toxin was absorbed into the tissue. After the injections were complete, the skin was sutured, and a topical antibiotic (Panolog cream) was applied to the sutured area to prevent infection.

Social transmission of food preference. All animals were trained to eat plain ground rat chow (Purina 5001) from two glass jars mounted on a Plexiglas platform that was placed in the animals' cages. Animals were then habituated to the testing room in the different building. Initially, the animals were less willing to eat in the new room, but after 2 (or sometimes 3) days they ate readily from the glass jars. The odor pairs and concentrations used were chosen based on pilot studies that suggested similar innate preferences for both members of a pair. Within each pair, trained odors were balanced across animals within groups. Each animal was tested on three odor pairs: cinnamon (1% by weight in ground chow) vs cumin (0.25%), basil (0.7%) vs thyme (2%), and oregano (0.7%) vs mint (0.5%).

Training. Training took place in the home room. A demonstrator animal that had just eaten ground chow laced with an odorant (e.g., cinnamon) was introduced into the subject's cage. Demonstrator animals were used only if they had eaten at least 1 g of odorant-laced chow within the last 30 min. Subjects were allowed to interact with the demonstrator for 20 min, after which the demonstrator was removed. This procedure was repeated twice at 1 h intervals, using different demonstrators and the same odorized food each time.

Testing. Subjects were tested 2.5 h after the third interaction. One group of rats was tested in the home environment (SAME) and a second group was tested in another room that differed in many aspects of its visual appearance and illumination and was located in a different building (DIFFERENT). The testing environment (SAME/DIFFERENT) was balanced across animals, lesion groups, and odor pairs. The animals were tested in

TABLE 1
Lesion Coordinates and Volumes for HS Lesions

HS lesions														
Posterior from bregma	2.4	3.0	3.0	3.0	4.0	4.0	4.0	4.8	4.8	4.8	5.7	5.7	5.7	5.7
Lateral from bregma	± 1.0	± 1.4	± 1.4	± 3.0	± 2.1	± 2.1	± 3.7	± 5.1	± 4.1	± 4.1	± 4.5	± 5.1	± 5.1	± 5.1
Ventral (from dura)	3.2	2.8	3.4	3.0	2.3	3.3	3.0	8.0	3.5	7.2	3.6	4.5	5.5	7.4
Volume (μl)	0.18	0.09	0.09	0.18	0.09	0.09	0.09	0.2	0.1	0.2	0.2	0.16	0.16	0.16

their home cages in both conditions. Following a 10-min habituation period, subjects were presented with two glass jars containing ground rat chow, both mounted on a Plexiglas platform. The chow in one cup was laced with the same odorant that the three demonstrators had eaten (e.g., cinnamon) while the chow in the second cup was laced with a different, novel odorant (e.g., cumin). Position of the trained odor (left vs. right) was balanced across animals. The subject was allowed to eat for 45 min, after which cups were weighed to determine how much the rat had eaten from each cup. A percentage preference score for the trained odor (e.g., cinnamon) was calculated as follows:

$$(\text{weight of food laced with cinnamon eaten} / \text{weight of all food eaten}) \times 100.$$

The total amount of food eaten was also calculated. Each subject was trained and tested three times using three different odor pairs. For half of the animals, two tests were done in the SAME condition and one in the DIFFERENT condition, and for the other half this pattern was reversed.

Histology. Animals were deeply anesthetized with an overdose (100 mg/kg) of sodium pentobarbital and perfused transcardially with 0.9% saline followed by 10% buffered formalin. Brains were removed and postfixed in formalin for at least 24 h and then submerged in a 20% glycerol solution 24 h prior to sectioning. Coronal 50- μm slices were cut on a freezing, sliding microtome; mounted; and stained with thionin. For each animal, the lesions were traced on plates from a rat brain stereotaxic atlas (Paxinos and Watson, 1998) spaced 1 mm apart from bregma -1.3 to bregma -8.3.

Figure 1 presents tracings of the largest and smallest lesions. The hippocampus and subiculum suffered almost complete damage in all cases. In addition, in four of the cases there was some damage to the cortex overlying the hippocampus. In all cases, there was at least some unilateral damage to portions of medial entorhinal cortex.

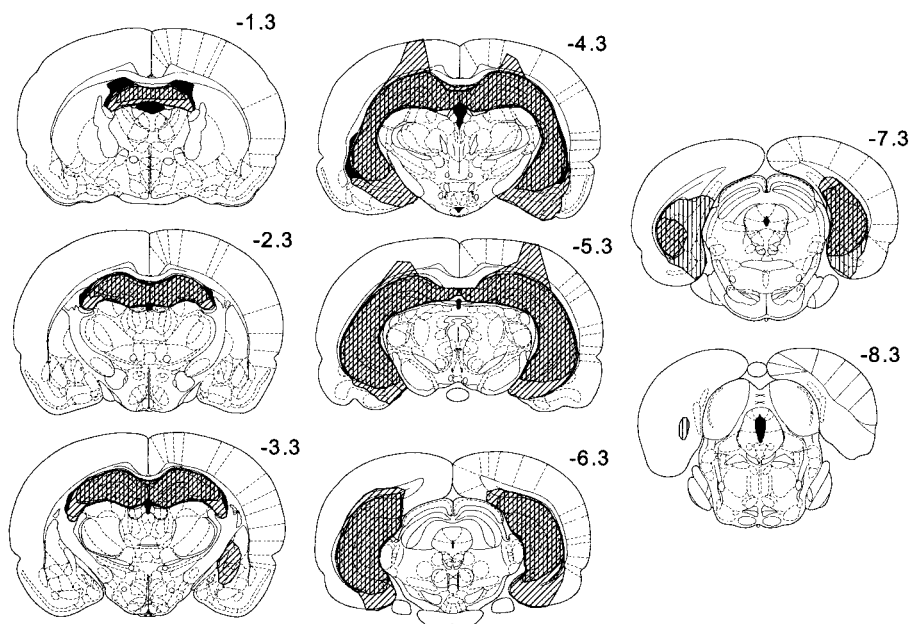


FIG. 1. Extent of the smallest (vertical hatching) and largest (diagonal) lesions in each group. Numbers indicate millimeters posterior to bregma.

Behavioral performance. For each animal, when tested twice within a SAME or DIFFERENT condition, the percentage preference scores and the total amount of food eaten were averaged across the repeated test condition, so that each animal had two sets of scores, one set for the SAME environment and one set for the DIFFERENT environment. Figure 2 presents the data for percentage food preference. A mixed-design ANOVA with a between-groups condition (subject group) and a within-group condition (test environment) was used to analyze the data. As expected, the HS group showed an impairment in memory for social transmission of food preferences, reflected in a significant group effect [$F(1, 10) = 8.5, p < 0.05$]. However, performance was not influenced by the test environment, as indicated by the absence of a significant effect of test condition [$F(1, 10) = 0.43, p > 0.5$]. In addition, the magnitude of the deficit did not vary between the test environments, as indicated by the absence of a significant interaction between lesion group and test condition [$F(1, 10) = 0.60, p > 0.4$]. Separate *t* tests again confirmed that neither group performed differently between the two test environments ($t_s < 1, p_s > 0.3$). In addition, sham-lesioned animals performed significantly better than chance in both environments ($t_s > 5, p_s < 0.005$), whereas HS animals performed not significantly different from chance level (50%) in both environments ($t_s < 1.2, p_s > 0.3$). Thus, testing of memory retention in an environment different from the training context did not affect the performance of either group.

A similar analysis for the amount of food eaten showed no differences between the subject groups [$F(1, 10) = 0.38, p > 0.5$] or between the test environments [$F(1, 10) = 2.2, p > 0.1$]. In addition, differences in the amount of food eaten by the two groups did not differ between the two test environments [$F(1, 10) = 0.22, p > 0.5$]. Thus, neither HS damage nor the test environment affected the animals' appetite or willingness to eat the odorized chow.

As in previous studies from our laboratory (Bunsey & Eichenbaum, 1995; Alvarez, Lipton, Melrose, & Eichenbaum, 2001), combined lesions of hippocampus and subiculum produced a severe deficit in memory for socially acquired food preferences. These results add to a body of evidence showing that different perturbations of hippocampal function lead to deficits in memory on this task (Rampon et al., 2000; Winocur, 1990; Winocur, McDonald, & Moscovitch, 2001; Clark et al., 2002; but see Burton, Murphy, Qureshi, Sutton, & O'Keefe, 2000). In addition, the present study provides clear evidence that the spatial context at the time of testing does not affect memory performance on the task. Normal animals performed equally well in both contexts, suggesting that the odor memory

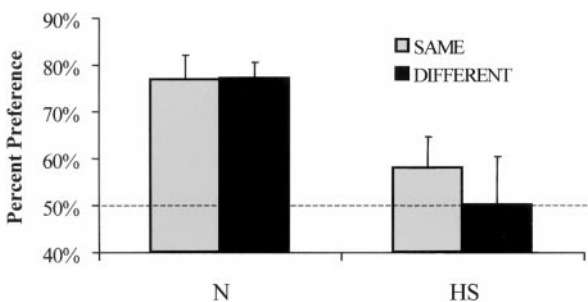


FIG. 2. Performance on social transmission of food preference. Asterisks indicate a significant difference from the N group. All data are presented as mean + SEM. The dotted line represents chance performance (50%).

did not depend on a representation of the training context. In addition, HS animals performed equally poorly in both environments, suggesting that any impairment in spatial representation resulting from hippocampal damage does not influence the odor memory deficit.

The present study confirms that lesions of hippocampus and subiculum produce a deficit in social learning of an odor association and shows for the first time that the spatial context of the testing environment does not affect performance in either normal control animals or animals with hippocampal damage. These findings are consistent with the idea that the critical memory requirement in the social transmission of food preferences involves the flexible expression of a learned association between the food odor and a conspecific's breath odor (Galef, Mason, Preti, & Bean, 1988) and not the memory for the spatial context in which the learning occurred.

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