

BRIEF REPORT

A Role for Olfaction in Object Recognition by Normal and Hippocampal-Damaged Rats

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To examine how olfactory and visual factors affect object discriminations in rats with and without hippocampus damage, the authors trained Long–Evans rats on simple object discriminations. They then examined how these discriminations were affected by rotations of the objects, by coating the objects in a transparent acrylic coating, or by both rotating and coating the objects in acrylic. The rats displayed no impairments when the objects were only sprayed in acrylic, and they displayed minor impairments when the objects were rotated. However, when the objects were both rotated and acrylic coated, the rats displayed severe impairments. This suggests that the rats are solving the rotated-only phase based on surface features of the objects (probably odor) and not based on the visual information. Such species' biases for obtaining object information are important factors to consider when designing and interpreting visual memory experiments across species. There was no difference in performance between the rats with hippocampus damage and the sham rats, and this is consistent with the literature on similar tasks. © 2001 Elsevier

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Initial reports of the amygdala and hippocampus being jointly involved in object memory in monkeys (Mishkin, 1978) were subsequently questioned when aspirations of the cortex

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overlying the hippocampal formation (i.e., rhinal cortex) resulted in impairments of equal magnitude as the hippocampal ablations (Murray & Mishkin, 1986). More recently, Murray and others have built a strong case that the hippocampus plays a nonessential role in simple object discriminations (Mumby, Astur, Weisend, & Sutherland, 1999; Murray & Mishkin, 1998; Nadel & Moscovitch, 1997). The rodent literature also suggests that, at least in simple object discriminations, the hippocampus is not part of the essential neural memory system (Glenn & Mumby, 1996; Kesner, Bolland, & Dakis, 1993; Mumby, Pinel, Kornecook, Shen, & Redila, 1995; Rothblat & Kromer, 1991).

Rarely discussed when comparing data from primate and rodent memory research are their sensory differences. Visual cues are a major source of sensory information for primates, whereas rodents are very sensitive to olfactory cues. Hence, what may seem to a human as a simple object discrimination because of very discrete visual cues may in fact be a relatively difficult discrimination for a rat due to very similar olfactory cues between the stimuli. Certainly, rodents can solve pure visual discriminations, as evidenced by various touchscreen experiments (Astur et al., 1996; Bussey et al., 1997), just as primates can solve discriminations based on haptic, auditory, or olfactory cues (Nordin & Murphy, 1998; Somers, Das, Dell'Osso, & Leigh, 2000; Zhou & Fuster, 1996). However, it is not clear how easily and what factors affect rodents when using these secondary sensory dimensions to solve discriminations. It is the goal of this report to examine how olfactory and visual factors affect object discriminations in rats with and without hippocampus damage.

A total of 8 adult male Long-Evans rats (Charles River, MA) that were used in a previous experiment examining retrograde amnesia for object and place information (Mumby et al., 1999) were used. All rats were previously trained on seven different simple object discriminations. In addition, 4 of the rats had previously received bilateral ibotenic acid lesions of the hippocampus (as described below), and 4 rats received sham lesions (as described below). They were housed individually with continuous access to water under a 12:12 light-dark cycle, with light onset at 7:00 a.m. Their body weights were reduced to approximately 85% of their free-feeding weights.

Surgery was performed under pentobarbitol anesthesia (65 mg/kg). Rats in group HPC ($n = 4$) received intrahippocampal injections of ibotenic acid (Sigma Chemical, St. Louis, MO) at 10 sites bilaterally (for details, see Mumby et al., 1999). Rats in group SHAM ($n = 4$) received sham lesions; their scalps were incised and sutured, but they sustained no damage to the skulls or brains. Immediately after surgery, each rat received diazepam (approximately 2 mg, i.m., Hoffmann-La Roche, Mississauga, Ontario), as a prophylaxis against seizures, and an antibiotic (penicillin G, 15,000 units, i.m., G.C. Hanford, Syracuse, NY). The experimenters who collected the behavioral data were blind to the group assignment of individual rats.

The apparatus for object discrimination training has been described in detail elsewhere (Mumby, Pinel, & Wood, 1990). Briefly, it consisted of an elevated runway that was separated from identical goal areas at each end by opaque guillotine doors. Each goal area contained two food wells into which food pellets (45 mg, Bio-Serv, Frenchtown, NJ) could be delivered by hand through plastic tubes that were mounted on the outside of the apparatus. A short divider wall protruded from the center of the end wall to separate the two food wells.

The test stimuli for the object discrimination problems were six objects of various

shapes, sizes, textures, and colors. The specific objects used were a plastic dinosaur, a small liquor bottle, a multicolored three-tiered Lego block, a brown jar cap, a Lego with a swinging door, and a flat pink sled. Each object was large enough to cover a food well but small and light enough to be easily displaced by the rats. No objects with obvious scents were included. The objects were washed with soap and water after every other completed testing session, and at the end of the day they were washed with a solution of diluted bleach to remove any extraneous scents. The rats had been previously trained with these object pairs in a previous experiment, and each S+ and S- valence was kept consistent with past experiences for each rat.

Procedurally, at the start of each session, each rat was placed into the center of the apparatus and allowed to explore for approximately 1 min. To begin the first trial, one of the guillotine doors was closed, and the experimenter positioned S+ and S- over the food wells on the other side of the door from the rat. The experimenter opened the door, and the rat approached and displaced one of the objects. If it displaced S+, then a food pellet was delivered to that food well; if it displaced S-, then no food pellet was delivered. A rat was considered to have made a choice if the object was displaced enough to expose the food well. The experimenter then closed the far door and positioned S+ and S- over the food wells on the other side of it in preparation for the next trial. The intertrial interval was approximately 15 s within each block of object discriminations. An intertrial interval of 60 s was used in between the end of one discrimination block and the start of the next discrimination block. There were 24 trials per session (one 8-trial block with each of the three object pairs). The order of object discriminations was counterbalanced pseudorandomly.

There were four phases of testing. During each of the phases described below, training continued until the rats correctly chose S+ on at least 20 of 24 trials with each object pair concurrently.

Phase 1: Original orientation. On each trial, the objects were positioned in the same single orientation as in the rats' previous training.

Phase 2: Rotated. Each object was positioned in one of four different orientations, rotated along the horizontal axis, on each trial. For example, the original orientation (Phase 1) of the liquor bottle was lying on its side with its top pointing at the rat. For the rotated positions, one of the orientations was with the bottle top pointing directly to the side, whereas another rotation was having the top point directly away from the rat.

Phase 3: Acrylic/Original orientation. A clear acrylic coating was sprayed over the surface of each object to attenuate distinguishing the objects on the basis of odor. The objects were positioned in the same single original orientation as in Phase 1. The acrylic coating added no obvious odor detectable by the experimenter, although presumably it did change some of the odor characteristics detectable by the rats.

Phase 4: Acrylic/Rotated. The acrylic-coated objects were positioned in one of the four different orientations as described in Phase 2.

For Phase 4, the upper limit of trials and errors was determined separately for each rat. The upper limit was calculated as three times the average number of trials to complete the previous three phases. For example, if Rat 3 reached criterion on an average of 112 trials for the first three phases, then its upper limit for Phase 4 was 336 trials (3×112 trials).

At the completion of behavioral testing, the rats were sacrificed using a lethal dose of sodium pentobarbital and then were perfused, and the brains were frozen-sectioned and stained with cresyl violet. There was extensive loss (> 90%) of cells in all principal subfields of the hippocampus and dentate gyrus, with some minor sparing of dentate granule cells and CA1 pyramidal neurons in the most temporal portions of the hippocampal formation. There was no evidence of damage to the thalamus or rhinal cortex (for a more extensive description and photographs of the histological results, see Mumby et al., 1999).

The rats with hippocampal lesions did not differ from the operated controls in trials to criterion, $F(1, 5) = 1.03$, $p = 0.36$, or in errors to criterion to learn the discriminations, $F(1, 5) = 2.55$, $p = 0.17$. There was a significant effect for phases for both trials to criterion, $F(3, 15) = 32.60$, $p < .001$ (Fig. 1), and errors to criterion $F(3, 15) = 33.00$, $p < .001$ (Fig. 2). Comparisons of the means using a Scheffé comparison revealed that performance during Phases 2 and 4 was poorer than during Phases 1 and 3 ($p < .05$). Performance during Phase 4 was also worse than during Phase 2 ($p < .01$).

These data indicate that rats can distinguish between two objects when they are in a fixed orientation whether or not they have distinctive odors (Phases 1 and 3). The data from Phase 2 revealed that the rats could distinguish between two objects even when they were rotated between trials. The poor performance of the rats during Phase 4 suggests that the successful performance observed in Phase 2 might be attributed to the rats discriminating between the two objects on the basis of odor or some other surface feature given that the visual information is identical between the two phases. It is improbable that the poor performance during Phase 4 can be attributed to the disruptive effect of

Trials to Criterion for Each Testing Phase

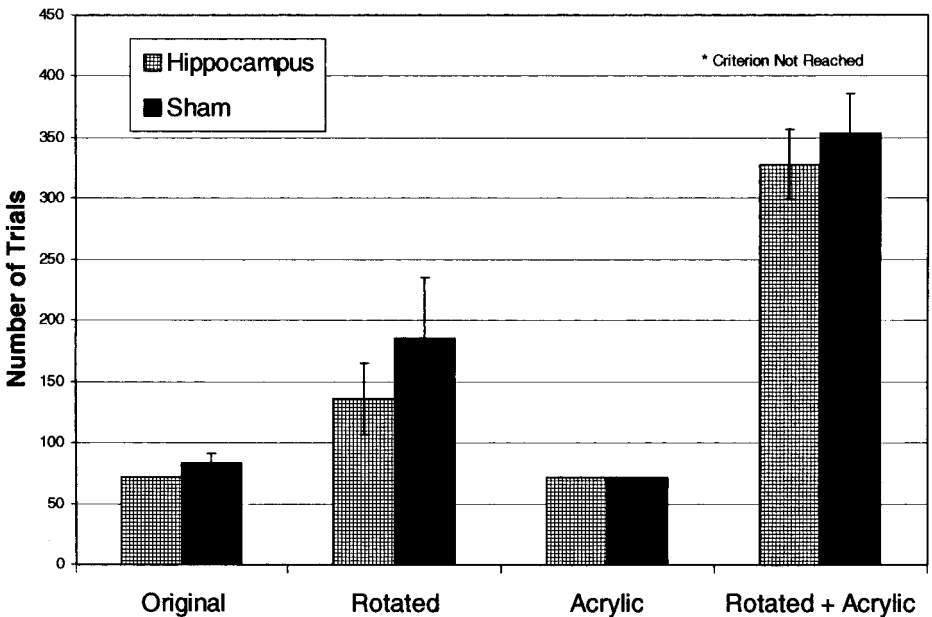


FIG. 1. Trials to criterion for each testing phase. Note that all rats solved Phase 1 and Phase 3 with little difficulty. However, Phase 2 required more trials to reach criterion, and neither group was able to reach criterion in Phase 4.

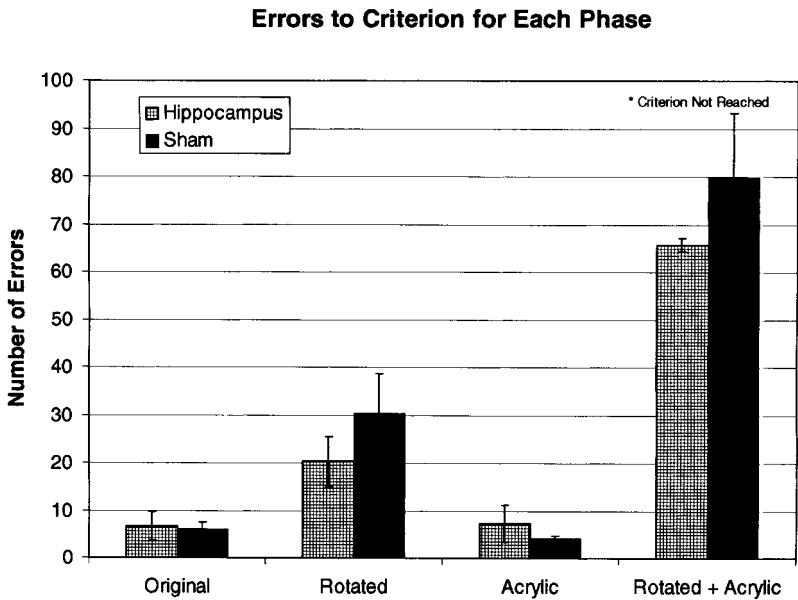


FIG. 2. Errors to criterion for each testing phase. Note that all rats solved Phase 1 and Phase 3 with little difficulty. However, Phase 2 resulted in more errors to reach criterion, and neither group was able to reach criterion in Phase 4.

coating the objects in acrylic given that this manipulation did not affect performance during Phase 3. Hence, it must be that the strategy adopted in the rotated-only phase was disrupted by the masking of surface features.

The result that the rats with hippocampus damage did not differ reliably from the sham rats in performing this task is consistent with other experiments (Mumby et al., 1995, 1999), and it provides additional evidence that the hippocampus is nonessential for simple object discriminations.

These results suggest that rats do not readily discriminate between objects when more than one orientation is used unless the objects have some surface feature that distinguishes them. The most probable distinguishing feature is stimulus odor because of rodents' high reliance on olfactory information. It has been shown that rats are capable of using odor to solve a number of complex tasks such as delayed matching to sample (Lu, Slotnick, & Silberberg, 1993), transitive inference (Bunsey & Eichenbaum, 1996), and spatial navigation (Whishaw & Gorny, 1999). Hence, it is possible that rats could solve the simple discriminations in these experiments based strictly on olfactory cues.

Admittedly, coating the objects in acrylic also homogenizes other surface features such as surface texture and surface glare. Currently, it is not clear which surface features are used preferably by rodents during object discriminations. Further work on rats' capacity to discriminate between objects should allow us to specify whether features other than olfactory cues were used.

These data suggest that under certain circumstances, various task demands may alter rats' strategies so as to bias them to pursue non-visual-based solutions, even when visual solutions would suffice. Moreover, because of species differences and preferences, these exact same task demands might not elicit the same strategies in other species such as

primates because of differences in sensory preferences. This is an important factor to consider in designing and interpreting visual memory experiments across species.

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