Hippocampal Lesions Modulate Both Associative and Nonassociative Priming

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In associative priming, rats are more likely to orient to a visual stimulus whose memory has not been recently activated (V1) than to one whose memory has been recently activated (V2). However, rats with excitotoxic hippocampal lesions are more likely to orient to the primed V2 than to the unprimed V1. This study investigated the influence of hippocampal lesions on nonassociative priming. Rats received presentations of 2 visual stimuli, V1 and V2, that had been presented more (V2, primed) or less (V1, unprimed) recently. Control rats oriented to V1 rather than to V2, whereas hippocampal rats oriented to V2 rather than to V1. These results parallel those observed in an associative priming procedure and thereby suggest that the role of the hippocampus in priming is general.

The memory of a stimulus can be activated or primed in two ways: by the recent presentation of the stimulus itself (nonassociative priming) or by the presentation of another stimulus with which it has an association (associative priming). Our recent results show that the hippocampus modulates associative priming (Honey & Good, 2000a, 2000b; see also Honey, Good, & Manser, 1998; Honey, Watt, & Good, 1998). In those studies, we monitored the rats’ tendency to orient to two visual stimuli (V1 and V2) as a function of whether their memories had been associatively primed by an auditory stimulus (for V2) or not (for V1). Control rats were more likely to show a behavioral orienting response (OR) to the unprimed V1 than to the associatively primed V2. This effect suggests that when a memory of a stimulus has been associatively primed, it can reduce the impact of the presentation of that stimulus on performance (Wagner, 1981). Rats with hippocampal lesions were more likely to orient to the associatively primed V2 rather than the unprimed V1. This effect suggests that the presentation of an associative prime augments the impact of the stimulus in rats with hippocampal lesions. In the present study, we investigated whether hippocampal lesions modulate nonassociative priming in a similarly striking manner. Thus, rats were presented with V1, followed some time later by V2, and shortly after were given a choice between V1 and V2. If the recent presentation of V2 reduces the impact of its subsequent presentation, then control rats should orient to V1 rather than V2 during the choice trials. If lesions of the hippocampus influence nonassociative and associative priming in the same way, then rats with hippocampal lesions should orient to V2 rather than to V1. Moreover, one influential model of animal memory (see Wagner, 1981) proposes that associative and nonassociative priming effects are based on similar mnemonic processes, and provides a theoretical basis for predicting that hippocampal lesions will have comparable effects on both forms of priming. However, this prediction does not follow from a recent psychobiological model of recognition memory (Aggleton & Brown, 1999; Brown & Aggleton, 2001). This model adopts the suggestion that (recognition) memory is supported by two processes, recollection and familiarity (Yonelinas, 2002), and assumes that only recollection depends on the integrity of the hippocampus. It is reasonable to suppose, as Aggleton and Brown (1999) did, that the associative priming effects that we have observed in control rats reflect recollective processes; and the observation that associative priming is influenced by hippocampal lesions (Honey, Watt, & Good, 1998; Honey & Good, 2000b) is broadly consistent with their model. However, on an a priori basis, the model is constrained (J. A. Aggleton, personal communication, 2003) to predict that lesions of the hippocampus will have no effect on nonassociative priming, because (recency-based) familiarity processes should adequately support such priming. That is, this model predicts that whereas the pattern of results in an associative priming procedure should be different in control and hippocampal rats, the pattern of results in a nonassociative priming procedure should be comparable in control and hippocampal rats.

Method

Subjects, Surgery, and Histology

Forty-seven adult hooded Lister rats served in Experiment 1 and 92 served in Experiment 2 (all were supplied by Harlan Olac Ltd., Oxon, United Kingdom). Twenty-three rats in Experiment 1 and 39 rats in Experiment 2 received ibotenic acid lesions of the hippocampus (Group Hippocampal). Experiment 1 was conducted in two replications, and Experiment 2 was conducted in four replications. The histological and behavioral analyses that follow were pooled across replication in order to simplify presentation, because the nature of the lesions was consistent across replications and because the pattern of behavioral results did not interact with replication (Fs < 1). Whereas all of the remaining rats in Experiment 1 received sham operations, only 41 of the remaining rats in Experiment 2 received sham operations, and the rest served as unoperated controls (Group Control); because the behavior of the sham-operated rats and the unoperated rats did not differ, they were pooled for the purpose of statistical analysis. The surgical procedures were closely modeled on those...
used by Honey and Good (1993). Briefly, the rats were first anaesthetized with Isoflurane and then placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). After a scalp incision, the bone overlying the area of neocortex directly above the hippocampus was removed, and injections of ibotenic acid (concentration 10 μg/ml; supplied by Biosearch Technologies, Novato, CA) were made with a 2-μl Hamilton (Reno, NV) syringe mounted on the stereotaxic frame. Injections of 0.05–0.10 μl were made at 28 sites with a KD Scientific electronic pump (Model 310; Boston, MA) at a rate of 0.03 μl/min. After each injection, the needle was left in position for 2 min to encourage diffusion of the ibotenic acid and to limit the spread of the drug into overlying cortical areas. Sham-operated rats received an identical treatment, with the exception that the passage of the needle was limited to the cortex and no drug was infused. Following a minimum of 2 weeks postoperative recovery, rats were gradually reduced to 80% of their ad-lib weights (Experiment 1: M = 481 g, range = 396–584 g; Experiment 2: M = 477 g, range = 356–596 g), at which they were maintained throughout Experiments 1 and 2. Rats were housed in pairs and had free access to water when resident in their home cages. The colony room was illuminated between the hours of 8 a.m. and 10 p.m., and testing began at approximately 9 a.m. As a part of our ongoing analysis of the role of the hippocampus in different forms of spatial learning, we trained rats in a water maze, using a variety of procedures, either before or after they participated in Experiments 1 and 2. The hippocampal rats from Experiments 1 and 2 exhibited a consistent deficit in the water maze procedures, but a detailed presentation of these results will be reported elsewhere.

Following the completion of behavioral testing, the lesioned rats were administered injections of Euthatal and perfused through the heart, after which their brains were removed. A Cresyl violet stain was used to determine the extent and location of cell loss.

**Behavioral Procedures and Apparatus**

All experimental sessions were conducted in a pair of standard experimental chambers (internal dimensions: 24.5 cm wide × 23 cm deep × 21 cm high; supplied by Campden Instruments Ltd., Loughborough, United Kingdom) that were identical to those used by Honey and Good (2000b). Briefly, both chambers had three aluminum walls, an aluminum ceiling, and a Perspex door that served as the fourth wall. The floors were constructed from stainless steel rods. The visual arrays (V1 and V2) were created with two 3-W panel lights that were operated at 24 V and faced the back wall of the chamber. The lights could be illuminated either throughout the duration of a trial (constant) or presented intermittently (flashing: 0.25 s on and 0.25 s off). The centers of the lights were mounted 15 cm apart, 5 cm from the closest aluminum wall and approximately equidistant (10 cm) from the floor and ceiling. Rats’ orienting behavior was recorded with a Hiwa VHS movie camera (Model TH-650).

On each of the first 2 days of Experiments 1 and 2, rats were placed in the operant chamber for 30 min to habituate them to the apparatus. Experiment 1 consisted of a single test session in which rats received eight presentations of the two lights that were illuminated throughout their 10-s duration. The onset of the first presentation was 70 s after the rats had been placed in the chamber. For half of the rats in each group (hippocampal and control), the interval between the offset of one presentation of the lights and the onset of the next was 70 s (for rats in Condition V1), and for the remainder it was 10 s (for those in Condition V2). Our aim in this preliminary experiment was to examine, using a simple between-subjects design, whether these two intervals would reveal differences in the behavior of the control and hippocampal rats and, if so, to then use them in Experiment 2. In Experiment 2, rats received more extensive testing in a within-subjects design. In this design, rats received choice trials involving two stimuli, V1 and V2, that had been presented more (V2) or less (V1) recently. Rats were given a single 11-min training session on each of 3 days. Each session consisted of four sets of three trials. The first trial of each set consisted of the presentation of V1 (e.g., constant illumination of the two lights), the second trial was the presentation of V2 (e.g., flashing illumination of the two lights), and the final choice trial involved the presentation of components of V1 and V2 (e.g., flashing light on the left and constant light on the right). Each trial was 10 s in duration. The interval between the offset of V1 and the onset of V2 was 50 s, and that between the offset of V2 and the choice trial was 10 s; the intervals between the presentation of V1 and the choice trial and V2 and the choice trial matched the intervals used in conditions V1 and V2 in Experiment 1 (i.e., 70 s and 10 s, respectively). The interval between the offset of the choice trial and the next presentation of V1 was 50 s, and the first presentation of V1 within each session occurred 50 s after the rats were placed in the chamber. The identity of the stimulus (constant or flashing) that served as V1 or V2 alternated, within a session, from one set of trials to the next, and similarly, the position of V1 and V2 on the choice trials (left or right) alternated from one choice trial to the next. For half of the rats in each group, on the first set of trials the constant light served as V1 and the flashing light served as V2; and for the remainder, the identity of the stimulus that served as V1 and V2 was reversed. For half of the rats in the two resulting counterbalanced subconditions, on the first choice trial V1 was presented on the right and V2 was presented on the left, and for the remaining rats this arrangement was reversed.

Experimental sessions were recorded with a video recorder and subsequently scored by an observer (V. J. Marshall) who was blind with respect to the rats’ group membership. An OR was defined in the same manner as in Honey and Good (2000b): as the tip of the rat’s snout turning toward one of the localized lights. In Experiment 1, we monitored the trials on which an OR was observed, and in Experiment 2 we monitored whether or not rats oriented to each of the first two elements of each set of trials (V1 and V2) and whether they oriented to V1 or V2 on the choice trials. Interobserver concordance for both of these measures of the OR (between V. J. Marshall and R. C. Honey) was ~95%.

**Results**

**Histological Analysis**

Three rats from Group Hippocampal in Experiment 1 and 8 rats from Group Hippocampal in Experiment 2 showed extensive sparing of hippocampal cells in the dorsal and ventral levels of this structure and were therefore excluded from the analysis. Figure 1 shows reconstructions of the maximum (gray) and minimum (black) extent of damage in the remaining rats that had acceptable hippocampal lesions in Experiments 1 (left-hand panel) and 2 (right-hand panel). In all cases, the rats showed extensive cell loss in the dorsal hippocampus (maximum cell loss = 100%, minimum cell loss = 70%). The majority of rats showed near-complete cell loss in all subfields of the hippocampus. Cell loss was also evident in the subiculum region in the majority of rats, but there was complete sparing of the pre- and parasepticulun and the medial and lateral entorhinal cortices in all cases. In ventral regions of the hippocampus, the extent of the cell loss was more variable (maximum = 100%, minimum = 40%). However, inspection of the behavioral data did not reveal a systematic relationship between the extent of the lesion in this region and the behavioral measures.

One lesioned rat in Experiment 1 showed unilateral and minimal cell loss in the temporal association cortex and the entorhinal cortex. Inspection of this rat’s behavioral scores revealed that they did not differ in any systematic way from the scores from the remaining rats in Group Hippocampal. This rat was not, therefore, excluded from the analysis.
Figure 1. Histology from Experiments 1 (left-hand panel) and 2 (right-hand panel). The maximum (gray) and minimum (black) extent of the lesion in Group Hippocampal at horizontal sections taken through the dorso-ventral extent of the brain. The depicted sections are in millimeters from bregma (clockwise from top left: 3.1, 3.6, 5.6, 7.6, 8.6, 6.6, and 4.6). Reprinted from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Figures 3.1, 3.6, 4.6, 5.6, 6.6, 7.6, and 8.6, Copyright 1998, with permission of Elsevier.
**Behavioral Analysis**

Panel A of Figure 2 depicts the mean percentages of trials with an OR in the control rats and hippocampal rats from Experiment 1. Inspection of this panel suggests that although there was some tendency for control rats to show greater orienting when the visual stimulus was presented every 70 s (in Condition V1) than when it was presented every 10 s (in Condition V2), the most striking effect is that hippocampal rats were far more likely to orient in Condition V2 than in Condition V1. Moreover, whereas there was little difference in the levels of orienting between Groups Control and Hippocampal when the visual stimulus was presented every 10 s (in Condition V2), there was a marked difference in their tendency to orient when the interval was 70 s (in Condition V1).

Analysis of variance (ANOVA) revealed that there was no effect of group (Control vs. Hippocampal), $F(1, 40) = 2.71, p > .10$, and no effect of condition (V1 or V2), $F(1, 40) = 1.95, p > .16$, but that there was an interaction between these two factors, $F(1, 40) = 5.53, p < .05$. An analysis of simple main effects revealed an effect of group (Control vs. Hippocampal) in Condition V1, $F(1, 40) = 7.91, p < .01$, and an effect of condition (V1 vs. V2) in Group Hippocampal, $F(1, 40) = 6.37, p < .02$. The finding that rats with hippocampal lesions show reduced levels of orienting when the interval between presentations was relatively long, an effect that was ameliorated when this interval was shorter, is consistent with other aspects of the results reported by Honey and Good (2000b) and by Oswald et al. (2002). Moreover, the results

![Figure 2](image_url)

*Figure 2.* Experiment 1 (Panel A): Mean ($\pm$ SEM) percentages of trials with an orienting response to a visual stimulus in control rats and those with hippocampal lesions, where the interval between stimulus presentations was either 70 s (V1) or 10 s (V2). Experiment 2 (Panels B, C, and D): Mean ($\pm$ SEM) percentages of trials on which there was an orienting response to the elements of a visual array, V1 and V2, which had been presented 10 s (V2) or 70 s (V1) before, in control rats and those with hippocampal lesions. Panel B shows first choice trials (pooled across test sessions), and Panels C and D show the four test trials within a session (again pooled across test sessions) for the control rats (Panel C) and hippocampal rats (Panel D).
from Experiment 1 are, in general terms, consistent with the theoretical analysis and results described in the introduction and provided the grounds for conducting a more extensive experimental analysis in Experiment 2. This experiment made use of a more elegant within-subjects assessment of nonassociative priming and used a procedure that more closely paralleled that used in the study of associative priming by Honey (2000b).

In Experiment 2, rats in Group Hippocampal also showed a lower level of orienting on the initial V1 (32.53%) and V2 (37.36%) elements of the sets of trials than did those in Group Control (V1 = 44.97%, V2 = 46.70%). An ANOVA revealed a main effect of group, F(1, 82) = 12.68, p < .01, but no effect of V1 or V2, F(1, 82) = 2.96, p > .08, and no interaction between these factors (F < 1). The results of the choice trials are shown in Panels B, C, and D of Figure 2 and are of more direct theoretical significance. Panel B shows the results from the first choice trials pooled over the three sessions. Inspection of this panel reveals a pattern that was very similar to that observed in Experiment 1; however, it also confirms that rats with hippocampal lesions can discriminate between our two visual stimuli (flash- and constant; see also Honey & Good, 2000b). Panels C and D show the orienting behavior of Groups Control and Hippocampal, respectively, across the four choice trials within a session (pooled over the 3 days of testing in Experiment 2). Inspection of Panel C reveals that control rats showed a clear preference to orient to V1 rather than to V2, an effect that was most evident toward the end of the test sessions. Inspection of Panel D indicates that the initial tendency for hippocampal rats to orient to V2 rather than V1 (reproduced from Panel B of Figure 2) dissipated over the course of the test sessions. An ANOVA conducted on the scores summarized in Panels C and D revealed an effect of group (Control vs. Hippocampal), F(1, 82) = 3.99, p < .05; no effect of stimulus (V1 or V2), F(1, 82) = 1.20, p > .27; and an interaction between these factors, F(1, 82) = 6.79, p < .02. Analysis of simple main effects revealed that there was an effect of group on choices of V1, F(1, 145) = 4.86, p < .03, and an effect of stimulus (V1 vs. V2) in Group Hippocampal, F(1, 82) = 6.02, p < .02. The latter finding has important theoretical implications that we will consider below; however, it also confirms that rats with hippocampal lesions can discriminate between our two visual stimuli (flash- and constant; see also Honey & Good, 2000b). Panels C and D show the orienting behavior of Groups Control and Hippocampal, respectively, across the four choice trials within a session (pooled over the 3 days of testing in Experiment 2). Inspection of Panel C reveals that control rats showed a clear preference to orient to V1 rather than to V2, an effect that was most evident toward the end of the test sessions. Inspection of Panel D indicates that the initial tendency for hippocampal rats to orient to V2 rather than V1 (reproduced from Panel B of Figure 2) dissipated over the course of the test sessions. An ANOVA conducted on the scores summarized in Panels C and D revealed an effect of group (Control vs. Hippocampal), F(1, 82) = 3.99, p < .05; no effect of stimulus (V1 or V2), F(1, 82) = 1.20, p > .27; and an interaction between these factors, F(1, 82) = 6.79, p < .02. Analysis of simple main effects revealed that there was an effect of group on choices of V1, F(1, 164) = 10.60, p < .01, and an effect of stimulus (V1 vs. V2) in Group Control, F(1, 82) = 9.27, p < .01. Although the overall ANOVA also revealed an effect of choice trial, F(3, 246) = 5.81, p < .01, there was no three-way interaction between choice trial, group, and stimulus (F < 1).

The observation that the tendency for control rats to orient to V1 (rather than to V2) on the choice tests seemed to become more marked over the course of test sessions whereas the tendency for hippocampal rats to orient to V2 (rather than V1) dissipated is intriguing and requires detailed consideration. One possibility, developed further in the Discussion section, is that in control rats there are two opposing priming processes that influence whether it is V2 (rather than V1) that elicits an OR during the choice tests: The recent presentation of V2 might (a) raise the level of activity within its memory and increase the likelihood that the presentation of V2 will generate an OR, or (b) result in a refractory process that makes V2 less able to reactivate its memory. These different processes would clearly cancel one another out if both had effects on performance that were of a similar magnitude. However, if the influence of the first process declined over the course of a test session relative to that of the second process, then the impact of the second process might only become evident during the later test trials within a session. Moreover, once it is assumed that this refractory process does not operate in hippocampal rats, then the transient influence of the first process would be revealed in these rats.

Discussion

Our previous research showed that hippocampal lesions modulate associative priming in rats. Thus, when faced with a choice between orienting to a stimulus whose memory has recently been associatively activated (V2) and a stimulus whose memory has not (V1), control rats oriented to V1, whereas hippocampal rats oriented to V2 (Honey & Good, 2000a, 2000b; see also Honey, Good, & Manser, 1998; Honey, Watt, & Good, 1998). The results of Experiments 1 and 2 show that hippocampal lesions also modulate nonassociative priming in rats: Control rats were less likely to orient to a recently presented visual stimulus (V2) than to one that had not been recently presented (V1; Experiment 2), whereas rats with hippocampal lesions were, at least initially, more likely to orient to a visual stimulus that had been recently presented (V2) than one that had not (V1; Experiments 1 and 2). The observation that both associative and nonassociative priming are influenced in a similar way by lesions of the hippocampus is consistent with the notion that the role of the hippocampus in priming is general and with models of memory that assume that these priming effects have a common origin (e.g., Wagner, 1981). Moreover, the observation that nonassociative (recency-based) priming is influenced by hippocampal lesions is also consistent with results showing that rats with fornix lesions show a deficit in temporal processing (Meck, Church, & Olton, 1984). However, this observation is inconsistent with a recent psychobiological model of recognition memory (Aggleton & Brown, 1999; Brown & Aggleton, 2001) that predicts that hippocampal lesions should have dissociable effects on associative and nonassociative priming.

There are two issues that deserve some further comment. The first is the way in which the priming effects seen in control and hippocampal rats can be captured at a theoretical level, and the second is how a disruption to the mechanisms underlying priming might provide an analysis for other deficits associated with hippocampal damage in rats.

If one accepts the general notion that the recent activation of the memory of stimulus by an associative or nonassociative prime can reduce the impact of the presentation of that stimulus (Experiment 2; see also, Honey & Good, 2000b; Honey, Good, & Manser, 1998; Honey, Watt, & Good, 1998), then one still needs to specify why this is the case. One suggestion, based on an influential theory of animal memory, rests on two principles: First, the activity that reflects the recent activation of a memory by a prime is qualitatively different, and less able to provoke responding, than that produced by the immediate impact of the stimulus; second, this
prime-induced activity can limit the extent to which the presentation of the stimulus can fully activate its memory and provoke responding (Wagner, 1981). Without these or similar principles, then one could only expect that our associative and nonassociative priming manipulations would augment responding: The activity produced by the prime would simply add to that produced by the stimulus itself and provoke more behavior relative to a condition in which the prime was absent. Of course, this is exactly the pattern of results that we have observed in our hippocampal rats. The arguments just presented, therefore, lead rather naturally to the suggestion that in hippocampal rats the activity produced by an associative or nonassociative prime does not differ from that produced by the presentation of the stimulus itself. That is, it suggests that for hippocampal rats mnemonic activity is source independent.

The results from our studies of associative and nonassociative priming in control rats indicate that when the memory of a stimulus has recently been activated, it can reduce the impact of the presentation of that stimulus. If we assume that these priming effects reflect the operation of some general processing principles (Wagner, 1981), then their influence should be evident (if not directly assessed) under a wide range of conditions, and for the present purposes, this influence should also be modulated by hippocampal lesions. For example, it seems reasonable to assume that exploratory behavior would be affected by whether or not the memory of a particular spatial location or configuration of cues has been recently activated (by association or a recent visit to that location). Lesions of the hippocampus might then affect exploratory behavior, not, or not only, because they affect some component of spatial navigation (Morris, Schenk, Tweedie, & Jarrard, 1990; O’Keefe & Nadel, 1978) or contextual learning (Good & Honey, 1997) per se, but rather because they modulate the way in which recently activated memories of components of an environment influence subsequent behavior. Notwithstanding this speculation, it is undoubtedly the case that any general understanding of memory and of the function served by the hippocampus will need to provide an account for the strikingly different (associative and nonassociative) priming effects that we have observed in control rats and those with hippocampal lesions.

References


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