Retention of concurrent conditional discriminations in rats with ibotenate lesions of hippocampus

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We studied the effects of removing the hippocampus on the retention of appetitive Pavlovian conditional discrimination. In Experiment 1, rats were trained preoperatively on two concurrent conditional discrimination problems in which a stimulus (i.e., a facilitator) signaled the reinforcement of another stimulus (i.e., a target). The results indicated that performance was poorer when a "trace" interval separated the facilitator and target than when the facilitator overlapped with the target. Data obtained from transfer and probe trials yielded results compatible with the idea that the problems were solved using "if-then" rules rather than by either direct associations between facilitators and reinforcement or unique configural associations. In Experiment 2, half of the rats had the hippocampus removed using multiple injections of small amounts of ibotenic acid, and after recovery all rats were tested for retention of the preoperatively learned conditional discriminations. In contrast with previous reports, the results in this study showed that lesions of the hippocampus did not disrupt conditional performance. Thus, rather than supporting the hypothesis that the hippocampus plays an important role in the control of conditional behaviors, our results indicate that the structure is *not* necessary for the performance of complex Pavlovian conditional discriminations.

Animals readily learn to solve Pavlovian conditional discriminations in which a stimulus (A) is reinforced (+) with food only when it is preceded by another stimulus (B). Learning is demonstrated to the extent that animals respond more to A when A is accompanied by B than when A is presented alone (Holland, 1983; Rescorla, 1985). Within this paradigm, we shall refer to B as a "facilitator," and call A a "target" (e.g., Rescorla, 1985).

It has recently been suggested that the hippocampus may be the neural substrate for this type of learning. Ross, Orr, Holland, and Berger (1984) gave rats concurrent training on two Pavlovian discrimination tasks. One task was a nonconditional A-, B+ discrimination, in which a 5-sec B stimulus (a clicker) was followed by a food unconditioned stimulus (US) and a 5-sec A stimulus (a noise) was not. The second task was a conditional A-, B-A+ discrimination, in which a 5-sec diffuse light B facilitator followed by a 5-sec trace period signaled that a 5-sec tone A target stimulus would terminate with food delivery. The target was not followed by food when it was presented alone. Incidence of headjerking (rapid, jerky movements of the rat's head and neck, usually oriented

toward the food magazine) served as the primary index of learning in both discrimination tasks.

Ross et al. (1984) reported that aspiration lesions of the hippocampus produced deficits in both the acquisition and retention of the A-, B-A+ conditional discrimination, but had no effect on nonconditional A-, B+ discrimination performance. This finding was seen as support for the hypothesis that the hippocampus is crucial to an animal's ability to perform conditional "if-then" operations. Thus, the hippocampus was viewed as playing a critical role in the use of such rules as "if B has been presented, only then will A retrieve the memory of food" (Hirsh, 1974, 1980), and "if B has been presented, only then will the association between B and food (i.e., the B-US association) be activated" (Olton, Becker, & Handelmann, 1979). However, the structure was not seen as being essential for the use of simple or direct associations (e.g., B-US) between a stimulus and reinforcement.

The present research focused on the role of the hippocampus in the retention of conditional discrimination, and addressed three questions which complicate the interpretation of Ross et al.'s (1984) results. First, Ross et al. inserted a 5-sec trace period between the presentations of B and A on reinforced trials. It seemed possible that hippocampectomy hindered the rats' ability to retain the memory of the B stimulus during this trace interval. That is, rather than directly rendering rats incapable of per-

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forming conditional "if-then" operations, hippocampectomy may have interfered with their ability to remember whether or not B had been presented. This type of memory disruption is predicted by some views of hippocampal function (e.g., Rawlins, 1985). From this perspective, hippocampectomy might interfere with conditional discrimination performance only if that performance required the retention of memory traces.

To evaluate this hypothesis, we gave rats concurrent training on two Pavlovian conditional discrimination problems, using a design similar to that employed previously by Davidson and Rescorla (1986). One problem was identical in form to the A-, B-A+ problem used by Ross et al. (1984). The other problem was of the same form as the first, except that it employed no trace interval. Instead, B (the facilitator) remained on during the trace interval and overlapped with A (the target) before both stimuli coterminated with food reinforcement. With this design, rats could solve both problems by using a form of the conditional rule "if B has been presented, A will be reinforced." However, only the first problem required rats to remember the B stimulus during the trace interval in order to perform appropriately. Hence, if hippocampectomy disrupted performance on both problems, this would suggest a disruption in the ability to perform conditional operations. If performance was disrupted on only the first problem, this would indicate an interference with the ability to retain information.

A second question pertained to whether or not the ability to perform conditional if-then operations was necessary for the rats in the Ross et al. (1984) experiment to solve the A-, B-A+ discrimination. We considered two alternative possibilities which did not seem to be ruled out by their data. First, the rats may have treated the combined presentation of the target and the memory trace of the facilitator as a "configural" or "unique" cue (e.g., Rescorla, 1973) that emerged to have its own direct association with food. Hence, hippocampectomy may have disrupted discrimination performance by interfering with the rats' ability to form configural cues, rather than by hindering their performance of conditional operations.

To assess this hypothesis, we sought to determine whether or not the respective B facilitator cues from each discrimination could promote responding to a previously trained and extinguished target cue different from the targets with which the facilitators were originally trained (see Holland, 1986; Rescorla, 1985). If such transfer were to be obtained, it would be inconsistent with the idea that discriminative responding is based on associations involving configural cues unique to the originally trained BA compounds. Transfer would not contradict the hypothesis that the rats used conditional "if-then" operations to solve the conditional discrimination problems, since facilitators may have the capacity to activate the association between any stimulus and reinforcement (see Davidson, Aparicio, & Rescorla, 1988; Davidson & Rescorla, 1986; Ross & LoLordo, 1986a).

We also assessed the hypothesis that discriminative responding in both problems was based on simple direct associations between their respective facilitators and reinforcement. To do this, we gave the rats probe trials in which each facilitator was presented alone. For the 15-sec facilitator from the problem without the trace interval, responding was monitored during each of the three 5-sec periods it was presented. For the facilitator from the trace problem, responding was monitored during the 5-sec facilitator, the 5-sec trace interval, and the 5 sec in which the target was normally presented (i.e., the target period). Ross et al. (1984) did not directly assess the ability of the facilitator alone (i.e., in the absence of the target) to produce conditioned behavior during the target period. Substantial responding to a facilitator alone would suggest that it was directly associated with reinforcement, and therefore not likely to be acting as a conditional cue.

The third problem concerns the nature of the brain damage in the rats resulting from the use of aspiration lesions of the hippocampus. Damage resulting from aspiration lesions is not limited to the hippocampus, but also includes adjacent areas (usually the subiculum) and both rostral and caudal directed fibers passing through the alveus and fimbria (Jarrard, 1986). Moreover, there is an unknown amount of damage to the vasculature.

In the present research, multiple injections of ibotenic acid were used to remove cells in the hippocampus (Jarrard, 1989). This procedure results in damage that is more selective than can be obtained with conventional lesion techniques (aspiration, electrolytic), and therefore provides a better measure of the effects of removing the hippocampus on the retention of learning.

EXPERIMENT 1

Experiment 1 was carried out on nonhippocampectomized rats which were trained on two different, concurrent Pavlovian conditional discrimination problems. One problem (no-trace) was of the form T1-, F1T1+, in which a 15-sec visual facilitator stimulus (F1) overlapped with a 5-sec auditory target stimulus (T1) before both stimuli coterminated with food reinforcement (+). No reinforcement was given when T1 was presented alone. The second problem (trace) was of the form T2-, F2-T2+, in which a 5-sec visual facilitator (F2), different from F1, was followed by a 5-sec trace before a 5-sec auditory target stimulus (T2), different from T1, terminated with food reinforcement. Reinforcement was withheld when T2 was presented alone. Discrimination learning would be demonstrated to the extent that greater responding was observed on reinforced facilitator/target compound trials than on nonreinforced target-alone trials.

After the rats solved each problem, a probe test was used to assess the nature of their solutions. This test measured the amount of responding to F1 and F2 when each was presented alone, when each was presented with its original target (T1 or T2), and when each was

presented in conjunction with a previously reinforced then partially extinguished white noise (N) transfer target. Previous data (e.g., Rescorla, 1985) had suggested that facilitators promote responding by lowering the threshold for activation of the excitatory connections among a US and its associates. Therefore, facilitators should not promote responding to stimuli that have no excitatory connection with reinforcement. Pretraining with the transfer target would serve to supply it with this excitatory history. Partial extinction could then be used to reduce responding to N and thereby allow assessment of the degree to which responding to the transfer target could be augmented by either facilitator.

Method

Subjects and Apparatus. The subjects were 16 naive male Sprague-Dawley rats, about 100 days old at the start of the experiment. Food deprivation was used to maintain the rats at 85% of their ad-lib body weight. Each animal's food ration was adjusted to allow for a weekly weight gain of approximately 1.5%. The rats had free access to water throughout the experiment except during experimental sessions. All rats were maintained throughout the experiment on a day/night cycle in which a 12-h day period began at 0600 h and ended at 1800 h.

The apparatus included four identical 28.3×21.6×21.5 cm conditioning chambers. The front and back walls were aluminum; the side walls were clear acrylic plastic. The floor consisted of .48-cm stainless steel rods, spaced 1.5 cm apart. A pellet dish was located approximately 5 cm to the right of the vertical midline of the front wall. A 6-W bulb, which provided constant, low-level chamber illumination, was located at the vertical midline of the front wall, approximately 5 cm from the top. The chambers were enclosed in a sound-resistant shell with a clear Plexiglas front to permit behavioral observations. A 25-W bulb located 13 cm above the approximate center of each chamber provided a 3 Hz flashing-light stimulus. A tubular 40-W bulb, 14 cm in length, and located parallel to and slightly below one Plexiglas wall of the chamber, was enclosed in a housing that was opaque except for a 4.45-cm slit on the side facing the chamber, through which the bulb could provide a steady illumination stimulus. A speaker, which provided auditory stimuli (i.e., white noise, and a 1500 Hz tone) was mounted on the interior shell wall. A cooling fan mounted on this wall also provided masking noise. A 10-Hz clicker was mounted at the approximate center of the back wall of the shell.

The behavior of the animals was recorded with a video camera located 2.09 m from the chambers, so that activity in all four chambers could be recorded simultaneously. Activity was videotaped by a videocassette recorder connected to a monitor. Videotapes were played back for scoring.

Procedure. The rats were assigned to two groups of 8 rats each, matched on ad-lib body weight, and each group was subdivided into two squads of 4 rats each. The rats in each squad were assigned to different experimental chambers and were run in their assigned chamber throughout the experiment. The squads were run in the same order each day, between 0800 and 1600 h.

Each rat was magazine trained, for one session, with food pellets (45 mg, P. J. Noyes Co.) automatically delivered on a variable-time 1-min schedule. With this schedule, a pellet was delivered on the average of one per minute for 10 min.

Pavlovian conditional discrimination training began on the day following magazine training. All rats were concurrently trained on two discrimination problems. One problem was of the form F1T1+, T1-. On F1T1+ trials a diffuse visual stimulus (F1) was presented for 15 sec. An auditory tone stimulus (T1) overlapped the final 5 sec of the light, with reinforcement immediately following cotermination

of both stimuli. On T1- trials, the 5-sec tone was presented by itself, without reinforcement. The other problem was of the form F2-T2+, T2-. On F2-T2+ trials, a 5-sec illumination of a different diffuse light stimulus (F2) was followed by a 5-sec trace interval, which was followed in turn by the presentation of a 5-sec clicker (T2) that terminated with reinforcement. The 5-sec clicker was presented alone without reinforcement on T2- trials. For half of the subjects, a flashing houselight served as F1, and a steady light served as F2. The F stimuli (flashing or steady light) were reversed for the remaining rats. The identities of F1 and F2 were also counterbalanced with respect to experimental chamber. Pilot research has indicated that the rats could easily discriminate between the tone and clicker stimuli used in this experiment, and that these stimuli elicited similar patterns of headjerking when followed by food reinforcement.

All rats received three presentations of each of the four trial types, for a total of 12 trials during each session. The intertrial interval (ITI) was variable around a mean of 2.5 min. The trials were presented in two different quasi-random sequences, which were alternated once every 3 days. All rats received one session per day for 30 days.

Beginning the day after the conclusion of conditional discrimination training, the rats were given 2 days of training in which the presentation of a 5-sec white noise (N) terminated with food reinforcement. This was followed by 3 days of extinction in which the 5-sec N was presented without reinforcement. The rats received 12 presentations of N on each day of training and extinction. Mean ITI was 2.5 min.

Transfer test sessions, which consisted of two presentations each of nine different trial types, took place during the next 2 days. During each session, the rats received two 5-sec presentations each of T1-, T2-, and N-. In addition, each rat was given two 15-sec presentations of F1- alone. F1- was also presented in combination with N such that the 5-sec N overlapped with the last 5 sec of the 15-sec F1 (F1N-). The rats also received two 5-sec presentations of F2- alone, and two presentations of a 5-sec F2 followed by a 5-sec trace interval, followed by a 5-sec presentation of N (F2N-). All of the above trials were nonreinforced. Finally, during each session all rats received one reinforced F1T1+ trial and one reinforced F2-T2+ trial. Two quasi-random sequences of trials were used, with half of the rats receiving each sequence. The sequences were the same except that the positions of trials involving F1 and T1 were interchanged with the positions of trials involving F2 and T2. The mean ITI was 2.5 min during each test session.

Scoring. The behavior of each subject on each trial was videotaped. Learning was assessed using a behavioral observation technique similar to that described by Davidson and Rescorla (1986). Three observations were made for each rat during each 15-sec trial period. This trial period began with the onset of the light (F1 or F2) on F1T1+ and F2-T2+ trials, and began 10 sec before the onset of T1- and T2- when these cues were presented alone. Paced by a one-per-1.25-sec auditory signal recorded on the tape, the observer shifted his/her gaze from chamber to chamber, observing each of the 4 rats once every 5 sec during the trial period. Only one behavior was recorded on each observation.

The six categories of behavior recorded were: (1) headjerking—short, rapid, horizontal and/or vertical movements of the head, usually directed toward the food magazine; (2) rearing—standing on the hind legs with both forepaws off the grid floor (this category excludes grooming movements performed while the rat was standing on its hind legs); (3) magazine—standing motionless in front of the food magazine, with the nose or head close to or in the magazine; (4) locomotion—movement of all four feet in a forward direction; (5) magazine-oriented activity—movement of the head and neck oriented toward the food magazine (this movement was slower and/or traversed a smaller distance, than headjerking); and (6) other—all behaviors other than those described above.

Results and Discussion

Analyses of variance were used to evaluate both training and transfer test data. To ensure that rats that were subsequently assigned to control and hippocampal lesion groups did not differ preoperatively, group (control vs. hippocampal lesion) served as a between-subjects variable in all analyses. No reliable main effects or interactions involving this variable were obtained. Hence, rats that subsequently received hippocampal lesions did not differ from controls during preoperative discrimination training and transfer testing. Because of its lack of effect, this preoperative variable will not be discussed further.

Conditional discrimination training. As reported in other studies that observed behavior to food-reinforced auditory cues (e.g., Ross et al., 1984), headjerking emerged as the primary index of conditioning. Figure 1 shows the mean percentage of observations of headjerking during the target period (i.e., the last 5 sec) of each trial type of both discrimination problems during the 1st, 6th, 12th, 18th, 24th, and 30th training sessions. Performance is shown separately for each of the trial types that constituted the two discrimination problems. Trial types designated by a 1 constituted the discrimination problem without a trace period. Trial types designated by a 2 constituted the trace problem.

As can be seen in Figure 1, the rats solved both discrimination problems, as indicated by headjerking, more on reinforced F1T1+ and F2_T2+ trials than on non-

Figure 1. Mean percentage of headjerking during acquisition of the no-trace and trace problems in Experiment 1. Responding is shown separately for reinforced (F1T1+, F2_T2+) and nonreinforced (T1-, T2-) trials.

reinforced T1- and T2- trials. However, Figure 1 also shows that the magnitude of the discrimination was greater for the F1T1+, T1- problem (without the trace) than for the F2_T2+, T2- problem, which involved the trace. Figure 1 indicates that this effect was largely due to greater headjerking to T2- than to T1- on all except the first day of training.

The training data were evaluated by analyses of variance in which sessions, problem type (trace vs. no trace), and trial type (compound vs. target alone) served as within-subjects factors. These analyses revealed a significant main effect of trial type [$F(1,14) = 487.51, p < .001$], confirming that the rats headjerked more on F1T1+ and F2_T2+ compound trials than on T1- and T2- target-alone trials. This effect of trial type grew over the testing sessions, as indicated by a significant trial type $\times$ sessions interaction [$F(5,70) = 50.194, p < .001$]. A significant problem $\times$ trial type interaction was also obtained [$F(1,14) = 34.072, p < .001$]. This finding confirmed that the differences between compound trials and target-alone trials were smaller for the trace problem than for the problem without the trace. Therefore, although the rats solved both problems, poorer performance was obtained on the trace problem.

Transfer testing. The purpose of the transfer test was to assess what the rats had learned when they solved the conditional discrimination problems. One hypothesis is that the rats solved these problems by learning simple facilitator-US associations. A second hypothesis is that the rats learned to associate configural or unique cues emerging from the facilitator/target compounds with reinforcement. The data shown in Figure 2 address both of these possibilities.

Figure 2 shows the mean amount of headjerking for each trial type presented during transfer testing. The left panel of Figure 2 depicts mean percent transfer-test headjerking when F1 was presented in combination with its original target (F1T1+), in compound with the transfer target (F1N-), and when presented alone (F1-). Headjerking to the original and transfer targets when they were presented alone is also shown (T1- and N-, respectively). The right panel of Figure 2 shows the comparable data for F2 when it was presented with its original target (F2_T2+), with its transfer target (F2_N-), and when it was presented alone (F2-). Headjerking is also shown to T2- alone, and is shown again, for ease of comparison, to N alone (N-*).

As illustrated in the left panel of Figure 2, F1 elicited substantially less headjerking when it was presented alone than when it was presented in combination with either T1 or N. Analyses of variance confirmed that both of these differences were statistically significant [$F(1,14) = 35.06, p < .01$, for F1 vs. F1T1+; $F(1,14) = 51.09, p < .01$, for F1 vs. F1N]. The same pattern of results was obtained for F2. That is, the rats headjerked reliably less when F2 was presented alone than when F2 was presented in compound with either T2 [$F(1,14) = 42.10, p < .01$] or N [$F(1,14) = 43.94, p < .01$].

Figure 2. Mean percentage of headjerking during transfer and probe trials in Experiment 1. The left panel shows responding on the no-trace problem when F1 was presented with the original target (F1T1+), in compound with the transfer target (F1N-), when presented alone (F1-), and when the original and transfer target were presented alone (T1-, N-, respectively). The right panel shows responding on the trace problem when F2 was presented with its original target (F2_T2+), with the transfer target (F2_N-), when presented alone (F2-), and when the original target was presented alone (T2-). Note: For ease of comparison, headjerking to the transfer target alone is repeated (N-*).

The relatively low levels of headjerking elicited by F1 and F2 when they were presented alone suggest that discriminative performance during original training was not the result of direct associations between these stimuli and the US. Rather, these facilitators seem to have augmented responding most when acting in concert with their target stimuli. The results of the transfer test also show that this capacity for augmentation was not limited to target stimuli (T1, T2) originally trained with F1 and F2, but extended as well to the white-noise transfer target (N).

Figure 2 shows large differences in headjerking to the transfer compounds (F1N- and F2_N-, respectively) relative to the transfer target (N-) alone. The magnitude of these differences is comparable to those obtained when the originally trained compounds (F1T1+ and F2_T2+) were compared to their respective targets (T1- and T2-) alone.

Analyses of variance assessed differences in headjerking due to trial type (compound vs. target alone) as a function of target (original vs. transfer). Responding to F1 compounds (i.e., F1T1+; F1N-) and F1 targets alone (T1-; N-) was analyzed separately from that to F2 compounds (F2_T2+; F2_N-) and F2 targets alone (T2-; N-). Although the main effect of trial type was highly

reliable in both analyses [smallest $F(1,14) = 218.94$, $p < .01$, for F2 compound vs. target-alone comparisons], in neither analysis was this factor found to interact reliably with target [largest $F(1,14) = 1.47$, $p > .20$, for F1 analysis]. Hence, it appears that discriminative performance involving the transfer stimulus was equivalent to that involving the originally trained stimuli.

The finding that F1 and F2 both promoted patterns of responding to the transfer target that were similar to patterns of responding to their originally trained targets provides evidence against the idea that discrimination performance was based on learning about configural cues. Instead, this outcome, taken together with evidence against a primary role for learning of simple facilitator-US associations, is more consistent with the view that the rats solved the discrimination problems by using "if-then" rules, as suggested by Ross et al. (1984), or by relying on other conditional mechanisms.

EXPERIMENT 2

Ross et al. (1984) reported that aspiration lesions of the hippocampus abolished the rats' ability to perform conditional "if-then" operations, whereas discriminative per-

formance based on simple associations was left intact. However, on the basis of their data, it seemed possible that "if-then" operations were not required to solve their conditional discrimination problem. The results of Experiment 1 above strengthened their argument by reducing the plausibility of two alternative interpretations of how rats solved that problem; viz., the rats learned a simple facilitator-US association or the rats learned to associate reinforcement with configural or unique cues arising from the facilitator-target compound.

Experiment 2 assessed the effects of ibotenate lesions of the hippocampus on retention of the conditional discrimination learning acquired in Experiment 1. In addition to testing whether or not hippocampal lesions abolished the ability to perform conditional operations, we were especially concerned with whether or not the effects of these lesions on the capacity to perform the memory trace problem were different from those produced in the problem without the memory trace. If hippocampectomy were to be found to disrupt retention of only the trace problem, this would suggest that the rat's ability to retain the memory of the facilitator during the trace interval was degraded. If both problems were disrupted to a similar degree, this would provide more converging evidence for the notion that hippocampectomy disrupts the performance of conditional "if-then" operations.

Method

Subjects and Apparatus. The subjects and apparatus were the same as those employed in Experiment 1.

Surgical procedure. The rats were divided into two groups of 8 animals each. The two groups were counterbalanced with respect to squad, experimental chamber, and identity of F1 and F2 stimuli. One group received ibotenate lesions of the hippocampus (HIP); the second group served as controls (C). Four of the 8 control rats received control operations.

The operative procedures used to remove the hippocampus were similar to those described elsewhere (Bouffard & Jarrard, 1988). The animals were anesthetized with a mixture of chloral hydrate and sodium pentobarbital. The animals were placed in a Kopf stereotaxic apparatus, an incision was made in the scalp, and holes were drilled in the skull to allow microinjections of ibotenic acid (IBO; Biosearch, San Rafael, CA). Injections were made with a 2- μ l Hamilton syringe mounted on the stereotaxic frame and held with a Kopf microinjector (Model 5000). A glass micropipette was glued onto the end of the needle of the syringe in order to minimize damage to the overlying neocortex.

The IBO was dissolved in phosphate buffered saline (final pH 7.4) at a concentration of 10 mg/ml. Injections of .10 μ l (.05 μ l at several sites) were made over 1 min at each of 26 sites in the HIP group (Jarrard, 1989). The pipette was left in place an additional 1 min to prevent spread up the pipette track. The operated control rats were subjected to the same surgical procedures, with the pipette being lowered into the neocortex but not deep enough to penetrate the hippocampus, and no IBO was injected.

Following behavioral testing, all lesioned animals were sacrificed and perfused with physiological saline and formalin. The brains were removed from the skull, embedded in egg yolk, and cut horizontally on a microtome into 40-micron sections. A cresyl-violet stain was used to determine cell loss due to the lesion, and selected sections were stained with the Fink-Heimer silver staining procedure (Fink & Heimer, 1967).

Retention testing. Following a 7-day postoperative recovery period, the rats were again placed on the restrictive feeding schedule, and their body weights were returned to their preoperative levels. Retention testing began 17 days after the completion of surgery. The rats were given 6 days of retention testing with the same procedure as that used during acquisition training; that is, each rat received three trials each of the form F1T1+, T1-, F2-T2+, T2- on each day. The scoring procedure was the same as that described for Experiment 1. The person scoring the videotaped behavior of the rats was unaware of the group designations and treatments received by each rat prior to scoring.

Transfer testing. Transfer test sessions, consisting of two presentations each of eight different trial types, took place during the next 2 days. During each session, the rats received two 5-sec presentations each of N-, the stimulus used as the transfer test target in Experiment 1, and of a novel transfer target, T3 (a 5000-Hz tone). The rats had not been exposed to T3 prior to this transfer test. In addition, each rat was given two 15-sec presentations of F1- alone. F1- was also presented in combination with N such that the 5-sec N overlapped with the last 5 sec of the 15-sec F1 (F1N-), and was presented in the same manner with T3 (F1T3-). Two 5-sec presentations of F2- alone, and two presentations of a 5-sec F2, followed by a 5-sec trace, followed by a 5-sec presentation of N (F2-N-) were also given. In addition, T3 was presented in this manner with F2 (F2-T3). All of the trials during this postsurgery transfer test were nonreinforced. Two quasi-random sequences of trials were used, with half of the rats receiving each sequence. The sequences were the same except that the positions of trials involving N and T3 were interchanged. The mean ITI was 2.5 min during each test session.

Results and Discussion

Retention testing. Removing the hippocampus with IBO had little effect on the performance of either conditional discrimination problem. Figure 3 shows the mean percentage of headjerking for the HIP and C groups, respectively, during each of the six sessions of postlesion testing. The left panel of Figure 3 shows mean headjerking during the target period (i.e., the last 5 sec) on each of the trial types comprising the no-trace discrimination. The right panel of Figure 3 shows performance on the trial types making up the trace problem. Headjerking for sham-operated and unoperated controls did not differ, so their data have been combined.

Little difference between the HIP and C groups can be seen in Figure 3. More headjerking occurred on the facilitator-target compound trials than on target-alone trials for both groups. For both groups, trace problem performance was initially disrupted before returning to pretest levels (see right panel). Furthermore, for both groups, the magnitude of the compound vs. target-alone difference was smaller for the trace problem than for the no-trace problem, as was observed in original training. Figure 3 also shows that each of these differences was of similar magnitude for Groups HIP and C. In short, retention of the capacity to perform conditional discriminations was not disrupted by ibotenic lesions of the hippocampus.

Analysis of variance confirmed reliable differences in headjerking between reinforced compound and nonreinforced target-alone trial types [$F(1,14) = 958.06$, $p < .01$], and the magnitude of these differences varied

Figure 3. Mean percentage headjerking for the hippocampal (HIP) and control (C) groups during the no-trace (left) and trace (right) problems in Experiment 2.

over test sessions [$F(5,70) = 16.77, p < .01$, for the trial type $\times$ sessions interaction]. Furthermore, the difference between problem types (trace vs. no trace) was also significant [$F(1,14) = 146.14, p < .01$], as was the trial type $\times$ problem type interaction [$F(1,14) = 90.38, p < .01$], confirming the reliability of the difference in discrimination performance between the trace and the no-trace problems. However, analysis of variance found no reliable differences in headjerking between groups (HIP vs. C), or any reliable interactions involving groups with trial type, problem type, or sessions [largest $F(5,70) = 1.49, p > .20$, for groups $\times$ trial type $\times$ sessions interaction].

The performance of Groups HIP and C were also compared during each of the two 5-sec periods that preceded the target period of each compound trial. It has been suggested (e.g., Ross & Holland, 1981, 1982) that conditioned behavior during the facilitator in the first 5 sec of a compound trial reflects the formation of a direct facilitator-US association, whereas conditioned responding during the 5 sec immediately preceding the onset of the target indexes a "within-compound" association between the facilitator and the target. Ross et al. (1984) reported that direct facilitator-US associations were intact in rats with aspiration lesions of the hippocampus, whereas performance based on within-compound associations was impaired (due to neocortical rather than hippocampal damage).

Figure 4 shows the effects of ibotenate lesions of the hippocampus on the mean percentage of headjerking during the first 5 sec (left panel) and the second 5 sec (right panel) of each facilitator-target compound trial for the trace and no-trace conditional discrimination problems. The data are presented separately for Groups HIP and C. The figure reveals little difference between the groups in headjerking performance on either problem during either 5-sec period. Neither group showed much headjerking in the first 5-sec period, indicating little direct association between the facilitator and reinforcement (but see below). However, both groups showed substantial headjerking during the second 5-sec period, indicating strong anticipation of the target (i.e., strong within-compound association). Analyses of variance using group as a between-subjects factor and problem type (trace vs. no trace) and sessions as within-subjects factors revealed no significant main effects due to group and no reliable interactions involving group during either the first [largest $F(1,14) = 1.976, p > .15$ for group] or second [largest $F < 1$] 5-sec period.

It has been reported that the conditioned response to visual stimuli associated with food takes the form of rearing and not headjerking (Holland, 1983; Ross & LoLordo, 1986b; but see Davidson & Rescorla, 1986; Kaye & Pearce, 1984). Since we employed visual facilitators in each of our discrimination problems, we also compared Groups HIP and C with respect to the incidence of rear-

Figure 4. Mean percentage headjerk for the HIP and C groups during the first 5 sec (left) and second 5 sec (right) of each facilitator-target compound trial for the no-trace and trace conditional problems.

ing behavior during the first 5 sec of each facilitator-compound trial. (Ross et al., 1984, used rearing to assess the strength of the direct facilitator-US association.) Rearing during the first 5 sec of the facilitator used in the no-trace problem (F1) was 38.8% for the rats of Group HIP; it was 41.7% for Group C rats. Rearing during the first 5 sec of the facilitator used in the trace problem (F2) was 43.9% for Group HIP and 55.6% for Group C. Analysis of variance found neither the main effect due to group nor the interactions involving group with sessions or problem type to be reliable [largest $F(5,70) = 1.91$, $p > .10$, for the group $\times$ session $\times$ problem type interaction].

Transfer testing. Table 1 shows the mean percentage of target period headjerk during the transfer test for each type of postoperative transfer test trial. As illustrated in that table, Group HIP and Group C showed similar headjerk performance on each type of test trial. Analysis of variance revealed no reliable difference due to groups for any trial type [largest $F(1,14) = 3.43$, for Trial Type N]. Table 1 also shows that, as in preoperative transfer testing, more responding was exhibited when N was presented in combination with either F1 or F2 than when N was presented alone. These differences were highly reliable [$F(1,14) = 85.61$, $p < .01$, for F1N vs. N; $F(1,14) = 76.26$, $p < .01$, for F2_N vs. N]. Hence, both F1 and F2 continued to promote responding to a previously trained and then extinguished test target. Also, as found during preoperative transfer testing, F1 and F2, respectively, elicited much less responding when presented alone than when presented in combination with N [smallest $F(1,14) = 35.46$, $p < .01$, for F1 vs. F1N]. This result indicates that the postoperative response-promoting capacity of F1 and F2 was not based

solely on a direct association with reinforcement. Furthermore, it was found that both F1 and F2 promoted more headjerk to N than to the previously untrained transfer target T3 [smallest $F(1,14) = 16.61$, $p < .01$, for F2_T3 vs. F2_N]. In fact, neither F1 nor F2 elicited reliably more responding when presented in combination with T3 than when they were presented alone [largest $F(1,14) = 3.11$]. This finding indicates that the capacity of F1 and F2 to promote responding to a target depended upon whether or not the target had a history of association with reinforcement.

We also compared lesioned rats and controls with respect to the amount of headjerk during the 5-sec interval immediately prior to the target period, on trials in which F1 and F2, respectively, were presented alone. We found headjerk during this interval to be 21.9% on both F1 and F2 trials for control rats, 28.1% on F1 trials, and 21.9% on F2 trials for hippocampectomized rats. Neither the difference between hippocampals and controls nor the differences between the trial types were reliable [all $F(1,14) < 1$]. It should be noted that the amount of "within-compound" headjerk observed in this postoperative transfer test was substantially lower than that observed during postoperative retention testing. Reinforced trials were given during retention testing but not during the postoperative transfer test. It is possible that responding based on within-compound associations was reduced by extinction during the postoperative transfer test (see Ross, 1983).

The transfer test data also speak to the possibility that responding to the facilitator/target compounds was not based on conditional "if-then" operations, but was due instead to summation of the associative strengths of each of the elements. This account is difficult to rule out because the rules for combining the associative strengths of different stimuli are presently unknown. This difficulty is compounded in both our study and that of Ross et al. (1984), because stimuli of different associative strengths might also elicit responses with different topographies. However, at least one aspect of our transfer data seems to reduce the plausibility of the summation hypothesis. For Group HIP, both N and T3 elicited very similar low levels of responding when they were presented alone. Nonetheless, both F1 and F2 promoted significantly more responding when presented in combination with N than when presented in combination with T3. Such large differ-

Table 1
Mean Percentage of Headjerk (Target Period)
for Each Group on Each Type of Transfer Test Trial

Group	F1N	F2_N	N	F1T3	F2_T3	T3	F1	F2
HIP	71.9	59.4	6.3	34.4	28.1	3.1	18.8	15.6
C	75.0	68.8	21.9	34.4	31.3	3.1	25.0	18.9

Note—Headjerk of rats with ibotenate lesions of the hippocampus (HIP) and of controls (C) on trials involving presentation of the facilitators from the problem without the trace (F1) and from the trace problem (F2), and on trials involving the presentation of target stimuli that had been previously trained then extinguished (N) or had no prior training history (T3).

Figure 5. Photomicrographs of horizontal, cresyl-violet stained sections at four dorsal-ventral levels of the brain for an unoperated control rat (C, left) and a rat with ibotenate hippocampal lesions (HIP, right).

ences in responding to the compounds, in spite of similarities in responding to the elements alone, challenges a broad range of summation rules.

Our results, like those of Ross et al. (1984), provided no evidence that hippocampectomy disrupted direct associations between the facilitator and the US. However, unlike Ross et al., we found that an intact hippocampus was *not* necessary for the performance of conditional discriminations. Furthermore, headjerking during the 5-sec interval immediately preceding the target period was not disrupted for our lesioned rats as compared with non-lesioned controls. Ross et al. interpreted responding during this interval as an index of within-compound association between the facilitator and the target. They suggested that their finding of disruption of within-compound associations was due to neocortical damage concomitant with hippocampal aspiration. Our chemical lesion procedure presumably involved less neocortical damage than the aspiration procedure, and this may be why our rats showed no deficits in responding based on within-compound associations.

Anatomical evaluation. Examination of cresyl-violet-stained brain sections revealed an extensive loss of cells in the hippocampus (see Figure 5). The damage was similar to that obtained in several recent experiments in which multiple injections of small amounts of IBO were used (see Bouffard & Jarrard, 1988; Jarrard, 1986). As illustrated in Figure 5, removing the hippocampus resulted in considerable overall shrinkage of the posterior cortex and distortion of the remaining areas of the hippocampal formation. The cells in the dorsal hippocampus were removed in all animals, with the exception of a few dentate granule cells adjacent to the dorsal thalamus and close to the midline. The brain from the HIP animal shown in Figure 5 showed some sparing of dentate granule cells on the right side at the second dorsal-ventral level. Otherwise, damage to the hippocampus was complete and selective at the midlevel. The only sparing of pyramidal cells was at the most ventral level, and this was limited to some CA1 and CA3 cells and several dentate cells. The surviving cells at the most ventral level were usually unilateral, but the brain pictured in Figure 5 shows small bilateral sparing. In 2 rats, all of the cells in the ventral hippocampus were removed. A comparison of the performance of completely hippocampectomized rats with that of rats that had some sparing of the hippocampus did not indicate any differences.

In other research, we have found it important that structures adjacent to the hippocampus, especially the subiculum, not be damaged (Jarrard, 1986). In 2 rats there was some loss of subicular cells in the dorsal area, but the amount was small and limited to one side, as seen in the brain in Figure 5. The other extrahippocampal damage was at the most ventral level, but this also involved few subiculum cells. Other brain structures adjacent to the hippocampus were not damaged. Thus, the overall damage to the hippocampus in the present study was not only essentially complete, but was also more selective than can be obtained using other lesion techniques.

Histological examination of the brains of operated control animals indicated only minimal damage, which was limited to the cortex overlying the hippocampus through which the pipette penetrated. In many areas where the pipette was lowered, there was no obvious loss of cells.

GENERAL DISCUSSION

Our results do not support the hypothesis that the hippocampus is the necessary substrate for the performance of complex Pavlovian conditional discriminations. In Experiment 1, the rats solved two concurrent Pavlovian conditional discrimination problems which appeared to differ with respect to the memory load required for their appropriate performance. Test trials indicated that discrimination performance was not based on either the formation of direct associations between facilitators and reinforcement or on the direct association of reinforcement with configural cues that were unique to the respective facilitator-target compounds. The test trial results were more consistent with the idea that the rats used conditional operations to solve both problems; for example, if the facilitator has been presented, then the target will be followed by reinforcement.

Experiment 2 then showed that ibotenate hippocampal lesions did not disrupt conditional discrimination performance, relative to that of control rats, of either the trace or the no-trace problem. Presuming that the rats were using conditional operations to solve both problems, performance should have been disrupted on both problems in Experiment 2 if the hippocampus was the substrate for the execution of conditional operations. A second transfer test yielded no evidence that either lesioned or control rats were using direct facilitator-US associations or configural cues to solve either discrimination problem. In addition, this test indicated that both facilitators promoted more responding to a transfer target that had been previously trained and extinguished, than to a novel transfer target that had not been trained as a signal for food. In fact, neither facilitator elicited reliably more responding when presented in combination with the novel transfer target than it did when it was presented alone. This pattern of results is consistent with the view that the response-promoting power of the facilitators was not based on their direct association with reinforcement, but depended instead on the facilitator's capacity to modulate the associative strength of the target.

Furthermore, our findings yielded no evidence that the hippocampus is necessary either for the performance of conditional mnemonic operations or for the retention of information for brief durations. Although the results of Experiment 1 indicate that the memory load involved with the trace problem was greater than that involved with the no-trace problem, performance of both hippocampal and control rats in Experiment 2 was equally affected when the 5-sec trace separated the facilitator and the target. If a functioning hippocampus was necessary for retention of the memory of the facilitator during the trace period,

performance of the hippocampectomized rats should have been disrupted, relative to controls, on the trace problem. This result was not obtained.

In summary, we found no disruption in conditional discrimination performance as a result of hippocampal lesion. This finding suggests that the hippocampus may not be a necessary substrate for the performance of conditional operations. This conclusion is made more plausible in light of the fact that neither preoperative nor postoperative tests indicated that conditional discrimination performance was based on simple associative processes.

Ross et al. (1984) reported that aspiration lesions of the hippocampus had no effect on a simple nonconditional discrimination, but did interfere with both the acquisition and retention of a conditional discrimination similar to the one used in the present study. Their results were interpreted to indicate that "the conditional control of learned behavior(s) can be attributed to normal hippocampal functioning" (p. 221).

What could account for the differences between the results obtained in the Ross et al. (1984) study and the results of the research reported here? First, there are obvious design differences in the two studies. Ross et al. trained their rats concurrently on one nonconditional and one conditional discrimination task, whereas we trained our rats concurrently on two different conditional discriminations. Perhaps learning two concurrent conditional discrimination problems is "easier" than learning one. Of course, one might also suppose that the opposite could be true. In any event, it would be difficult to explain how requiring a rat to solve two conditional discriminations instead of one would eliminate the need for a hippocampus. This type of argument is complicated by the fact that the data from our study seem consistent with Ross et al.'s interpretation that the rats used conditional operations to solve their conditional discrimination problems. Therefore, even though our rats learned two conditional discriminations, whereas the rats in Ross et al.'s experiment learned only one, there is no evidence that learning in either experiment was based on different processes. The same kind of analysis applies to differences between the studies in terms of the number of trials per day and the intertrial interval.

A second, and probably more important, difference between the Ross et al. (1984) study and the present research is the nature and extent of the rats' brain damage. In the earlier study, an aspiration approach was used to remove the hippocampus, whereas in the present research the cells comprising the hippocampus were removed with a neurotoxin. As described in the paper by Ross et al., the aspiration approach resulted in damage that included not only extensive bilateral lesions of the hippocampus, but also involved the adjacent subiculum and presubiculum, the cingulate cortex and cingulum, and, in some rats, the entorhinal cortex and the gray matter of the temporal neocortex. The aspiration approach also interrupts the axons, many of which are extrahippocampal in origin and termination, that pass in both rostral and caudal directions

through the alveus and the fimbria (see Jarrard, 1986). The operated control rats that were included in the Ross et al. study had the neocortex overlying the hippocampus removed, but the resulting damage did not include much of the extrahippocampal damage noted above. That this extrahippocampal damage can be important is indicated in other research (see Jarrard, 1986). In those experiments, performance of a preoperatively learned complex spatial task was found to be minimally affected when the hippocampus alone was removed using IBO, but was similar to that found with aspiration lesions when the ibotenate lesions also included loss of cells in the subiculum. In the present research, histological examination revealed not only that the loss of hippocampal cells was extensive, but also that the damage was limited to the hippocampus. Hence, while the results of the Ross et al. study were obtained following widespread damage to the hippocampal formation, our data were collected from animals with more selective damage to the hippocampus.

The results of our experiments pose difficulties for several theories of hippocampal function. In the theory advanced by Hirsh (1974, 1980), the hippocampus is seen as being essential for conditional retrieval when the meaning of some stimulus varies, depending on the presence or absence of some other stimulus. Nonetheless, our hippocampectomized rats showed no deficit in their ability conditionally to retrieve the information needed to perform the discriminations.

In the theory of hippocampal functioning proposed by Olton et al. (1979), "working memory" is seen as being involved in retaining information that is used within a trial, while "reference memory" is seen as containing information that has utility across trials. Under this theory, the hippocampus is viewed as being necessary only for working memory. As described by Olton et al., both working and reference memory processes would be involved in the performance of the conditional discrimination containing the memory trace. Specifically, information concerning the presence of the facilitator would have to be held during the trace interval in order to know whether to headjerk upon presentation of the auditory stimulus (e.g., working memory), whereas the "if-then" rules, which could be used on all trials, would comprise the reference memory components of the problem. Related to Olton et al.'s theory is Rawlins's (1985) view that the hippocampus serves as a temporary memory buffer for all types of information. Certainly, the relevant stimuli used in our trace discrimination were temporarily discontinuous, and yet the hippocampectomized rats were able to perform at a level comparable to that of the controls. Thus, there is little support in the present research for either the "working memory" or "temporary memory buffer" theories of hippocampal functioning.

In view of previous research in the area, it is surprising that the hippocampectomized rats were able to perform the complex conditional discriminations without impairments. Although the hippocampus may be involved in carrying out such complex tasks, our results indicate

that the structure is not necessary for such performance if the tasks are learned before hippocampectomy. Other research will be needed to determine if complex conditional tasks can be learned without an intact hippocampus.

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