Hippocampal lesions and negative patterning in pigeons

NICOLA BROADBENT, STEPHEN GALLAGHER, and MICHAEL COLOMBO
University of Otago, Dunedin, New Zealand

A number of studies have shown that rats with hippocampal lesions are impaired on the negative patterning task, thereby supporting the view that the hippocampus may be important for configural learning. We trained 8 control sham-operated pigeons and 9 pigeons with bilateral hippocampal (hippocampus and area parahippocampalis) lesions on a negative patterning task to respond in the presence of a houselight or tone presented individually, and to withhold responding in the presence of the houselight and tone presented simultaneously. Pigeons with hippocampal lesions learned the task at the same rate as did control unoperated pigeons. This finding is in line with recent reports that the hippocampus is not critical for performance of the negative patterning task.

There is considerable evidence to support the view that the avian hippocampus is a functional homologue of the mammalian hippocampus (Bingman, 1993). For example, damage to the hippocampus in mammals results in severe impairments in spatial behavior (Squire, 1992), and the same is true for pigeons (Bingman, 1993; Colombo, Cawley, & Broadbent, 1997; Good & Macphail, 1994). Whether hippocampal lesions result in nonspatial memory impairments, however, is an issue of considerable debate. Although early accounts viewed the hippocampus as important for both spatial and nonspatial memory (Squire, 1992), recent studies suggest that the nonspatial impairments previously attributed to hippocampal damage in mammals were in fact due to inadvertent damage to the tissue adjacent to the hippocampus (Murray, 1996; Nadel, 1992, 1994). Indeed, when damage is restricted to the hippocampus, there is little evidence that mammals are impaired on nonspatial tasks such as visual delayed matching-to-sample and visual concurrent discrimination learning (Aggleton, Hunt, & Rawlins, 1986; Alvarez, Zola-Morgan, & Squire, 1995). In line with these findings, we have also found that hippocampal lesions in pigeons have no effect whatsoever on visual delayed matching-to-sample or visual concurrent discrimination learning (Colombo, Cawley, & Broadbent, 1997; Colombo, Swain, Harper, & Alsop, 1997).

Recently there has been considerable interest in the notion that the hippocampus is involved in relational or configural learning (Eichenbaum, Otto, & Cohen, 1994; Rudy & R. J. Sutherland, 1989, 1995). A common procedure used to assay configural learning is the negative patterning task. In this procedure, the animal is typically

trained to make a response in the presence of a light or a tone and to refrain from making a response when both the light and tone are presented concurrently. To solve this task, the animal must learn that the compound stimulus (light and tone) represents a configuration that is distinct from the component elemental stimuli (light or tone). A number of studies have shown that rats with hippocampal lesions are severely impaired in the acquisition of a negative patterning task (Alvarado & Rudy, 1995b; McDonald et al., 1997; Rudy & R. J. Sutherland, 1989; R. J. Sutherland & McDonald, 1990), fueling the notion that the hippocampus is critical for configural learning.

That rats with hippocampal lesions are impaired on the negative patterning task reopens the possibility that hippocampal damage can cause nonspatial impairments and suggests that the critical sensitivity to hippocampal lesions lies not in the spatial/nonspatial dimension but rather the configural/nonconfigural dimension. Given that pigeons are extremely competent at processing visual information, it seemed reasonable to examine what effect hippocampal damage in pigeons would have on learning a negative patterning task.

METHOD

Subjects

Seventeen pigeons (*Columba livia*) served as subjects. Nine had bilateral hippocampus and area parahippocampalis (hereafter referred to as *hippocampal*) lesions and 8 served as sham-operated controls. All the subjects had previously participated in a number of different experiments investigating the effects of hippocampal lesions on the learning, retention, and reversal of visual discriminations (Broadbent & Colombo, 1997). Each pigeon was housed within the colony in individual wire mesh cages with free access to water and grit. The colony was maintained on a 12:12-h light:dark cycle, with lights on at 0700 h. The pigeons were maintained at 80%–85% of their free feeding weights on a diet of wheat, peas, and corn.

Apparatus

The negative patterning task was conducted in standard sound-attenuated pigeon operant chambers. Situated on the front panel of

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Figure 1. Reconstructions of the smallest (dark) and largest (striped) hippocampal lesions. A, archistriatum; AV, archistriatum pars ventralis; Cb, cerebellum; CDL, area corticoidea dorsolateralis; DA, tractus archistriatalis dorsalis; E, ectostriatum; HA, hyperstriatum accessorium; HD, hyperstriatum dorsale; HV, hyperstriatum ventrale; N, neostriatum; NC, neostriatum caudale; V, ventricle.

each chamber were three circular translucent plastic response keys, each 2.5 cm in diameter. All three keys were mounted 22 cm above the chamber floor and were 10 cm apart, center to center. Behind the keys were stimulus projectors (IEE Model 1071) used to deliver the visual stimuli. Only the left key was used in the present experiment. Food reward was delivered via a lighted magazine situated below the center projector.

The stimuli consisted of a tone (2000 Hz) generated by a PC computer and a houselight (28 V, 2.8 W) positioned in the center of the roof of the operant chamber. All extraneous noise was masked by both a white noise generator situated in the room and a ventilation fan situated in the rear of each operant chamber, which together measured 83 dB as measured on the C scale (slow-setting) of a Simpson sound level meter (Model 886). The programming of trial events, presentation of stimuli, and recording of data were controlled by IBM-compatible computers.

Behavioral Procedure

Shaping. Because of their prior experience, it was necessary to only briefly shape the pigeons to peck the left key when lit with a white light until they responded on 30/30 trials with a latency averaging less than 10 sec.

Acquisition. The left key was continuously illuminated with a white light for the duration of the session. At the end of a 45-sec intertrial interval (ITI), a houselight (L+), tone (T+), or houselight/tone (LT-) stimulus was presented for a duration of 10 sec. On S+ trials (L+ or T+), a response to the left key within the 10-sec period resulted in termination of the stimulus and 2 sec of access to wheat, followed by the ITI. Failure to respond on S+ trials resulted in termination of the stimulus, which was followed by the ITI. On S- trials (LT-), the pigeons were required to withhold responding for the duration of the stimulus presentation. Such correct responses did not result in reward and were followed by the ITI. Responding on S- trials resulted in termination of the stimulus, followed by a 45-sec timeout period, which was then followed by the ITI. Conditions during the timeout period were identical to the ITI.

A daily session consisted of 90 trials, with 30 presentations of each of the L+, T+, and LT- trials presented pseudorandomly within a session. Pigeons were trained until they reached a criterion of two successive days at 27/30 (90%) correct responses for both the S+ and S- trials. Testing was continued for a maximum of 90 sessions.

Surgery and Histology

Surgery was conducted under ketamine hydrochloride anesthesia (100 mg/ml). The head was immobilized using a Revzin stereotaxic adapter. A topical anesthetic (Xylocaine, 10%) was applied to the scalp, which was then retracted to expose the skull. Rongeurs were used to expose the dura above the left and right hippocampal regions. Nine animals received bilateral aspiration of the hippocampus and area parahippocampalis according to the locations given by Karten and Hodos (1967). The remaining 8 sham-operated animals were treated in an identical fashion, with the exception that the bone overlying the hippocampus and area parahippocampalis was not removed, and no tissue was aspirated. The incision was then sutured, Xylocaine (10%) was applied to the wound margins, and the animals were allowed to recover until they were alert and mobile. Surgery was conducted 14–20 months prior to the start of the present experiment.

Upon completion of behavioral testing, the pigeons were deeply anesthetized with Halothane anesthesia and perfused intracardially with physiological saline followed by 10% formalin. The head was then stored in 10% formalin. The brain was then removed and stored in 30% sucrose and 10% formalin until it sank. The brain was embedded in paraffin, sectioned at 10 μ m, and every 10th section was stained with cresyl violet.

Figure 2. Number of sessions required by the 3 control and 3 hippocampal pigeons that satisfied the acquisition criterion. On the right of the figure is the average number of sessions collapsed across all three animals in each group.

RESULTS

Histology

Reconstructions of the largest and smallest hippocampal lesions are shown in Figure 1. All birds sustained complete hippocampal lesions that included all of the medially located hippocampus and laterally situated area parahippocampalis. In all cases, the lesions extended beyond the hippocampus and area parahippocampalis to include minor encroachment anterior into the posterior hyperstriatum accessorium, and encroachment bilaterally into the corticoidea dorsolateralis in the posterior extent of the lesion. There was also occasional minor damage to the neostriatum.

Negative Patterning Performance

One pigeon received an incomplete lesion to the hippocampus and was therefore excluded from the analysis. A second hippocampal bird was dropped from the study because it failed to respond to either of the elemental stimuli. Of the remaining 7 hippocampal and 8 control pigeons, 6 birds (3 hippocampal and 3 control) were able to satisfy the 90% acquisition criterion. The number of sessions to learn the negative patterning task for these 6 birds is shown in Figure 2. A two-sample *t* test revealed no difference in the number of sessions required by the 3 control pigeons (38, 51, and 68 sessions) and 3 hippocampal pigeons (34, 36, and 55 sessions) to learn the negative patterning task [$t(4) = .97, p = .39$].

The performance of the remaining 5 control and 4 hippocampal pigeons on the L+, T+, and LT− stimuli averaged across blocks of five sessions is shown in Figure 3A. The data were subjected to a three-way analysis of variance (ANOVA) with group (2: control and hippocampal), stim-

ulus type (3: L+, T+, and LT−), and blocks of five sessions (18: Blocks 1–18) as variables. It is clear from the figure that although these animals failed to satisfy the acquisition criterion, both control and hippocampal pigeons showed a gradual improvement across blocks [$F(17,119) = 25.0, p < .01$]. Indeed over the last 5 sessions, control and hippocampal animals were performing 82.0% and 78.3% correct. There was also a significant effect of stimulus type [$F(2,14) = 39.5, p < .01$], which was due to the fact that performance on the elemental stimuli was superior to performance on the compound stimulus, and a significant stimulus type $\times$ block interaction [$F(34,238) = 14.4, p < .01$], suggesting that the improvement across blocks was attributable to an improvement in performance on the compound stimulus. Despite this improvement in performance, there was no main effect of group [$F(1,7) = 0.57, p = .47$], no group $\times$ block interaction [$F(17,119) = 1.04, p = .42$], no group $\times$ stimulus type interaction [$F(2,14) = .12, p = .89$], and no group $\times$ stimulus type $\times$ block interaction [$F(34,238) = 1.33, p = .12$], indicating that the control and hippocampal animals learned the negative patterning task at the same rate.

We also examined the performance of control and hippocampal animals in terms of latency to make a response; the results are shown in Figure 3B. It is clear that the results expressed in terms of latency were virtually identical to the results obtained in terms of percent correct. A three-way ANOVA with group (2: control and hippocampal), stimulus type (3: L+, T+, and LT−), and blocks of five sessions (18: Blocks 1–18) as variables was calculated. Of critical interest, there was no main effect of group [$F(1,7) = .05, p = .83$], no group $\times$ stimulus type interaction [$F(2,14) = .31, p = .74$], and no group $\times$ block interaction [$F(17,119) = 1.54, p = .09$]. We did note a significant group $\times$ stimulus type $\times$ block [$F(34,238) = 1.64, p < .05$]. To confirm the source of this interaction, we conducted separate two-way ANOVAs for the L+, T+, and LT− data with group (2: control and hippocampal) and blocks (18: Blocks 1–18) as factors. There was a significant group $\times$ blocks interaction for the LT− stimulus [$F(17,119) = 2.08, p < .05$], but not for the L+ ($p = .55$) or T+ ($p = .52$) stimuli. It is likely that this significant effect was due to the fact that early in training the hippocampal animals had somewhat longer latencies to respond to the LT− stimulus, whereas later in training they had somewhat shorter latencies to respond to the LT− stimulus. A two-way ANOVA conducted for the last 20 sessions with group (2: control and hippocampal) and blocks of five sessions (4: Blocks 15–18) as factors, however, revealed no significant difference between the control and hippocampal pigeons in terms of latency to respond on the LT− stimulus [$F(1,7) = 0.02, p = .89$].

DISCUSSION

The negative patterning problem has been described as a task that is particularly sensitive to hippocampal damage (Rudy & R. J. Sutherland, 1995). In the present ex-

Figure 3. (A) Performance on the L+ (left panel), T+ (center panel), and LT- (right panel) stimuli across the 90 sessions for the 5 control and 4 hippocampal animals that did not satisfy the acquisition criterion. The dotted line represents chance levels of performance. (B) Latency to respond to the L+ (left panel), T+ (center panel), and LT- (right panel) stimuli across the 90 sessions for the 5 control and 4 hippocampal animals that did not satisfy the acquisition criterion.

periment, hippocampal lesions had no effect on the ability of pigeons to learn a negative patterning task. We can dismiss the possibility that the absence of any effects was due to incomplete lesions, since in all animals the lesions not only completely removed the hippocampus and area parahippocampalis, but also tended to encroach on adjacent areas. We can also dismiss the possibility that our results differ from those obtained with rats because the avian hippocampus has evolved to perform a different function than the mammalian hippocampus. Although much research still needs to be done to settle the issue of functional homology, there is considerable evidence to suggest that the pigeon hippocampus is a functional as well as a structural homologue of the mammalian hippocampus (Bingman, 1993).

It is also difficult to account for the absence of any effects on the grounds that the type of negative patterning procedure adopted was not sensitive enough to be affected by hippocampal lesions. First, although the literature is far from clear on this issue (Alvarado & Rudy, 1995b), there is some evidence to suggest that when performance is measured in terms of rates of responding

rather than simply making a go/no-go response, animals with hippocampal lesions perform the negative patterning task as well as do controls (Davidson, McKernan, & Jarrod, 1993). However, we actually used the go/no-go procedure, and required the animals to withhold making a response for as long as 10 sec, and yet there was no evidence that animals with hippocampal lesions behaved any differently from control animals. Second, Alvarado and Rudy (1995a; Rudy & R. J. Sutherland, 1995) have argued that the effects of hippocampal lesions could be ameliorated by organizing the T+, L+, and LT- stimuli to occur across sessions rather than within a session. Again, this situation does not apply to our experiment since the T+, L+, and LT- stimuli always occurred within a session, which according to Rudy and R. J. Sutherland (1995) ought to increase the chances of obtaining a negative patterning deficit following hippocampal lesions. Third, Rudy and R. J. Sutherland (1995) have stated that by varying the ratio of elemental to compound stimulus presentations, the negative patterning task can be made more or less sensitive to the effects of hippocampal lesions. In this regard, our present design, in which we em-

played an unequal number of S+ and S- stimuli (30 L+, 30 T+, and 30 LT-), should have been more sensitive than designs with equal numbers of S+ and S- stimuli (Alvarado & Rudy, 1995a; McDonald et al., 1997), and yet again we failed to find any effect of hippocampal lesions. In summary, our task parameters, which were modeled after those of Rudy and Sutherland (1989), were such as to maximize the chances of obtaining a deficit following hippocampal lesions, yet there was no evidence that such lesions impaired negative patterning performance.

One criticism of the present study is that because we did not conduct a transfer test, we were unable to eliminate the possibility that our animals had solved the task using slower nonconfigural means. Although it is true that in the present study, in comparison with other negative patterning studies (Alvarado & Rudy, 1995b; Rudy & R. J. Sutherland, 1989; R. J. Sutherland & McDonald, 1990; see also Rescorla, Grau, & Durlach, 1985, for behavioral work with pigeons), our subjects required a large number of sessions to learn the negative patterning task, and although we cannot dismiss the possibility that our subjects may have been solving the task by using a nonconfigural strategy (e.g., numerosity, stimulus intensity, or stimulus complexity; cf. Davidson et al., 1993), we believe that certain procedural variables contributed to the lengthy acquisition period. Clearly, learning to attend to the relevant cues is a critical component of any discrimination task (N. S. Sutherland & Mackintosh, 1971). In this respect, our animals were at a tremendous disadvantage, for not only did they have to learn that the relevant cues were diffuse presentations of a houselight and a tone, they also had to overcome over 2 years of experience in which they were specifically trained that the relevant cues appeared presented onto the response keys. Thus when confronted with a white light presented on the left response key, our animals likely attempted to solve the negative patterning task on the basis of this information, rather than on the basis of the diffusely presented houselight and tone stimuli. Only after failing at this would they have then shifted their attention to the diffusely presented houselight and tone cues. Although we have no way of knowing this for certain, it seems more likely that the lengthy acquisition period was a result of such procedural factors rather than the result of the animals' attempting to solve the task by using nonconfigural means.

Although variations in parameters such as the ratio of S+ to S- presentations (Rudy & R. J. Sutherland, 1995), split or mixed designs (Alvarado & Rudy, 1995a), stimulus salience (Redhead & Pearce, 1995), spaced or massed sessions and ITI length (Han, Gallagher, & Holland, 1998), and the type of response measure utilized (Davidson et al., 1993) may play a role in whether or not hippocampal lesions will impair negative patterning performance, another interpretation of the negative patterning literature and the present study is possible. As we (Colombo, Cawley, & Broadbent, 1997; Colombo, Swain, et al., 1997) and others (Jarrard, 1993, 1995; Nadel, 1991, 1992, 1994) have argued in the context of certain visual tasks thought

at one time to be sensitive to "hippocampal" damage, it is the situation in most cases (but see Alvarado & Rudy, 1995b) that when impairments have been noted on the negative patterning task, the damage often extended beyond the hippocampus to include adjacent tissue, and it is in fact damage to this adjacent tissue that might be causing the negative patterning deficits. In the end, understanding what the hippocampus contributes to learning the negative patterning task will surely require more detailed behavioral studies of the sort conducted by Alvarado and Rudy (1995a; Rudy & R. J. Sutherland, 1995). These data are a first step to study some of these factors in the pigeon brain.

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