Discrimination learning in turtles after lesions of the dorsal cortex or basal forebrain

ALAN BLAU and ALICE SCHADE POWERS
St. John's University, New York, New York

The basal forebrain of turtles contains cholinergic neurons that project to the dorsal cortex, a three-layered structure on the surface of the cerebral hemispheres. This experiment was designed to examine whether the basal forebrain is involved in associative function in reptiles, as has been shown in mammals. Three groups of turtles—a sham-lesioned control group, a group given aspiration lesions of the dorsal cortex, and a group given ibotenic acid lesions of the basal forebrain—were trained in an operant chamber on a simultaneous pattern discrimination and its reversal. The stimuli to be discriminated were horizontal and vertical stripes. Relative to controls, lesions of either the dorsal cortex or the basal forebrain disrupted acquisition and reversal of the discrimination. The results suggest that the basal forebrain systems of reptiles and mammals function in a similar way.

Recent research has revealed an important role for the cholinergic basal forebrain in learning and memory in mammals. The nucleus basalis of Meynert, which consists of cholinergic cells projecting to the neocortex (Aggleton, Burton, & Passingham, 1980; Saper, 1984), seems to participate in both the acquisition and the retention of a large number of different sorts of learning (Dunnett et al., 1985; Lerer, Warner, Friedman, Vincent, & Gamzu, 1985; Murray & Fibiger, 1985, 1986). Anatomical studies in reptiles have identified a similar cholinergic projection from the basal forebrain to the telencephalic surface. Reptiles do not have a true six-layer neocortex, but the dorsal surface of the hemisphere is covered with a three-layered cortex, termed the *dorsal cortex* (Powers & Reiner, 1979, 1980). Cells in the basal forebrain, chiefly in a region called the ventral paleostriatum, react positively with an antibody for choline acetyltransferase (Mufson, Desan, Mesulam, Wainer, & Levey, 1984). These cells project to the dorsal cortex (Bruce & Butler, 1984; Desan, 1984; Ouimet, Patrick, & Ebner, 1985).

The present experiment was undertaken to determine whether the basal forebrain participates in learning in turtles. Prior research in our laboratory had demonstrated that lesions of the dorsal cortex impaired reversal learning (Cranney & Powers, 1983), and we wished to replicate that result here and to determine whether basal forebrain lesions would have the same effect. Reiner and Powers (1980) had previously demonstrated that electrolytic lesions of the basal forebrain severely impair the retention of simultaneous visual discriminations learned preoperatively. Those lesions cut the lateral forebrain bundle, however, which carries visual information from the

thalamus to the telencephalon, and we attributed the severe loss in that study to disruption of those connections. In this experiment, we made lesions of the basal forebrain with injections of ibotenic acid in order to destroy cells in that region without destroying the thalamotelencephalic fibers that pass through the region.

Three groups of turtles were first pretrained to press keys in an operant-discrimination chamber, then given sham lesions or lesions of the dorsal cortex or basal forebrain. After recovery from surgery, they were trained to criterion on a horizontal-vertical discrimination, then on the reversal of that discrimination.

METHOD

Subjects

Fifteen naive adult painted turtles (*Chrysemys picta*) of both sexes served as subjects. They were maintained in individual open aquaria in a room with controlled light (14 h on, 10 h off) and temperature (30°C). They were run in the experiment during the light portion of the light-dark cycle.

Apparatus

The apparatus consisted of a water-tight black Plexiglas enclosure with a hinged top; its floor measured 21.2×23.0 cm, its height 20.5 cm. A houselight mounted in the center of the front wall of the chamber 18.8 cm above the floor provided general illumination of 64.8 lx, measured in the center of the floor of the chamber with a Simpson illumination meter (Model 408). Centrally located on the front wall of the chamber, 7 cm above the floor, was a translucent disk (the food magazine), 2.2 cm in diameter with an aperture of 1.0 cm in its center. Food reward (beef baby food) was delivered through the aperture in the center of the disk. The food-delivery system consisted of a surgical rubber hose attached to a syringe whose plunger was driven by a Davis LR-130 liquid pump.

Flanking the food magazine were two response keys, each a transparent plastic disk, 3.0 cm in diameter, through which stimuli were presented via rear-mounted IEE projectors. Depressions of the response keys were relayed to standard programming equipment that controlled and recorded the events in the experimental chamber. The stimuli were 2.5 cm in diameter. Each consisted of three

Correspondence concerning this article should be addressed to Alice S. Powers, Department of Psychology, St. John's University, Jamaica, NY 11439.

white lines, 2 mm wide and separated by 3 mm from one another, on a black background. The lines were either horizontal or vertical, and the intensities of the stimuli were equated.

Because turtles take their food underwater to eat, the chamber contained water to a depth of 2.5 cm when the experiment was in progress.

Procedure

Preoperative training. All turtles were trained initially to eat from the food magazine and to press the response keys, illuminated with a red light. There were 20 trials per day with an intertrial interval of 15 sec. Each reinforcement consisted of the delivery of 0.3 ml of beef baby food, accompanied by a 15-sec illumination of the food magazine. Once the turtle had learned to press both keys, presented in a random order, the response requirement was increased to FR-2. Pretraining continued until a turtle completed 20 trials on FR-2 in 30 min or less.

Surgery. Within 24–48 h of the completion of pretraining, the turtles were anesthetized with Equithesin (0.20 ml/100 g body weight i.p.). Five turtles received bilateral lesions of the dorsal cortex made by aspiration. These lesions extended from A2.0 to A4.0 in the stereotaxic atlas of Powers and Reiner (1980). Five received bilateral lesions of the basal forebrain made by injection of 0.1 μ l of a 1% solution of ibotenic acid in phosphate-buffered saline (pH 7.4) over a period of 15 min. The coordinates for the basal forebrain lesions were A3.4, L1.8, V2.4 (also from the Powers & Reiner atlas). Sham lesions were made by opening the skull. Drill holes were not covered after either sham or actual lesions: the openings healed rapidly on their own. Five to 7 days were allowed for postoperative recovery.

Postoperative training. All turtles were trained on a pattern discrimination between horizontal and vertical stripes. Three turtles of each group were reinforced for going to vertical and two for going to horizontal. They were run on the problem for 20 trials per day until they reached a criterion of 85% correct. Then the discrimination was reversed and the turtles were trained to the same criterion on the reversal.

Each stimulus appeared equally often on the left and on the right. The sequence of trials was determined by Gellermann (1933) orders. Each session began with a 15-sec intertrial interval, during which responses on either key reset the timer. Once the keys were illuminated, reinforcement or timeout was delivered on an FR-2 schedule. Correct response was followed immediately by the extinction of the stimulus lights and delivery of the reinforcement at the magazine, which was illuminated for 15 sec before the intertrial interval began again. An incorrect response led to a 15-sec timeout followed by the 15-sec intertrial interval; the keys were both dark during these periods. After an error, a limited correction procedure was used. The same trial was repeated four more times. If, after these repetitions, the turtle still had not chosen correctly, it was guided to the correct side by illuminating only the correct target. Thus, each trial ended with reinforcement. Initial and repetitive errors were recorded separately. In addition, total time per session was recorded.

Histology. Upon completion of postoperative training, the turtles were anesthetized and perfused via the innominate artery with saline followed by Heidenhain's solution (without mercuric chloride). After several days of fixation in formalin, the brains were embedded in paraffin, sectioned at 15 μ m, and stained by the Kluver-Barrera method (luxol fast blue for fibers, cresyl violet counter-

Figure 1. Representative lesions from the two groups. The dorsal cortex lesion is Case 50; the basal forebrain lesion is Case 387. cd = dorsal cortex; cm = medial cortex; cp = pyriform cortex; d = Area d; CN = core nucleus of the dorsal ventricular ridge; CO = optic chiasm; DVR = dorsal ventricular ridge; GP = globus pallidus; NDB = nucleus of the diagonal band of Broca; PA = paleostriatum augmentatum; PT = pallial thickening; TO = optic tract; V = ventricle; VP = ventral pallidum.

stain for cells). The lesions were reconstructed by an observer blind to the outcome of the experiment on standard sections from the atlas of Powers and Reiner (1980).

RESULTS

Histology

Except for 2 subjects, Nos. 42 and 71, which sustained bilateral damage to the dorsal ventricular ridge as well as to the cortex, lesions of the dorsal cortex were restricted to that structure. A sample lesion of the dorsal cortex is shown in Figure 1.

Lesions of the basal forebrain were small and were located in the globus pallidus, the ventral paleostriatum, and the diagonal band of Broca. One case, No. 51, sustained very slight bilateral damage to the dorsal cortex. A sample lesion of the basal forebrain is also shown in Figure 1.

Figure 2 presents the median initial errors on acquisition and reversal for each group. Both groups with lesions were impaired on both acquisition and reversal. Mann-Whitney U tests revealed that, relative to the sham-lesioned controls, the dorsal cortex group had significantly more trials to criterion on both acquisition ($U = 2.5$, $p < .05$) and reversal ($U = 0$, $p < .05$). Similarly, the basal forebrain group also had significant deficits on acquisition ($U = 2$, $p < .05$) and reversal ($U = 1.5$, $p < .05$) when compared with controls. The two lesioned groups, however, did not differ significantly from each other.

To determine whether responding was slower for the lesioned groups than for the shams, we calculated the mean length of trial by dividing the total session length by the number of times the stimuli were presented (total number of initial and repetitive errors plus 20). We then compared these times in the three different groups. No significant differences were found on this measure among the groups.

Spearman rank-order correlations were calculated between the extent of damage to the target structure and deficits on acquisition and reversal. For the dorsal cortex group, we calculated $W\%$, an index of total bilateral destruction of a structure (Hodos & Bobko, 1984). To obtain this index, the percentage of damage to a given structure in one hemisphere is multiplied by the percentage of damage to the structure in the other hemisphere, and the product is divided by 100. This index has the advantage that it gives higher values for symmetrical lesions than for asymmetrical ones and a value of zero for unilateral lesions. Calculations of the percentage of damage to the dorsal cortex included the pallial thickening, which is now thought to be part of the dorsal cortex, since it receives a projection from the lateral geniculate nucleus of the thalamus, as does the lateral half of the dorsal cortex (Desan, 1984; Hall, Foster, Ebner, & Hall, 1977). There was a significant correlation between dorsal cortex damage and deficit ($\rho = .78$ for both acquisition and reversal, $p < .01$).

For the basal forebrain group, it was not possible to calculate the percentage of damage because the cholinergic

Figure 2. Performance of the three groups on acquisition and reversal. CD = dorsal cortex group; NBM = basal forebrain group.

cells we were trying to destroy are scattered in a wide area throughout the basal forebrain, not in one restricted structure. We therefore calculated these correlations on the basis of the total amount of destruction of the basal forebrain. Again, there were significant correlations between basal forebrain damage and deficit ($\rho = .84$ for acquisition, $\rho = .79$ for reversal, $p < .01$ in both cases). Correlations calculated across the groups between the total extent of brain tissue damaged and deficits on acquisition and reversal were significant ($\rho = .57$ for acquisition, $\rho = .64$ for reversal, $p < .05$ in both cases). Total tissue damage was correlated significantly with damage to either the dorsal cortex or the basal forebrain, the respective target areas ($\rho = .95$, $p < .01$).

We made the basal forebrain lesions by ibotenic acid to prevent damage to the thalamotellencephalic fibers that pass through the region via the lateral forebrain bundle. One way to examine whether the basal forebrain lesions damaged the lateral forebrain bundle is to look for retrograde chromatolysis or cell loss in the nucleus rotundus, a thalamic nucleus that projects to the core nucleus of the dorsal ventricular ridge in the telencephalon. Retrograde cell loss in the nucleus rotundus is a recognized concomitant of lateral forebrain lesions in reptiles (Kruger & Berkowitz, 1960; Powell & Kruger, 1960; Reiner & Powers, 1980). In this study, there was no evidence of degeneration in the nucleus rotundus.

DISCUSSION

The results of this study show that damage to the dorsal cortex or to the basal forebrain of turtles produces similar effects: deficits on both acquisition and reversal of a simultaneous pattern discrimination. These results are consistent with our hypothesis that associative functions are mediated by a system of cholinergic neurons in the forebrain of turtles.

The lack of an effect on response times suggests that the lesioned animals were no less eager to eat than were sham-lesioned subjects. Thus, the deficits we observed were not due to changes in motivation in the animals with lesions.

Although total size of lesion was correlated with deficit, total damage to the target structure was highly correlated with total lesion size. We believe, therefore, that the amount of damage to the target, rather than the total amount of damage to the brain, was responsible for the deficits we observed.

Although both lesions produced deficits in this experiment, the results of other studies from this laboratory show that not all forebrain lesions disrupt discriminative behavior. For example, medial cortex lesions do not affect acquisition or reversal of go/no-go pattern discriminations, whereas dorsal cortex lesions impair both (Grisham & Powers, in press). We believe, therefore, that the effects observed in this experiment were similar, not because any lesions would disrupt performance, but because the lesions disrupted a cholinergic system in both cases.

Dorsal Cortex Effect

The data presented here might appear to suggest that lesions of the dorsal cortex in turtles produce visual deficits. Such a conclusion would be consistent with the fact that the dorsal cortex receives a projection from the lateral geniculate nucleus of the thalamus (Hall & Ebner, 1970; Hall, Foster, Ebner, & Hall, 1977), but it would probably be erroneous. Taken as a whole, the results obtained in our laboratory regarding dorsal cortex function suggest that it mediates some sort of associative function, not visual ability. Lesions of the dorsal cortex do not disrupt retention of visual discriminations, for example (Grisham & Powers, in press; Reiner & Powers, 1983). They do disrupt acquisition of a simple keypress response (Grisham & Powers, in press) and retention of a maze habit (Petrillo & Powers, 1987), neither of which is a task that requires vision. We cannot completely rule out the possibility that the associative ability that is disrupted is specifically visual, but we believe that the pattern of results obtained after lesions of the dorsal cortex supports the notion that its function is more general.

The reversal deficit obtained after dorsal cortex damage is similar to that described by Cranney and Powers (1983) in a series of color and pattern problems. In the present experiment, we demonstrate that such a deficit occurs in a simple reversal of a pattern discrimination with no irrelevant color present.

Grisham and Powers (in press) examined a number of different hypotheses about what factor may account for the reversal deficit, and found no evidence that turtles with dorsal cortex lesions had difficulty withholding responses, extinguishing a learned response, or reacquiring an extinguished response. They concluded that the reversal deficit obtained after dorsal cortex lesions was due to a learning impairment, to which the reversal task was particularly sensitive.

Cranney and Powers (1983) and Reiner and Powers (1983) showed that lesions of the core nucleus of the dorsal ventricular ridge, a telencephalic visual area, impaired performance on visual discriminations. Since 2 subjects in the dorsal cortex group in this experiment sustained

bilateral damage to the core nucleus, we asked whether the deficits we observed in acquisition in this group were due to damage to the core nucleus. They were not. The 2 subjects with bilateral damage to the core nucleus performed in the middle range of the dorsal cortex group: they ranked second and third on errors in acquisition. The amount of damage to the core nucleus was slight and apparently did not contribute to the deficit we observed.

Basal Forebrain

Damage to the basal forebrain in this study was quite slight, but it was sufficient to produce deficits similar to those seen after dorsal cortex lesions. The deficits we observed were not due to disruption of thalamotelencephalic connections, but rather to damage to cells in the basal forebrain.

It seems likely that the damage that resulted in behavioral impairment was to cholinergic cells of the basal forebrain, which project to the dorsal cortex (Desan, 1984; Ouimet, Patrick, & Ebner, 1985). Since the results were similar after dorsal cortex and nucleus basalis lesions, these two structures appear to be part of a system that participates in learning and memory in turtles. Interrupting the system either in the basal forebrain or in the cortex causes the animals to experience associative deficits. Similar results have been obtained in rats given lesions of the nucleus basalis of Meynert (Olton & Wenk, 1987). We are currently undertaking experiments designed to investigate whether lesions of the basal forebrain are correlated with a loss of cholinergic cells.

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