BRIEF COMMUNICATIONS

Hippocampal Lesions Impair Memory for Location but Not Color in Passerine Birds

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The effects of hippocampal complex lesions on memory for location and color were assessed in black-capped chickadees (Parus atricapillus) and dark-eyed juncos (Junco hyemalis) in operant tests of matching to sample. Before surgery, most birds were more accurate on tests of memory for location than on tests of memory for color. Damage to the hippocampal complex caused a decline in memory for location, whereas memory for color was not affected in the same birds. This dissociation indicates that the avian hippocampus plays an important role in spatial cognition and suggests that this brain structure may play a role in working memory generally.

The hippocampus is clearly involved in memory, but it is difficult to characterize exactly the role of this structure in various types of memory (Macphail, 1993). It is widely thought that the rodent hippocampus is important in memory for spatial information (e.g., Nadel, 1991). The hippocampus has also been implicated in working memory (e.g., Wan, Pang, & Olton, 1994). Recently, a body of literature has developed that demonstrates enlargement of the hippocampal complex in animals subjected to unusually strong demands for good spatial cognition (Sherry, Jacobs, & Gaulin, 1992; Bingman, 1992; but see Squire, 1993). These specialized animals may be particularly good models for the study of hippocampal function.

On the basis of similarities in development (Källén, 1962), connectivity (Casini, Bingman, & Bagnoli, 1986), and neurotransmitters (Erichsen, Bingman, & Krebs, 1991; Krebs, Erichsen, & Bingman, 1991), the avian dorsomedial cortex is believed to be homologous to the mammalian hippocampus. We refer to this brain region as the hippocampal complex, to encompass the hippocampus proper and area parahippocampalis as defined by Karten and Hodos (1967). Substantial differences do exist between the mammalian and avian hippocampal complexes, however, including the absence of clear subdivisions such as the CA fields and entorhinal cortex (Macphail, 1993, p. 386), and the apparent absence of a mossy fiber system like that found in mammals (Erichsen et al., 1991). It is not known whether or not a trisynaptic path exists in the avian hippocampus. Comparisons of structure and function between the avian and mammalian hippocampal complexes may yield insights into the relationship between hippocampal function and structure; shared architectural features should map to shared functional properties while functional differences may be related to structural differences. If both the mammalian and avian hippocampal complexes are involved in spatial cognition, it may be the structural properties common to both classes of animals that give the hippocampus the ability to process spatial information.

Scatter hoarding birds place individual food items in a dispersed distribution and remember the locations of these cached items for days or months. Dependence on spatial memory for cached food may have resulted in specialization of memory in food-hoarding birds (e.g., Shettleworth, 1995). Previous studies have documented differences between food-storing black-capped chickadees (Parus atricapillus) and non-storing dark-eyed juncos (Junco hyemalis) in the use of spatial information in working memory tasks (Brodbeck, 1994; Brodbeck & Shettleworth, 1995; Hampton & Shettleworth, in press; see Shettleworth, 1995, for a recent review of studies comparing storing and nonstoring species). Species differences in the use of spatial information may represent a specialization of spatial cognition in food-storing birds that is related to dependence on memory for the location of scatter-hoarded food.

Food-storing birds have a relatively large hippocampal complex compared with nonstoring birds (Sherry, Vaccarino, Buckenham, & Herz, 1989; Krebs, Sherry, Healy, Perry, & Vaccarino, 1989), and this difference in relative hippocampal volume is evident between chickadees and juncos housed for extensive periods of time in the laboratory (Hampton & Shettleworth, in press). Hippocampal complex damage impairs the ability of chickadees to return accurately to locations in an aviary where they previously stored food (Sherry & Vaccarino, 1989). Sherry and Vaccarino (1989) also showed that hippocampal complex lesions impair learning of a spatial discrimination in the same environment, while sparing performance of a black–white discrimination. Pigeons with hippocampal complex lesions are impaired at delayed spatial matching...
to sample, whereas performance of two nonspatial tasks is unaffected (Good & Macphail, 1994a). Hippocampal-lesioned homing pigeons show deficits in the ability to learn the location of the home loft (Bingman & Yates, 1992). Together, these results suggest a specific role for the avian hippocampal complex in spatial memory.

We achieve three objectives with this study: (a) We make the first within-subject, within-task comparison of the effects of hippocampal complex damage on spatial and nonspatial working memory in birds. Previous studies have used between-subjects designs and/or distinct tasks to assess memory for spatial and nonspatial information (Sherry & Vaccarino, 1989; Good & Macphail, 1994a, 1994b). In the present experiment we use one testing paradigm and require animals to remember spatial information in some sessions and nonspatial information in other sessions. (b) We directly contrast working memory (Olton et al., 1979) and “declarative” memory (Squire, 1992) hypotheses of hippocampal function against the hypothesis that the avian hippocampus is uniquely involved in spatial memory. Chickadees with hippocampal damage make “working-memory errors” in a color discrimination task that requires birds to visit sites with cards of one color and avoid sites with cards of the other color (Sherry & Vaccarino, 1989). Working-memory errors in this case were defined as revisits to sites the bird had already visited and either retrieved the reward or found empty. This failure to avoid already visited sites can be explained by a deficit in spatial memory, separate from any impairment in working memory, however, as previously visited sites can be distinguished from other sites only on the basis of their location. We use a test of nonspatial working memory that is not confounded with a requirement for spatial memory. (c) We test the validity of our operant paradigm in the assessment of hippocampal function in chickadees and juncos. A major thrust of studies comparing storing and nonstoring species is to compare memory of intact animals that differ in relative hippocampal volume as well as in food-storing behavior in the wild. The current study tests the hypothesis that the operant tests used by Brodbeck and Shettleworth (1995) and other investigators (Olson, Kamil, Balda, & Nims, 1995; see Shettleworth, 1995) in their comparisons of storing and nonstoring species indeed measure hippocampal function.

**Method**

**Subjects**

Three black-capped chickadees and 5 dark-eyed juncos, captured and maintained as described in previous studies (e.g., Brodbeck & Shettleworth, 1995) were used in this study. Food was removed from the subjects' cages each night prior to testing the following day, as described previously (Brodbeck & Shettleworth, 1995). These birds had participated in studies comparing memory for the location of a stimulus with memory for the color of a stimulus (Shettleworth & Westwood, 1995). When the present study began, they had completed many thousands of trials matching both color and location. Each subject had experienced nearly the same number of trials of both types, and birds of both species had experienced equal numbers of trials.

**Apparatus**

Birds were tested in three similar cages, each containing a computer touch screen (27.25 cm wide by 20.25 cm high) on which stimuli were presented and responses registered, a hopper for the delivery of food rewards, and a rear perch with a photobeam used to localize the animal between trials and during retention intervals (RIs). The apparatus has been previously described (Brodbeck & Shettleworth, 1995).

**Tests of Memory for Space and Color**

Because subjects were experienced in matching both space and color, no further training was necessary. Following the expiration of an intertrial interval with a mean of 30 s (15 s minimum, 45 s maximum), perching between the photobeams on the rear perch caused a sample to appear on the computer monitor at the front of the box. Samples occupied one of 20 possible locations on the computer screen. Location samples were white squares (3.7 cm), color samples were circles (2.5 cm in diameter) of one of 14 colors. Pecking the sample five times (FR 5) made the sample disappear and initiated an RI of 5 s. Following expiration of the RI, perching on the rear perch resulted in presentation of a test. On location tests, the test array consisted of two white squares; one square occupied the studied location, and a distractor occupied an alternate location. On color tests, two circles were presented symmetrically placed about the location where the sample had been presented, separated by 1.9 cm. One circle was of the studied color, and the second circle was of an alternate color. Pecking the studied sample resulted in 1–3 s of feeder access, whereas pecking the alternate sample only extinguished the stimuli. Location and color tests were administered on alternate days during sessions of a maximum of 60 trials or 60 min, whichever occurred first.

Each bird was given 300 tests each of memory for location and color to establish a baseline level of performance on the two types of tests. Following collection of the baseline data, bilateral hippocampal complex lesions were administered to each bird. Performance of birds following the lesions was assessed during 500 tests each of location and color conducted as during collection of baseline data.

**Surgery and Histology**

Subjects were deprived of food for about 1 hr and anesthetized with 50 mg/kg xylazine followed after 5 min by 25 mg/kg ketamine injected into the pectoral muscle. Birds were placed in a stereotaxic holder modified following Vaccarino (1986), feathers were removed from the top of the head, an incision was made to expose the skull, and bone over the hippocampus was removed. Tissue composing the hippocampal complex was aspirated bilaterally through an 18-gauge needle. Dissolvable sponge was placed in the resulting cavity, and the skin was sealed over the wound using cyano-adhesive. Birds were allowed to recover for 5 days prior to the resumption of testing.

Following the completion of testing, birds were deeply anesthetized and perfused transcardially with 0.8% saline followed by 10% formalin in saline. The fixed brain was removed from the cranium and refrigerated in formalin. Prior to sectioning each brain was transferred to a 30% sucrose, 10% formalin solution and stored in the refrigerator until it sank. Brains were sectioned at 40-μm intervals, and every eighth section was mounted and stained with cresyl violet. Lesions were visualized under a light microscope, and with the aid of an enlarger, drawn onto images of sectioned brains previously prepared for both species from tissue from intact animals (Hampton & Shettleworth, in press).
and sometimes into the underlying neostriatum. Lesions typically extended farther anterior than the hippocampus, at the level of the surface of the brain. This resulted in more substantial damage to the anterior hyperstriatum accessorium. This area receives projections from the thalamofugal visual pathway, one of two visual pathways in birds (Horn, 1985). Lesions of hyperstriatum accessorium, used in the operated control group of Sherry and Vaccarino (1989), had no effect relative to unoperated controls on either spatial or nonspatial tasks. Effects of lesions to this region are generally subtle or nonexistent (Ebbesson, 1980). This evidence indicates that damage to the hyperstriatum accessorium in conjunction with damage to the hippocampus should not substantially complicate interpretation of any memory deficits observed following the lesions.

**Results and Discussion**

**Histology**

The hippocampus was extensively damaged in every subject, whereas caudal area parahippocampalis was substantially spared. Figure 1 depicts the largest and smallest lesion in each of the two species, indicated by the bold lines. There was some damage to hyperstriatum dorsale and hyperstriatum ventrale, near the midline. Lesions intruded into the lateral ventricles, and sometimes into the underlying neostriatum. Lesions typically extended farther anterior than the hippocampus, at the level of the surface of the brain. This resulted in more substantial damage to the anterior hyperstriatum accessorium. This area receives projections from the thalamofugal visual pathway, one of two visual pathways in birds (Horn, 1985). Lesions of hyperstriatum accessorium, used in the operated control group of Sherry and Vaccarino (1989), had no effect relative to unoperated controls on either spatial or nonspatial tasks. Effects of lesions to this region are generally subtle or nonexistent (Ebbesson, 1980). This evidence indicates that damage to the hyperstriatum accessorium in conjunction with damage to the hippocampus should not substantially complicate interpretation of any memory deficits observed following the lesions.

**Behavior**

The first 50 trials of baseline and the 250 trials of each type immediately following surgery were discarded as warm-up and recovery trials, respectively. The data were collapsed into blocks of 250 trials; for each type of trial there was therefore one baseline block and one block of trials following surgery. All percentage correct scores were arcsine transformed prior to analysis as a within-subjects analysis of variance (ANOVA), for unbalanced designs. Before surgery, both species performed well on tests for both location and color, and every bird but 1 did better on location tests than on color tests (Figure 2). We applied ANOVA (Species x Test Type) to the prelesion data to determine whether, in intact birds, there were differences between species. Only the effect of test type was significant, $F(1, 6) = 6.07, p < .05$; neither the effect of species nor the interaction of species with session type approached significance. Performance on tests for location dropped in all birds following surgery. In each case performance on color tests changed comparatively little following the lesions, and in some cases improved. Damage to the hippocampus therefore caused a selective impairment in spatial memory, while sparing memory for color. This conclusion is supported by ANOVA (Species x Session Type x Lesion Phase), which indicated a significant interaction between lesion phase and test type, $F(1, 6) = 28.27, p < .01$. No other effects were significant. While performance on both types of trials was depressed immediately after surgery (the first 250 trials of each type), greater impairment of spatial memory was still evident. Our use of the second block of 250 trials in this analysis ensures that the deficits are long-lasting, and that the observed impairments are not related to reacquisition of the task.

Our results suggest that the avian hippocampus is involved specifically in spatial cognition and does not serve short-term memory, working memory, recognition memory, or declarative memory generally. If the lesions impaired any of these types of memory, impairments should be observed in both location and color tests. Neither can these results be explained by a general decline in ability to match to sample. If the lesions produced a general decline in performance, one would expect the most dramatic change to occur in the more difficult task, and this is exactly opposite what was observed. Not only did the greatest decrements occur in the spatial task, but on average perfor-
Figure 2. Matching performance by juncos (J) and chickadees (C) on tests of memory for both location and color, before (PRE) and after (POST) bilateral hippocampal lesions.

Performance on the initially easier spatial task was poorer than performance on the harder color task in birds with hippocampal damage. This specificity in the memory deficit makes use of a lesioned control group unnecessary for interpretation of the results of this within-subject study. It is unlikely that surgical damage, per se, would selectively impair spatial memory and leave memory for color intact.

Other reports indicate a role for the avian hippocampus in working memory (Saghal, 1984; Sherry & Vaccarino, 1989; see Good & Macphail, 1994a, p. 294, for a criticism of this study), autoshaping (Good & Macphail, 1994a; Good & Macphail, 1994b), and differential reinforcement of low rates of responding (DRL; Reilly & Good, 1989, report effects on DRL opposite those reported by Reilly & Good, 1987). The present results provide evidence that the avian hippocampus is not involved in working memory overall but is important specifically in spatial cognition. Nonspatial working-memory deficits may not be apparent unless task parameters are manipulated in order to make correct responding more difficult (Wan et al., 1994). We did not manipulate RIs in this study, so it is possible that color performance would have been affected by the lesions at delays longer than 5 s. Nonetheless, it is clear from these results that memory for location is more vulnerable to hippocampal damage than is memory for color. We did not find a difference between the species prior to surgery, although such a difference might be expected. Studies using larger sample sizes are required to determine whether differences in spatial memory between the two species occur in the task used. Whether or not the function of the avian hippocampus will eventually be described in more general terms, the involvement of this brain region in spatial memory draws a clear parallel between mammalian and avian hippocampal function. Description of the similarities and difference in architecture between the mammalian and avian hippocampal complexes may shed new light on the structural features necessary to support spatial cognition.

References


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