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Species and sex differences in hippocampus size in parasitic and non-parasitic cowbirds

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To test the hypothesis that selection for spatial abilities which require birds to locate and to return accurately to host nests has produced an enlarged hippocampus in brood parasites, three species of cowbird were compared. In shiny cowbirds, females search for host nests without the assistance of the male; in screaming cowbirds, males and females inspect hosts' nests together; in bay-winged cowbirds, neither sex searches because this species is not a brood parasite. As predicted, the two parasitic species had a relatively larger hippocampus than the non-parasitic species. There were no sex differences in relative hippocampus size in screaming or bay-winged cowbirds, but female shiny cowbirds had a larger hippocampus than the male.

Key Words: Brood parasitism; Cowbirds; Hippocampus; Spatial memory; Sex differences

Introduction

The relationship between memory and the brain has been of great interest to neurobiologists for many years. Much of the research has focused on the hippocampus, a brain region which has been implicated in several types of learning, but is thought to be particularly concerned with memory for spatial aspects of the environment.[1] Studies performed under naturalistic conditions are important in establishing the role played by the hippocampus in memory, in the appropriate behavioural, evolutionary and ecological context.[2] Birds are particularly suited for such studies because they show the most sophisticated and complex forms of memory-based spatial behaviour including homing, migration, food storing and, for brood parasitic species, locating hosts' nests.[2,3]

A number of studies have found a positive correlation between the size of the hippocampus and the importance of these memory-based spatial behaviours in the wild.[4–14] For example, homing pigeons have greater hippocampal volumes than do closely related non-homing strains,[4] and the same is true for migratory warblers *versus* non-migratory warblers.[5] In birds which scatterhoard food the hippocampus is relatively larger, and contains more neurones, than is the case in those which do not show this behaviour.[6,7] Among closely related species of birds that engage in different amounts of food storing, relative hippocampal volume is correlated positively with the number and length of time over which caches are left[8–10] and experience of storing food triggers hippocampal growth.[15] In mammals, hippocampus size is correlated with food storing and home range size: rodents of the species *Dipodomys*, which scatterhoard food, have greater hippocampal volumes than do closely related species which larder-hoard,[11] and polygynous male *Microtus* voles have a larger hippocampus than do males which are monogamous.[12] A large hippocampus, relative to telencephalon and body size, is thought to reflect the increased demands on visuospatial cognition that may accompany these memory-based spatial behaviours.[13]

Sherry *et al*[13,14,16] predicted that sex differences in hippocampal volume would occur, but only in species which showed sex differences in memory-based spatial behaviours. One such example is the brown-headed cowbird (*Molothrus ater*), a species of brood parasite. Brood parasites lay eggs in the nests of other species (the host) which then incubate and rear their young.[17] The success of a brood parasite depends, to a large extent, on the parasite laying its eggs within the egg-laying period of the host so that, combined with a shorter incubation period than that expected for the size of its eggs[18] the parasite chicks have a developmental advantage over the host's offspring. By hatching earlier than their hosts, they can outcompete the host's chicks for food. Female parasitic cowbirds lay a large number of eggs during the breeding season:[19,20] they normally lay a single egg early in the day and spend the rest of the morning searching for nests in which to lay eggs on subsequent days.[21]

Female brown-headed cowbirds search for host nests in which to lay their eggs, and return to lay a single egg between one and several days after first location. Males do not assist females in this process. Sherry *et al*[14] found that females of this species have a larger hippocampus, relative to the size of the telencephalon, than do the males (but see Ref. 22). No sex difference was found in two non-parasitic species, the red-winged blackbird (*Agelaius phoeniceus*) and the common grackle (*Quiscalus quiscula*). Sherry *et al*[14] suggested that selection for spatial abilities which required the birds to locate, and return accurately to, host nests may have produced a sex difference in the size of the hippocampus.

The aim of this study was to test this hypothesis using three closely related species of cowbird that have very different breeding habits. Shiny cowbirds (*M. bonariensis*) are extreme generalists and parasitize more than 200 different hosts.[23] In this species, as in brown-headed cowbirds, the females search for host nests without the assistance of the male.[24] Screaming cowbirds (*M. rufoaxillaris*) are host specialists and parasitize the bay-winged cowbird (*M. badius*) almost exclusively.[23] Male and female *M. rufoaxillaris* inspect host nests together.[24] The third species, *M. badius*, is not a brood parasite. It shows biparental care and is sedentary, the nest sites and territories shifting only short distances between breeding seasons.[25]

On the basis of the hypothesis of Sherry *et al*,[14] two predictions can be made concerning species and sex differences in relative hippocampus size between the three species: the two parasitic species should have a relatively larger hippocampus than the non-parasitic species, and there should be no sex differences in hippocampus size in *M. rufoaxillaris* or *M. badius*, but female *M. bonariensis* should have a larger hippocampus than the male.

Materials and Methods

Thirty-six adult birds were used: nine male (51.6 ± 1.4 g body weight; mean ± s.e.m.) and eight female (46.8 ± 1.4 g) *M. bonariensis*; six male (55.5 ± 1.5 g) and six female (52.3 ± 1 g) *M. rufoaxillaris*, and four male (46.2 ± 1.5 g) and three females(41.5 ± 1.4 g) *M. badius*. They were caught by walk-in traps and mist nets at the ECAS Biological Station, Buenos Aires Province, Argentina, during the breeding season (9 December 1994 and 6 March 1995). Each bird was given a lethal overdose of sodium pentobarbital ($8\,\mu g\ g^{-1}$, i.p.) and perfused transcardially with heparinized 0.85% saline in 0.1 M sodium phosphate buffer followed by 4% paraformaldehyde solution in the same buffer. Following perfusion, the sex and reproductive state of the birds were determined by examination of their gonads. The brains were extracted and postfixed in 4% paraformaldehyde for at least 14 days. Brains were cut in 40 μm frozen coronal sections and treated in an identical way to control for shrinkage. Every fifth section was stained with cresyl violet. The volumes of the hippocampus (parahippocampus and hippocampus proper) and the remainder of the telencephalon were traced from the section using a 10× photographic enlarger. To calculate the areas of the hippocampus and the telencephalon (minus hippocampus) of each section, traced outlines were digitized using a WACOM graphics tablet and Image 1.51. The volumes of the total hippocampal formation and remainder of the telencephalon were computed using the formula for a truncated cone, as described in previous studies.[6] All measurements were made blind: the slides were coded by number and the codes were not interpreted until all the measurements had been completed. The estimated observer error in tracing hippocampal boundaries is < 4%.[6] Results are given as mean ± s.e.m.

Results

Absolute volumes: Figure 1 shows the volume of the hippocampus *vs* the telencephalon (minus hippocampus) for each sex and species. There are significant differences in volume of the telencephalon ($F_{2,33} = 6.64$, $p < 0.01$) and hippocampus between the three species ($F_{2,33} = 3.51$, $p < 0.05$). In spite of being the smallest species, *M. badius* had a larger telencephalon than *M. bonariensis* and *M. rufoaxillaris* (Scheffe F-test, $p < 0.01$ for both comparisons) but there was no significant difference in telencephalon size between the two parasitic species. *M. badius* had a smaller hippocampus than *M. bonariensis* ($p < 0.05$) and showed a weak tendency to have a smaller hippocampus than *M. rufoaxillaris* ($p < 0.10$). There was no significant difference in hippocampal volume between the two parasitic species ($p = 0.95$).

Relative volumes (species effects): In order to determine the relative size of the hippocampus while removing the effect of brain size across species, a two-way ANOVA was performed on the arsine transformation of the individual ratio of hippocampus over telencephalon volume, with sex and species as grouping variables. Overall, there was a significant effect of species ($F_{2,33} = 14.32$, $p < 0.001$), a marginal effect of sex ($F_{1,30} = 3.42$, $p < 0.07$) and a significant

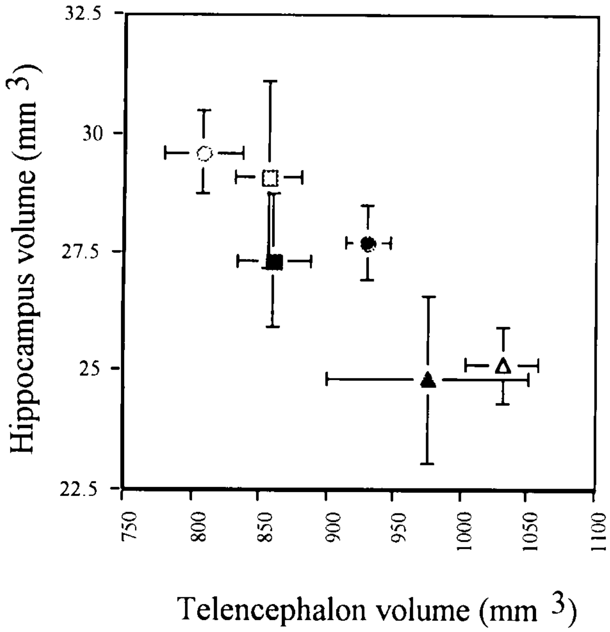

FIG. 1. Mean hippocampal volume (± s.e.) plotted against telencephalon volume for males and females of the three species. Open circles: *M. bonariensis* females ($n = 8$). Filled circles: *M. bonariensis* males ($n = 9$). Open squares: *M. rufoaxillaris* females ($n = 6$). Filled squares: *M. rufoaxillaris* males ($n = 6$). Open triangles: *M. badius* females ($n = 3$). Filled triangles: *M. badius* males ($n = 4$).

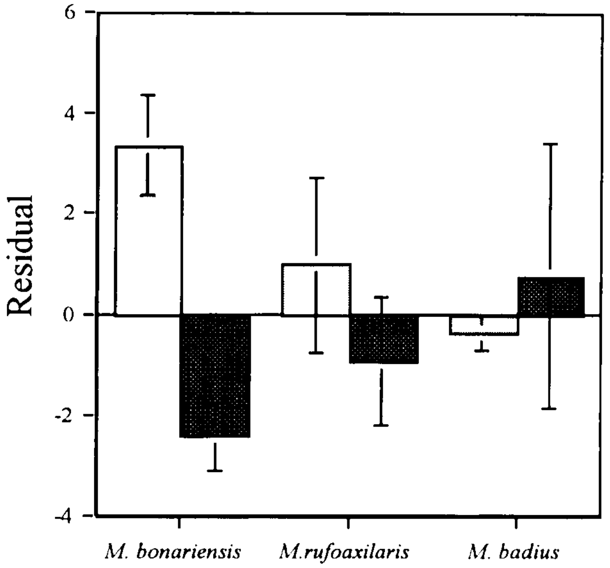

FIG. 2. Residuals of linear regressions of hippocampal *vs* telencephalic volume restricted to go through the origin. Stippled bars, females; black bars, males.

interaction between species and sex ($F_{2,30} = 3.52$, $p < 0.05$). *M. bonariensis* and *M. rufoaxillaris* had a larger relative hippocampal volume than *M. badius* (Scheffe F-tests, $p < 0.001$ for both comparisons) but there was no significant difference in hippocampal volumes between *M. bonariensis* and *M. rufoaxillaris* (Scheffe F-test, $p = 0.99$).

Sex differences: *M. bonariensis* males had a larger telencephalon than females (930.9 ± 16.39 mm^3 *vs* 808.1 ± 29.33 mm^3, $p < 0.01$), but there was no sex difference in the volume of the hippocampus (males: 27.7 ± 0.8 mm^3, females: 29.5 ± 0.9 mm^3, $p = 0.15$). There were no sex differences in the volume of either the hippocampus or the telencephalon in either *M. rufoaxillaris* or *M. badius* ($p = 0.43$ and $p = 0.67$, respectively).

We tested for sex differences in relative hippocampal size separately in each species, by calculating, for each species, a regression of hippocampus volume on telencephalon volume with the constraint of passing through the origin, and then using the residuals of these regressions as dependent variables. The results are shown in Figure 2. Unpaired *t*-tests indicated a significant difference between the sexes in *M. bonariensis* ($t = 4.90$, df = 15, $p < 0.0002$) but no significant differences for either of the two other species.

Discussion and Conclusion

Taken together with the results of Sherry *et al*,[14] our evidence suggests that the hippocampus is relatively larger in parasitic than in non-parasitic species, and that sex differences occur only in species that show sex differences in the spatial habits of males and females. In *M. bonariensis* and *M. ater*, the two species of the available set in which only the females search for nests, females have a relatively larger hippocampus than do males, while in *M. rufoaxillaris* and *M. badius*, species in which females and males have similar spatial habits, sex differences are not present. Both *M. bonariensis* and *M. rufoaxillaris*, the two parasitic species in the present study, have a relatively larger hippocampus than non-parasitic *M. badius*. Thus, the association between a relatively large hippocampus and nest searching in parasitic cowbirds is consistent with the hypothesis that searching for hosts' nests makes special demands on the processing of spatial information and that the hippocampus plays a specialized role in this respect.

It is possible, but unlikely, that other confounding variables are responsible for the species and sex differences reported in this study. Statistical control for telencephalon size eliminates the possibility that differences in body or brain size could be responsible for the differences in hippocampal volume. All three species live in semi-open or open habitats, are residents in the study site all year round, and form mixed feeding flocks during winter. Habitat use, feeding and non-migratory behaviours are shared by both sexes. These common features provide little reason to expect the species or sex differences observed. However, a statistically reliable attribution of these differences to parasitism or different life habits by the sexes requires a comparative analysis that would exclude lack of phylogenetic independence. There are not yet sufficient studies to allow such a comparative analysis to be performed with respect to parasitism, but the emerging picture is encouraging and suggests that there may be much to gain from further comparative work. In conclusion, our findings support the hypothesis that life habits that make strong demands on the processing of spatial information correlate with the evolution of relatively large hippocampal systems in birds. Detailed functional and histological implications of this enlargement as well as the direction of causality in this correlation are not yet known.

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General Summary

We study brain function by asking if species that differ in behaviour show differences in selected regions of the brain. Here we apply this technique to the hippocampus, a brain structure involved in spatial information processing, comparing these three species of South American cowbirds. Two species are brood parasites that search for and remember the location of hosts' nests, while the third is a normal breeder. The two parasitic species have relatively larger hippocampus than the non-parasitic one. In one of the parasitic species females alone show this specialised behaviour. Females of this species (but not of the other two) have larger relative hippocampus than males. Our results reaffirm the advantages of investigating a variety of species taking into account their ecology and life history.