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Seasonal changes of hippocampus volume in parasitic cowbirds

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Abstract

Avian brain plasticity has been demonstrated by seasonal variations in neuroanatomy correlated with changes in singing and hoarding behaviour. We report a new instance of plasticity. Brood parasitism in South American cowbirds involves memory for location of hosts' nests, and is associated with an enlarged hippocampus relative to telencephalon size. This effect holds between sexes and species during the breeding season. We report that for two parasitic species, relative hippocampal volume is smaller during the non-breeding than the breeding season, and that sexual dimorphism present in summer in one of the species is not found in winter. These results support the hypothesis that the avian hippocampal formation shows neuroanatomical plasticity associated with seasonal changes in spatial memory demands. © 1997 Elsevier Science B.V.

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1. Introduction

Several recent studies reported correlationsm between relative volume of brain structures andthe behavioural ecology of the species under investigation. The comparative neuroanatomical approach has been particularly successful in relation to the neural systems controlling bird song (De Voogd, 1994; Catchpole, 1996) and those associated with the performance of spatial tasks (Krebs et al., 1989; Clayton and Krebs, 1995). Here we report further evidence showing that neuroanatomical specialisations for habits requiring intensive use of spatial memory appear to follow seasonal variations in natural behaviour.

Spatial performance in birds is known to involve the hippocampus (defined as the dorso -medial cortex, including parahippocampus

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(Bingman, 1993)). This involvement is supported by the following observations:

(1) Selective damage of the hippocampus impairs performance in spatial tasks (Sherry and Vaccarino, 1989; Hampton and Shettleworth, 1996; Patel et al., 1997).

(2) Species which hoard food for later consumption (a task that relies on intensive and accurate use of spatial memory) have larger relative hippocampus size than non-hoarding related species (Krebs et al., 1989; Sherry et al., 1989; Healy and Krebs, 1992; Hampton et al., 1995; Basil et al., 1996).

(3) Avian species that breed parasitically (namely, lay eggs in nests of other species, a task that uses spatial memory to relocate potential hosts' nests) show larger relative hippocampal size than non-parasitic relatives (Reboreda et al., 1996).

(4) Parasitic species showing sexual differences in the performance of hosts' nest relocation also show sexual differences in relative hippocampal size, while non-parasitic species or species where males and females do not differ so markedly in ecology do not show these sexual differences in neuroanatomy (Uyehara and Nairns, 1992; Sherry et al., 1993; Reboreda et al., 1996).

(5) At least one hoarding species (the black-capped chickadee *Parus atricapillus*) which shows seasonal variations in hoarding intensity shows seasonal variations in relative hippocampus size (Smulders et al., 1995; but see Krebs et al., 1995) and in hippocampal neurogenesis (Barnea and Nottebohm, 1994).

In an earlier study (Reboreda et al., 1996), we presented the first part of a study of neuroanatomical specialisations in three species of South American cowbirds of the genus *Molothrus* (Icterinae). The system is attractive for comparative work because they constitute a set of phylogenetically closely related species with striking differences in natural behaviour, hence offering the opportunity for seeking neural differences in association with these behavioural differences. All three species are mostly granivorous and common in the grasslands and scrublands of Central–Eastern Argentina. During winter they have a similar ecology, but during the breeding season there are well-defined variations. The shiny cowbird, *M. bonariensis*, has ecological specialisations similar to those observed in another exclusively parasitic species, the North American brown-headed cowbird, *M. ater* (Hann, 1941; Rothstein et al., 1987). Typical behaviour consists of females searching for potential hosts' nests, to which they return for laying when their own condition (egg availability) and the state of the host's nest coincide. This habit makes very special demands on information processing because the performance of the parasitic offspring is heavily dependent on the relative timing of hatching in host's and parasite's chicks. At the time a parasite female is ready for laying an egg, she may face choices among a number of putative host nests at various stages within her home range. Since nests can only be parasitised successfully while the female host is laying her own eggs, each nest may be available only for brief periods and must be erased from the set of putative targets when it passes its vulnerable stage. This kind of process is similar to the demands of hoarders that have to update their inventory of remembered storage sites every time they store or remove an item. We shall refer to this special form of processing as book-keeping.

Similarly to *M. ater*, *M. bonariensis* is a generalised parasite, with approximately 200 host species (Friedmann and Kiff, 1985) and with a strong sexual dimorphism in behaviour, as the female seems to do the host location task unaided by males (Mason, 1987). The screaming cowbird, *M. rufoaxillaris*, is also an obligate parasite, but with a very different breeding ecology. This species differs in habits from both *M. ater* and *M. bonariensis* because males and females show similar participation in hosts' nests book-keeping (Fraga, 1986; Mason, 1987), and because mainly one species is parasitised. The main and nearly exclusive host of *M. rufoaxillaris* is the bay-winged cowbird, *M. badius*, which is the third species in our study. *M badius* in turn differs from all the previous species because it is non-parasitic and shows similar life style for both sexes (Fraga, 1991). Thus, within four species of a single genus, three of them living sympatrically, we have striking differences in parasitism-related memory dependency.

In our previous report of the three sympatric species (Reboreda et al., 1996), we found that during the breeding season the two parasitic species had larger hippocampus volume relative to the rest of the telencephalon than the non-parasitic *M. badius*, and that, as expected from the sexual dimorphism in behaviour, the female had larger relative hippocampus size than the male in *M. bonariensis* but not in *M. rufoaxillaris* or *M. badius*. These results are consistent with the report by Sherry et al. (1993) of sex differences in relative hippocampus volume in *M. ater* but not in two other Icterinae (the red-winged blackbird *Agelaius phoeniceus* and the common grackle *Quiscalus quiscula*), which are non-parasitic and give support for the hypothesis of Sherry et al. (1993) that the differences in relative hippocampus size are associated with the behavioural specialisations required for effective parasitism.

So far, however, studies of brain specialisations in parasitic birds were confined to the breeding season. On the bases of the plasticity found in the bird song and the hoarding neural systems, one might expect these differences to be absent in the non-breeding winter season when book-keeping of hosts' nests is not performed. Here we present data from a winter sample and analyse for seasonal differences in the two parasitic species in our set (not enough samples of *M. badius* were available for this comparison).

2. Materials and methods

Although the breeding season data have already been reported, this study presents a direct comparison between breeding and non-breeding neuroanatomy, and this requires information establishing that methodologies during both periods were standardised. We present below the methods used in both samples and we report the total number of brains available for comparison.

M. bonariensis: we used 29 adult, eight females and nine males caught during the breeding season (9 December–12 January) and five females and seven males caught during the non-breeding season (9–16 June). *M. rufoaxillaris*: we used 24 adult, six females and six males caught during the breeding season (5 December–12 January) and seven females and five males caught during the non-breeding season (9 June). *M. badius*: We used nine adult, three females and four males caught during the breeding season (5 December–3 March) and one female and one male caught during the non-breeding season (16 June). Although sample size precludes including this species in the seasonal comparisons, we report the values obtained for completeness, as this species' brains were processed as part of the summer and winter batches of all three species.

Birds were caught by walk-in traps and mist nets in ECAS Biological Station, Buenos Aires Province, Argentina. Each bird was given a lethal intraperitoneal overdose of sodium pentobarbital (8 μg/g) and perfused transcardially with heparinised 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 post-fixed in 4% paraformaldehyde for at least 14 days.

Brains were cut as 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 sections using a 10 × photographic enlarger. To calculate the areas of the hippocampus and the telencephalon (minus hippocampus) of each section, the traced outlines were digitised 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 (Krebs et al., 1989). All measurements were done blind with respect to sex and species: 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 less than 4% (Krebs et al., 1989). Results are mean ± S.E. of the mean.

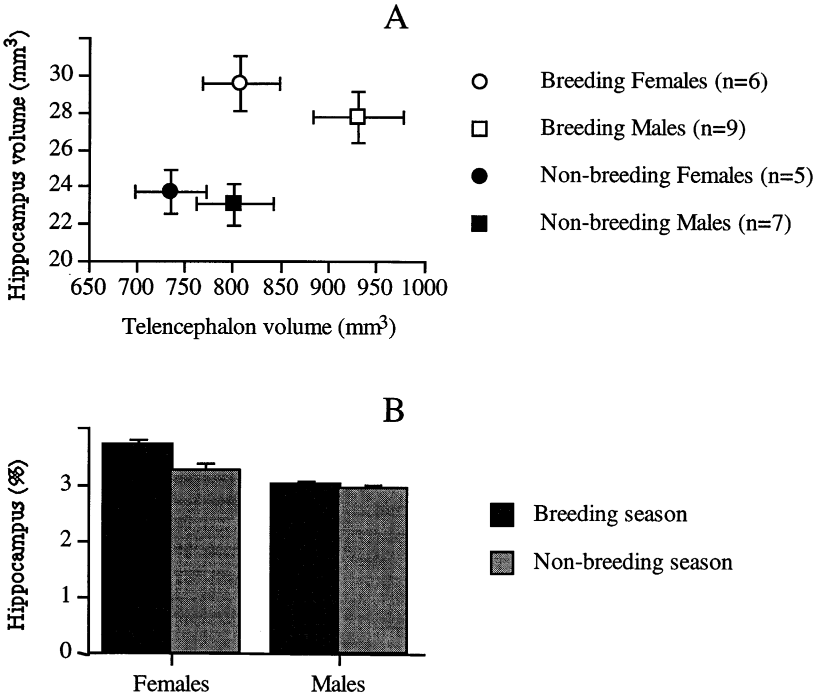

Fig. 1. (A) Shiny cowbird (*Molothrus bonariensis*). Mean (± S.E.) hippocampal volume plotted against telencephalon volume for males and females during the breeding and the non-breeding seasons. (B) Mean (± S.E.) of the individual ratio of hippocampus over telencephalon volume during the breeding and the non-breeding seasons.

3. Results

There were no significant differences in body weight between seasons in males of either *M. bonariensis* (breeding season 51.61 ± 1.40 g, non-breeding season 52.69 ± 1.25 g, $t = -55$, $P = 0.59$) or *M. rufoaxillaris* (breeding season 55.52 ± 1.53 g, non-breeding season 55.16 ± 2.34 g, $t = 0.13$, $P = 0.90$). Females of both species were heavier during the breeding season (*M. bonariensis*: 46.78 ± 1.39 vs. 40.68 ± 1.07 g, $t = 3.1$, $P = 0.01$; *M. rufoaxillaris*: 52.33 ± 1.05 vs. 46.54 ± 1.67 g, $t = 2.88$, $P = 0.017$). For male *M. badius*, the available body weights of breeding vs. non-breeding season were 46.20 ± 1.50 vs. 41.0 g, while for females they were 41.50 ± 1.4 vs. 40.0 g.

Fig. 1A shows the volume of the hippocampus vs. telencephalon (minus hippocampus) for each sex and season in *M. bonariensis*. We did a two-way analysis of variance (ANOVA) of telencephalon and hippocampus volumes with sex and season as grouping variables. Telencephalon volume was larger during the breeding season ($F_{1,25} = 9.72$, $P = 0.0045$) and males have a larger telencephalon than females ($F_{1,25} = 8.61$, $P = 0.0071$). There was no interaction between season and sex ($F_{1,25} = 0.75$, $P = 0.39$). Hippocampus volume was also larger during the breeding season ($F_{1,25} = 31.04$, $P = 0.0001$) but in this case there were no sex differences ($F_{1,25} = 1.69$, $P = 0.21$).

In order to test the relative size of hippocampus, removing the effect of brain size across seasons and sexes, we did a two-way ANOVA on the arcsine transformation of the individual ratio of hippocampus over telencephalon volume, with season and sex as grouping variables. In *M. bonariensis*, relative hippocampal volume was larger during the breeding season ($F_{1,25} = 5.08$, $P = 0.033$) and females have a larger relative hippocampus volume than males ($F_{1,25} = 19.93$, $P = 0.0001$) (Fig. 1B).

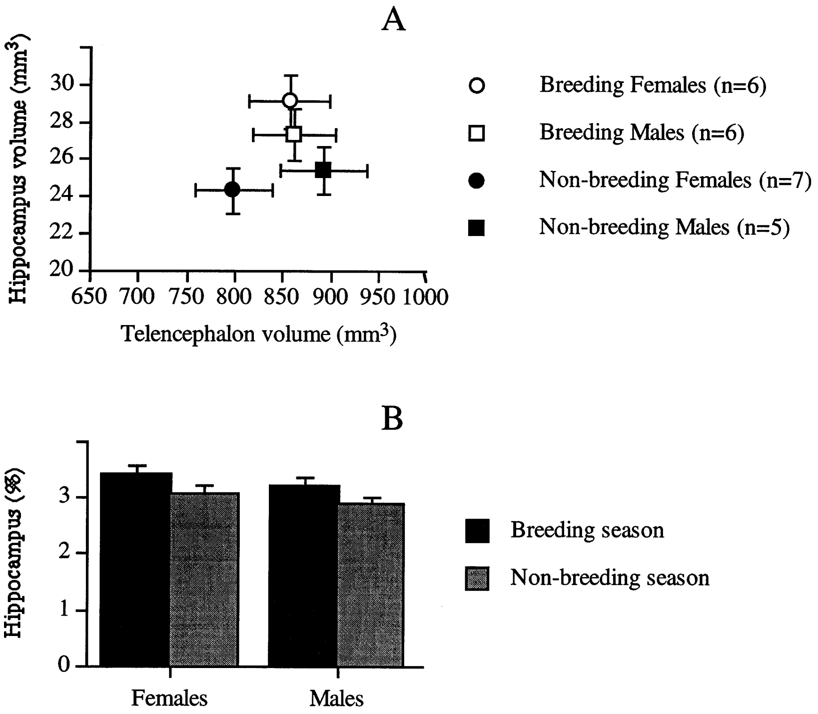

Fig. 2. (A) Screaming cowbird (*Molothrus rufoaxillaris*). Mean (± S.E.) hippocampal volume plotted against telencephalon volume for males and females during the breeding and the non-breeding seasons. (B) Mean (± S.E.) of the individual ratio of hippocampus over telencephalon volume of males and females during the breeding and the non-breeding seasons.

Fig. 2A shows the volume of the hippocampus vs. telencephalon for each sex and season in *M. rufoaxillaris*. There were neither seasonal ($F_{1,20} = 0.20$, $P = 0.66$) nor sexual ($F_{1,20} = 2.63$, $P = 0.12$) differences in telencephalon volume. Hippocampal volume was larger during the breeding season ($F_{1,20} = 4.51$, $P = 0.046$) but in this species there were no sex differences ($F_{1,20} = 0.054$, $P = 0.82$). The relative hippocampal volume was larger during the breeding season ($F_{1,20} = 4.23$, $P = 0.053$) but, again, there were no sex differences in relative hippocampal volume ($F_{1,20} = 1.51$, $P = 0.23$) (Fig. 2B).

Because the breeding and non-breeding season groups had to be processed as two separate batches, it is not possible to completely eliminate the possibility of a batch effect on the seasonal comparisons made in the two parasitic species. Future studies may handle this problem by statistical comparison with *M. badius*. Since this species is non-parasitic, the hypothesis of a causal role for parasitic behaviour would be consistent with lack of the seasonal effects observed in the other two species. The available values for the telencephalon volumes (breeding vs. non-breeding season, in mm^3) of *M. badius* are: males 975.86 ± 75.18 ($n = 4$) vs. 1155.64 ($n = 1$), and females 1030.56 ± 27.03 ($n = 3$) vs. 1264.6 ($n = 1$), whereas the values for the hippocampal volumes are: males 24.83 ± 1.75 ($n = 4$) vs. 33.61 ($n = 1$) and females 25.07 ± 0.79 ($n = 3$) vs. 27.55 ($n = 1$), so that so far there is no suggestion of a reduction in average relative hippocampal size of the winter vs. summer batches (2.52 ± 0.14%, $n = 7$ vs. 2.54 ± 0.37, $n = 2$).

4. Discussion

Differences in relative hippocampal volume of closely related species of cowbirds appear to mirror differences in habits in the following respects:

(1) Two species (*M. bonariensis* and *M. ater*) are generalised brood parasites in which the female (but not the male) is very active during the breeding season in locating potential hosts' nests, probably keeping a reference library of hosts' location and condition. In these two species, females during the breeding season have a larger relative hippocampal volume than males (Sherry et al., 1993; Reboreda et al., 1996). The sexual difference was analysed for *M. bonariensis* during winter, when the behaviour of both sexes is similar, and found to be absent (this study). Seasonal comparisons, though, are not yet conclusive because of the possible confounding of age distribution in our samples. The winter population is likely to contain a greater proportion of younger animals, born the previous summer and inexperienced with respect to breeding. Until the observations are repeated with subjects of known ages, it is not possible to establish if the seasonal variations are reversible or a developmental consequence of age and/or experience.

(2) One species (*M. rufoaxillaris*) is a specialised brood parasite that has mainly one host. During the breeding season (but not in winter), both males and females search and locate potential hosts' nests. There were no sexual differences in relative hippocampal size in this species in either season but there was a significantly larger relative hippocampal volume in the breeding season with respect to winter (Reboreda et al., 1996; this study).

(3) One species (*M. badius*) is non-parasitic and no sexual differences are known with respect to use of spatial memory in either season. No sexual differences in relative hippocampal volume were found in the breeding season (Reboreda et al., 1996), and no winter data are yet available for statistical comparisons.

In addition, two comparisons between species can be made at this stage. *M. ater* probably has a larger relative hippocampal volume than two confamilial non-parasitic species (*A. phoeniceus* and *Q. quiscula*). The data of Sherry et al. (1993) suggest this effect but no direct statistical test has yet been published. Both *M. bonariensis* and *M. rufoaxillaris*, which are parasitic, have significantly larger relative hippocampal volume during the breeding season than a congeneric species (*M. badius*) which is non-parasitic. Cross-species comparison has not yet been made in winter for either the North or South American sets of species.

Overall, the results confirm the picture emerging from both hoarding (Clayton and Krebs, 1995) and bird song studies (De Voogd, 1994) of a very dynamic structure of the avian nervous system. Both hoarding and parasitism studies support the view that the hippocampal area of the brain is associated with the performance of habits requiring enhanced or more intensively used spatial memory, in particular some form of bookkeeping in which the location of significant elements of the environment are remembered for a limited time and inventory up-dating is continuously required because sites lose significance after hoards are retrieved or host nests are used up.

In the case of parasitism, we do not yet have data on the histological correlates of these volumetric changes but, both in the song system and in hoarding, it is likely that active neurogenesis accompanies macroscopic changes in brain anatomy (Alvarez-Buylla et al., 1988; Nordeen and Nordeen, 1990; Barnea and Nottebohm, 1994). The most important pending developments in relation to neurobiological adaptations for parasitism are a better description of the differences in use of memory between the species and the sexes in the field, a laboratory analysis of their spatial memory performance and an elucidation of the cellular significance of volumetric variations.

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