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Andrea Alejandra Astié · Alejandro Kacelnik
Juan Carlos Reboreda

Sexual differences in memory in shiny cowbirds

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Abstract Avian brood parasites depend on other species, the hosts, to raise their offspring. During the breeding season, parasitic cowbirds (*Molothrus* sp.) search for potential host nests to which they return for laying a few days after first locating them. Parasitic cowbirds have a larger hippocampus/telencephalon volume than non-parasitic species; this volume is larger in the sex involved in nest searching (females) and it is also larger in the breeding than in the non-breeding season. In nature, female shiny cowbirds *Molothrus bonariensis* search for nests without the male's assistance. Here we test whether, in association with these neuroanatomical and behavioural differences, shiny cowbirds display sexual differences in a memory task in the laboratory. We used a task consisting of finding food whose location was indicated either by the appearance or the location of a covering disk. Females learnt to retrieve food faster than males when food was associated with appearance cues, but we found no sexual differences when food was associated with a specific location. Our results are consistent with the view that parasitism and its neuroanatomical correlates affect performance in memory tasks, but the effects we found were not in the expected direction, emphasising that the nature of avian hippocampal function and its sexual differences are not yet understood.

Key words Spatial memory · Hippocampus · *Molothrus bonariensis* · Sexual differences · Memory task

A. A. Astié · J. C. Reboreda (✉)
Instituto de Biología y Medicina Experimental-CONICET,
Vuelta de Obligado 2490, 1428 Buenos Aires, Argentina

A. Kacelnik
Department of Zoology, University of Oxford, South Parks Road,
Oxford OX1 3PS, UK

Present address:

J. C. Reboreda
Departamento de Ciencias Biológicas,
Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires, Pabellón II Ciudad Universitaria,
1428 Buenos Aires, Argentina
e-mail: reboreda@bg.fcen.uba.ar, Fax: +54-1-5763384

Introduction

Brood parasitism is a form of breeding in which the parasite lays its eggs in the nest of another species, the host, which incubates and rears the young (Rothstein 1990). The shiny cowbird *Molothrus bonariensis* is an obligate generalist brood parasite, with approximately 200 host species (Friedmann and Kiff 1985). Female cowbirds may lay a large number of eggs (from 40 to 120) during the breeding season (Scott and Ankney 1983; Kattan 1993). They 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 (Scott 1991). Shiny cowbird females seem to do the host location task unaided by males (Mason 1987).

The success of a brood parasite depends, to a large extent, on the parasite laying its eggs within the laying period of its host. Because parasitic cowbird eggs have a shorter incubation period than expected for their size (Briskie and Sealy 1990), they tend to hatch before host eggs. Therefore, during the nestling period, parasite chicks are favoured in competition for food against the host chicks (Massoni and Reboreda 1998).

Nest searching and re-location make very special demands on information processing, because when a parasite female is ready to lay an egg she may face choices within her home range among a number of putative host nests at various stages. Since nests can only be parasitized successfully during the laying period of the host, each nest may be available only for brief periods and must be erased from the set of putative targets when it passes the appropriate stage. This kind of process might require specialisations similar to those of species that hoard food for later consumption. The latter have to update their inventory of remembered storage sites every time they store or remove an item.

Spatial memory performance in birds is known to involve the hippocampus (Sherry and Vaccarino 1989; Hampton and Shettleworth 1996; Patel et al. 1997), 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 (O'Keefe and Nadel 1978).

The clearest evidence in support of the hypothesis of a specialised memory ability in parasitic cowbirds comes from neuroanatomical rather than behavioural work. This evidence can be summarised as follows. Parasitic cowbirds have a larger hippocampus relative to the rest of the telencephalon than related non-parasitic species (Reboreda et al. 1996). Species showing sexual differences in nest searching and nest re-location show sexual differences in relative hippocampal size, while non-parasitic species or species where males and females do not differ so markedly in nest searching behaviour do not show these sexual differences in neuroanatomy (Sherry et al. 1993; Reboreda et al. 1996). In two parasitic species, relative hippocampal volume is smaller during the non-breeding than during the breeding season, and sexual dimorphism present in the breeding season in one of the species is not found in the non-breeding season (Clayton et al. 1997).

In this study we test the hypothesis that shiny cowbirds *M. bonariensis* have sexual differences in memory abilities associated with their hippocampal dimorphism when they are performing tasks not associated with parasitism. To test whether or not the memory abilities of shiny cowbird females differ in quality or quantity from that of males, we compare the two sexes in tasks that do not involve searching for nests, but which may reveal putative spatial memory adaptations acquired by females through the demands of nest location in the wild. The tasks we chose (searching for food in a restricted space using either location or appearance cues) differ from nest finding in at least two substantial properties. First, "spatial" and "appearance" cues are quite distinguishable in the laboratory, but this distinction is not clearly discernible in the field. Candidate host nests differ in both appearance and location, and there is no reason to assume that birds use these two dimensions separately or to a different extent. A similar point applies to most food-hoarding studies. Second, the demands imposed by parasitism and the neuroanatomical correlates observed so far may correspond to differences in either working or reference memory. The two tasks we used were based on testing for performance depending on reference memory (that is cross-trials rather than trial-specific memory). It is not clear which memory

system is taxed more heavily by nest monitoring, but since each nest cycle lasts for at least several days and probably involves multiple visits, as a first guess a reference memory task seems a more appropriate start point. On the negative side, the majority of differences detected in relation to food hoarding used working memory tests designed around various versions of delayed matching to sample, suggesting that working memory tasks may be more sensitive as tools for exposing differences.

Methods

Subjects

The subjects were 12 wild-caught shiny cowbirds, *M. bonariensis*, 6 females (weight 42 ± 2.5 g, mean \pm SE) and 6 males (weight 51 ± 3.5 g). Birds were caught by mist nets between 23 and 26 August 1996 near the town of Cardales, 50 km northwest of Buenos Aires. They were housed in groups of four (two males and two females) in wire cages which measured $120 \times 40 \times 30$ cm. Cages were arranged so that they were visually but not acoustically isolated. Temperatures in the laboratory ranged between 23 and 26°C and the lights were on between 0600 and 2000 hours. The birds were deprived of food from 1800 hours until the start of each session at 0900 hours the following morning. During the experimental sessions the birds were rewarded with millet seeds and in the period between the end of the session and 1800 hours they were given millet seeds and balanced food for insectivorous birds (Henel) ad libitum. The weight of the birds remained stable over the course of the experiment. After the experiments had been completed the birds were released.

Apparatus, training and experimental procedures

We estimated the amount of food to be delivered during the experimental session through a satiation curve of birds deprived of food from 1800 hours to 0900 hours the following morning.

The birds were trained to retrieve food from an experimental patch which consisted of a wood board $40 \times 40 \times 1.5$ cm with 64 wells (1.3 cm in diameter, 0.5 cm in depth) distributed regularly in a 8×8 array. Each well was covered by a sliding white plastic disk 1.8 cm in diameter. Birds were trained to retrieve food following the scheme presented in Table 1. All females and five out of six males were retrieving food from the patch after ten sessions. We used these 11 birds for the experiment.

Training and experiments were conducted in the cage in which the birds were housed. At the beginning of each session the cage was divided into two halves using a white plastic partition. In one

Table 1 Scheme followed for training the birds to retrieve food from the experimental patch

Session	Trials per session	Trial length (min)	Wells with food	Seeds per well	Wells not covered	Wells partially covered	Wells covered
1	1	20	32	5	64	0	0
2	1	20	32	5	32	32	0
3	1	20	32	5	0	32	32
4	1	20	32	5	0	32	32
5	1	20	32	5	0	0	64
6	2	10	16	5	0	0	64
7	4	5	8	5	0	0	64
8	4	5	4	10	0	0	64
9	4	5	4	10	0	0	64
10	4	5	4	10	0	0	64

section (60 × 40 × 30 cm) we tested one subject while in the other we kept the three other birds. Each trial started with the introduction of the patch into the experimental section. The order in which birds were tested each session was counterbalanced.

The birds experienced treatments with appearance and location cues sequentially. In both treatments each trial started with the introduction of the patch with all its wells covered into the experimental section. Only one well contained food (ten seeds of millet). In the treatment with appearance cues the well with food was covered with a differently coloured disc (black) and its location was changed between trials in a semi-random order. In each trial we used a different quadrant (clockwise) and chose one well of that quadrant at random. In the treatment with location cues the well with food did not change between trials and there were no well-specific appearance cues (all disks were white). Each trial ended 30 s after the bird had found the well with food (this allowed enough time for the ten seeds to be consumed) or after 5 min, whatever happened before. Birds received 12 trials per session and the intertrial interval was 1 min. At the beginning of each session we rearranged all the caps to eliminate the possibility that the birds were using subtle differences in the caps as cues.

Birds were tested from 0900 to 1100–1200 hours. Because of temporal constraints on testing all the birds during the morning we divided the birds into three groups. Each group (two females and two males housed in the same cage) was tested (training and experiment) sequentially: group 1 on 3–27 October, group 2 on 28 October–19 November and group 3 on 20 November–13 December 1996. There were no significant differences between groups for the dependent variables considered (number of errors and latency) in either treatment. Therefore, for the analysis we pooled the data from the three groups.

Data collection and analysis

The experimental sessions were video recorded and later transcribed using EthoLog 1.0 software. The dependent variables measured were latency (time elapsed from the bird's first landing on the patch to the opening of the well with food) and number of errors (openings of wells without food). These two variables are highly correlated and do not show independent differences, so they are analysed separately to provide a more detailed description of the results. In a few trials some birds did not search for food or stopped searching for food before the end of the trial (5 min). In these cases, we assumed that the latency was 300 s and the number of errors 64. This value corresponds to the expected number of errors if the subjects search for food at random with replacement (i.e. they can open the same well more than once). To analyse changes within sessions, data were grouped in blocks of four trials. Data were analysed with a two-way repeated measures ANOVA with sex as independent variable and number of errors or latency as dependent variable. The criterion for ending the treatment was that number of errors and latency did not change significantly in three consecutive sessions (nine blocks of four trials).

Results are given as mean ± SE. All the *P* values reported are two-tailed.

Results

Appearance cues

Figure 1 shows the number of errors (Fig. 1 A) and the latency to finding the well with food (Fig. 1 B) for females and males in the appearance treatment. In both sexes the number of errors and the latency decreased rapidly, but females reached asymptotic behaviour faster than males. There were significant differences in the number of errors between sexes ($F_{1,9} = 6.4, P < 0.05$), blocks ($F_{11,99} = 31.4$;

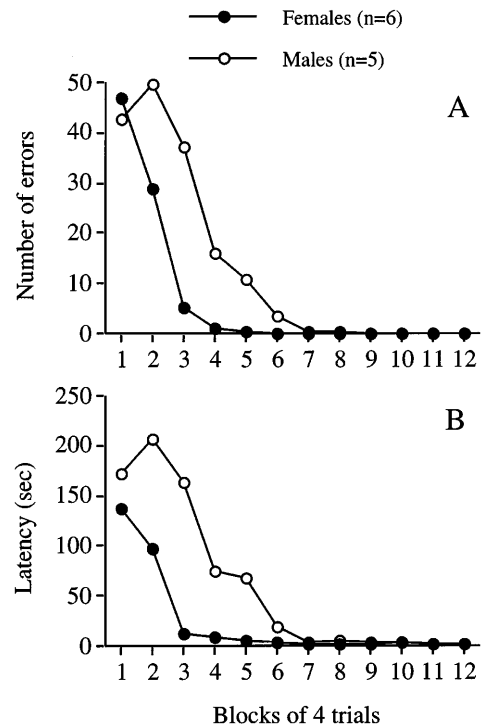


Fig. 1 **A** Number of errors and **B** latency to finding the well with food in treatment with appearance cues. Each symbol represents the mean of 4 trials

$P < 0.001$), and in the interaction between sex and blocks ($F_{11,99} = 3.24, P < 0.001$). The differences between blocks were significant in both females ($F_{11,99} = 16.9, P < 0.01$) and males ($F_{11,99} = 17.6, P < 0.01$). Simple main effects showed that there were significant sexual differences in blocks 2 ($F_{1,20} = 12.7, P < 0.01$), 3 ($F_{1,20} = 29.5, P < 0.01$) and 4 ($F_{1,20} = 6.7, P < 0.05$).

Similarly, there were significant differences in the latency between sexes ($F_{1,9} = 7.8, P < 0.05$), blocks ($F_{11,99} = 23.5, P < 0.001$) and in the interaction between sex and blocks ($F_{11,99} = 4.7, P < 0.001$). The sexual differences were significant in blocks 2 ($F_{1,20} = 17.7, P < 0.01$), 3 ($F_{1,20} = 33.9, P < 0.01$), 4 ($F_{1,20} = 6.5, P < 0.05$) and 5 ($F_{1,20} = 5.8, P < 0.05$).

Location cues

Figure 2 shows the number of errors (Fig. 2 A) and the latency to finding the well with food (Fig. 2 B) for females and males. Both sexes showed a similar pattern in the decrease of number of errors and latency. There were significant differences in the number of errors between blocks ($F_{20,180} = 15.3, P < 0.001$) and in the interaction between sex and blocks ($F_{20,180} = 1.8, P < 0.05$) but there were no differences between sexes ($F_{1,9} = 1.8, P = 0.22$). The differences between blocks were significant in both females ($F_{20,180} = 6.0, P < 0.01$) and males ($F_{20,180} = 11.1, P < 0.01$). Simple main effects showed that there was a significant sexual difference in block 3 ($F_{1,20} = 26.25, P < 0.01$).

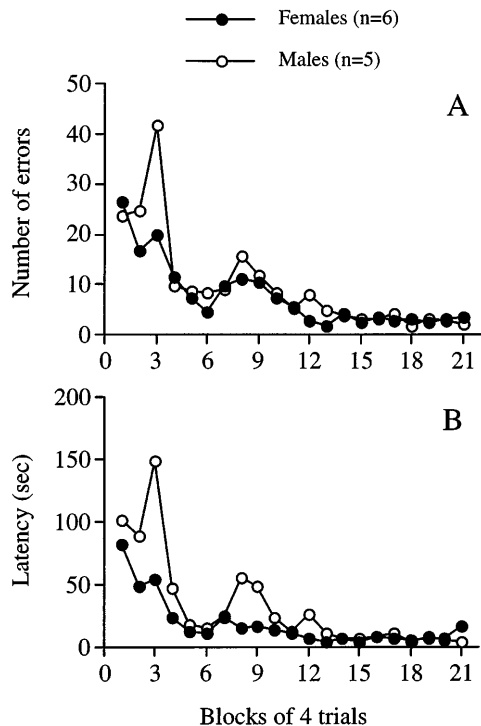


Fig. 2 **A** Number of errors and **B** latency to finding the well with food in treatment with location cues. Each symbol represents the mean of 4 trials

This difference was the result of one male that made a high number of errors in trials of block 3. If this subject was excluded the differences became non significant.

As regard latencies, there were significant differences between blocks ($F_{20,180} = 8.04$, $P < 0.001$) but there were no differences between sexes ($F_{1,9} = 1.44$, $P = 0.26$) or significant interaction between sex and blocks ($F_{20,180} = 1.48$, $P = 0.09$).

Discussion

The relationship between memory and the hippocampus is the target of growing interest in both mammalian and avian neuroethology.

In mammals, studies have shown a relation between some demands of the mating system and the development of the hippocampus. In the polygamous vole, *Microtus pennsylvanicus*, males range more widely than females (Gaulin and Fitzgerald 1989). In this species males have a larger hippocampus and perform better than females in laboratory measures of spatial ability. These sexual differences in hippocampal volume and spatial ability are absent in the closely related monogamous vole *M. pinetorum* which shows no obvious differences in the use of the environment between the sexes (Jacobs et al. 1990).

In birds, both food storing and brood parasitism have been useful in relating comparative results in neuroanatomy to the ecological habits of different species or sexes.

The hoarding studies have made use of the fact that some species of birds store a large number of food items, each in a separate place, and retrieve them accurately hours to months later. In these species, the hippocampus is enlarged relative to the rest of the telencephalon as compared to non-storing relatives (Krebs et al. 1989; Sherry et al. 1989), and lesions have shown that it must be intact for successful retrieval performance, but not for active storage activity or for performance of non-spatial tasks (Sherry and Vaccarino 1989). Laboratory studies showed that storing species have a better performance in spatial memory tasks than closely-related non-storing species (see Clayton and Krebs 1995 for a review), supporting the hypothesis that the larger hippocampus in food-storing birds is associated with an enhancement of either ability for or reliance on spatial memory.

The other ethological system in which an association between relative hippocampus volume and spatial memory demands has been found, the parasitic cowbirds, has not been studied with the same intensity. In this case, the only evidence in support of the hypothesis of a specialised neurobehavioural architecture comes from neuroanatomical work (Sherry et al. 1993; Reboreda et al. 1996; Clayton et al. 1997). Given the nature of the differences in neuroanatomy and natural behaviour, one might expect females to have better memory performance than males, and this difference to appear mainly in tasks requiring the use of spatial information.

We do acknowledge from the start that this working hypothesis is far from definitive, as this research is in its early days. Caution is essential because it is not obvious what sort of demands parasitism imposes on these animals. Potential hosts' nests (similarly to hoarding places) differ in position and appearance, and remembering the location of many individual hoards could be enhanced by a better memory of a large number of "snapshots" of the visual stimuli surrounding the place of each hoard, as much as by having an especially well-developed sense of location. Indeed, even from a neurobiological perspective, the specific role of the hippocampus (assuming it has a single specific role) is not yet certain. The hippocampus may be involved in spatial tasks simply because these are members of a broader class of problems which involve hippocampal activity (Eichenbaum 1996).

In this study we tested the hypothesis that parasitic shiny cowbirds have sexual differences in memory abilities associated with the hippocampal dimorphism that we found in previous work. We did find that females performed better than males, but the effect of the nature of the task was not as expected. Females learnt to retrieve food faster than males when food was associated with appearance cues, but we found no sexual differences when food was associated with a specific location. Both sexes reached asymptotic behaviour faster with appearance than with location cues, and the asymptotic number of errors was smaller with appearance (no errors) than with location cues (approximately 3 errors per trial). It appears that learning was easier when food was associated with appearance than with location cues. Future studies may

modify the procedures to use tasks which have comparable level of difficulty. However, since our main interest is the identification of sexual differences, this is not a serious problem here.

The failure to find differences between sexes in the treatment with location cues is only weakly indicative of the possibility that the null hypothesis of lack of sexual differences in this task may be true. There are various reasons why our subjects may have shown less extreme sexual differences than in nature. Our task does not exclude the possibility that behavioural effects are due to differences in motivation, rather than in mnemonic constraints. For instance, while we take our inspiration from a task involving searching for eggs and nests, we tested our animals using food as reinforcement. This translation from nature to the laboratory may have affected females more than males. However, the fact that we found an effect of treatment on the expression of sexual differences argues against pure motivational explanations.

The timing of our experiments may have also been a factor. As has been reported earlier the hippocampal region of shiny cowbird females is relatively larger than that of males only during the breeding season (Reboreda et al. 1996; Clayton et al. 1997). We conducted the experiments during the breeding season (November and December), but birds had been captured at the end of the non-breeding season (late August). Although birds were kept in a light-dark cycle corresponding to the one they experienced during the peak of the breeding season we cannot rule out the possibility that the sexual dimorphism in hippocampus volume had not developed in captivity to the same extent as in nature. Developmental data have shown that some of the hippocampal specialisations found in storing birds require hoarding for their expression (Clayton and Krebs 1994). Our birds were denied the possibility of searching for nests, and this may have hindered their differentiation. Since the birds were released after the experiment, we have no data on their neuroanatomy.

It is also possible that while brain dimorphism is related to behaviour in the wild, it is not simply a quantitative matter of spatial relative to non-spatial performance. Host nests (similarly to hoarding sites) display both appearance and location differences and we do not have strong reasons to exclude a female advantage in the former rather than the latter dimension, other than the usual association of the hippocampal complex with spatially oriented tasks. Hoarding and nest location for parasitism might make very different demands, and perhaps the enlargement of the hippocampus observed in both cases is associated with memory specialisations which are very different in nature. There may not be a simple link to a particular kind of experimental procedure, such as those designed to study spatial memory. Many dimensions of memory performance, including the exploration of working versus reference memory, remain unexplored. The specialisation of brain structure and function may be much more "modular", in the sense of there being a rather specialised ability for host nest location, as is often proposed for functional hypotheses in the framework of evo-

lutionary psychology (see for instance Cosmides and Tooby 1997) than implied by our simple testing of proficiency in remembering the site or appearance of a desired object, but we see our study as a first step in mapping behavioural differences onto structural differences in neuroanatomy.

In summary, we found evidence that anatomical differences between sexes in the avian hippocampus complex are somehow accompanied by differences in performance in laboratory memory tasks, but our results did not endorse the simple view that larger hippocampal volume parallels generally enhanced spatial performance. The exact significance of the neuroanatomical sexual differences is not yet understood. Indeed, since nothing is known as yet about potential differences in memory performance between species or times of the year, this opens a clear challenge for future research.

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