ELSEVIER

Behavioural Brain Research 81 (1996) 61–68

BEHAVIOURAL BRAIN RESEARCH

Research report

Hippocampal volume in migratory and non-migratory warblers: effects of age and experience

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Received 23 January 1996; revised 19 March 1996; accepted 19 March 1996

Abstract

We tested the hypothesis that experience of migration from Europe to tropical Africa by Garden Warblers is associated with changes in the relative volume of the hippocampus, a brain region thought to be involved in processing spatial information, including that used in navigation. Relative hippocampal volume was larger in birds at least one year old that had migrated to and from Africa, than in naive birds approx. 3 months old. Further comparisons between groups of differing age and experience of migration suggested that both experience and age during the first year have an effect of relative hippocampal volume. The increase in relative hippocampal volume was mainly due to a decrease in the size of the telencephalon; however, the comparison between young, naive birds and older, experienced birds also suggests a possible increase in absolute hippocampal volume. The latter is associated with an increase in number and density of neurons, whilst the former is associated with an increase in density but no change in total number of neurons. In a non-migratory close relative of the garden warbler, the Sardinian warbler, older birds had a smaller telencephalon but there was no change in hippocampal volume, which supports the view that changes in the hippocampus may be associated with migratory experience, whilst changes in the telencephalon are not.

Keywords: Avian; Development; Migration; Hippocampus

1. Introduction

Many species of birds undertake regular migrations which may cover distances of several thousands of kilometres. Often these long journeys are broken by stopovers en route, during which the birds replenish fat stores. It is known that not only do many species fly annually between the same breeding and overwintering sites, but also that they may use the same stopover sites on both journeys [13,24,25,34]. Accurate return to these several different locations (breeding, wintering and stopover sites) would appear to require that the birds learn landmarks or spatial co-ordinates associated with each of them. This view is supported by results of experiments indicating that experienced migrants, but not inexperienced first year birds, that have been experimentally displaced are capable of compensating for the displacement (summaries in Refs. [4] and [17]).

Memory for landmarks associated with familiar locations also appears to be used by homing pigeons [9,10]. A number of experiments suggest that the hippocampal region of homing pigeons is important in navigation involving learned landmarks [7,8]. Hippocampal lesions appear to impair performance in at least two ways: (a) the acquisition of the ability to home from distances greater than 30 km and (b) the ability of experienced birds to home from novel release sites within the familiar area close (<20 km) to the home loft [5,6].

Retrieval of caches by food-storing birds also involves hippocampus-dependent spatial memory [28]. Further, in food storers it appears that the hippocampal region grows as a result of food-storing experience. This has been shown both by direct manipulation of experience [11] as well as by comparison of storing and non-storing species of different ages [18,19].

Experienced migrants have been exposed to a greater

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PII S0166-4328(96)00044-7

number of spatial locations than naive conspecifics. Does this additional experience bring about changes in the hippocampus parallel to those observed in young food storers? Although two earlier, cross-species analyses showed no correlation between migration distance and variation in hippocampal volume [20,29], in this study we tested the hypothesis that long-distance migrating birds with migratory experience have larger relative hippocampal volumes than conspecifics before their first migration. We measured relative hippocampal volume in the garden warbler (*Sylvia borin*), a species that migrates from its breeding grounds in western Europe in the autumn to wintering grounds in tropical Africa [15,21,34]. We compared four groups of garden warblers. (group A) Birds ≥ 15 months old with at least one experience of migration to and from Africa; (group B) birds ≥ 3 months old with no migrating experience; (group C) birds equivalent to group A, but held in captivity for an additional year; (group D) birds equivalent to group B, but held for a year in captivity. The comparison between groups A and B confounds age and experience of migration. Therefore, groups C and D were included to examine effects of changes in age without changes in migratory experience.

An additional test of whether any differences between groups A and B in the garden warbler were related to migration was carried out by comparing two groups of Israeli Sardinian warblers (*Sylvia melanocephala*), corresponding to A and B. Sardinian warblers of this population are year-round residents and therefore effects of experience specifically associated with migration are excluded [2].

2. Materials and methods

2.1. Preparation of materials

Sixty-two garden warblers (30 juveniles and 32 adults) were captured either in August–September 1991, in July–August 1992, or in August 1994 in southern Germany. The birds were divided into treatment groups A–D and G–H as indicated in Table 1. Twenty Sardinian warblers (12 juveniles and eight adults) were captured in Beit–Jimal, Israel in July 1993 to form groups E and F in Table 1.

Birds in groups A, B and E–H were killed shortly after capture, whereas groups C and D were held in captivity for 12 months following capture in July–August 1992. The captive birds were held in two $320 \times 200 \times 260$ cm indoor aviaries and exposed to the photoperiodic conditions experienced by freeliving garden warblers that breed in southern Germany but spend the winter at the equator. Food and water were supplied ad libitum. On 8 December, 9 March, 22 April and 9 June testicular width of the males and the diameter of the largest follicle of the females were measured by laparotomy. At the same times birds were weighed and their clavicular fat content was scored using indices between 1 (no fat) and 5. Locomotor activity was continuously recorded in both aviaries with the help of infrared-detectors. All birds went through a cycle of gonadal growth and regression between April and August. Before and after this cycle the birds showed fall and spring nocturnal migratory restlessness accompanied by fattening. At the times of capture and collection the majority of birds were in postjuvenile and postnuptial moult respectively. These data indicate that – as in previous studies [16] – the caged birds went through the normal sequence of seasonal events.

All birds were killed with an intraperitoneal overdose of sodium pentobarbitone and birds of groups A–F were perfused transcardially with 0.75% saline followed by 10% formol saline. All brains were treated in an identical way to control for the effects of shrinkage. Following perfusion the brains were dissected out and postfixed for 7 days in 10% formol saline before being transferred to 30% sucrose formalin. The brains were subsequently cut as frozen sections of 25 μm thickness at a standard angle in the coronal plane. Every tenth section was stained with Cresyl violet. The telencephalic hemispheres and cerebellum from the brains of the birds in groups G and H were separated out and weighed to the nearest 0.0001 g to determine wet mass. Following drying to constant mass at 70°C, the brain regions were re-weighed to determine dry mass.

2.2. Volumetric analysis

The boundaries of the hippocampus were defined as described in Refs. [14] and [23]. The boundaries are shown with a range of staining methods by Clayton and Krebs [11]. Hippocampal and telencephalic (excluding the hippocampus) volumes were measured by magnifying each section $10\times$ with a photographic enlarger, tracing the outlines and calculating the area with a digitising tablet. Two other telencephalic regions, ectostriatum and paleostriatum, were also measured. Slides were coded such that all measurements were done 'blind'. Interobserver reliability has been shown by Krebs et al. [23] to be about 95%. The formula for a truncated cone was used to compute the volume of all brain regions. These volumes were logarithmically transformed and analysed by regression with hippocampal volume as the dependent variable and telencephalon volume as the independent variable. The residuals from this regression (relative hippocampal volume), as were absolute volumes of the two measures, were compared between treatment groups by analysis of variance. Post-hoc comparisons were made using Fisher's PLSD test. There were no sex differences in any measure; therefore in the Results section the two sexes were combined.

Table 1
The experimental groups

Experimental group		Date of capture	Date of collecting brain
A	Garden warbler > 1 year old, experience of migration	Aug–Sept 1991 (7)July 1992 (9)	within 5 days of capture
B	Garden warbler < 3 months old, no experience of migration	Aug–Sept 1991 (6)July 1992 (8)	within 5 days of capture
C	Garden warbler > 1 year old, experience of migration, 1 year in captivity	July–Aug 1992 (10)	Aug 1993
D	Garden warbler < 3 months old, no experience of migration, 1 year in captivity	July–Aug 1992 (11)	Aug 1993
E	Sardinian warbler > 1 year old	July 1993 (8)	within 1 day of capture
F	Sardinian warbler < 3 months old	July 1993 (12)	within 1 day of capture
G	Garden warbler > 1 year old	August 1994 (6)	within 10 days of capture
H	Garden warbler < 3 months old	August 1994 (5)	within 10 days of capture

2.3. Neuron size, density and number

Neuron density and number in the hippocampus of groups A and B were estimated by taking measurements from a random subsample of 22 birds (11 birds of groups A and 11 of group B). Neuron size was estimated in a subsample of 12 birds (six from each group) by measuring the diameter of 20 hippocampal neurons chosen at random from each individual under 400 × magnification. Neuron density was estimated by counting profiles at 400 × magnification in a single section measuring either 250 000 μm^3 or 500 000 μm^3. A correction factor for split nucleoli computed by the Abercrombie method was then applied to these measurements [1,22]. Neurons were identified by the presence of both the pale nucleus and more darkly stained nucleolus. In terms of both cell types and cell density the hippocampus is a heterogeneous structure and therefore neuron density estimates were obtained from three regions in the hippocampus: the medial arm of the V-shaped structure of densely-packed cells (area 2 in Ref. [14]), the dorsomedial region (area 4 in Ref. [14]) and the dorsolateral region (area 6 in Ref. [14]). In each of these areas, the cells in three adjacent fields were counted (Fig. 1). As each field contained approximately 100 cells, about 900 cells were counted per section. Cell counts were made in the three areas at three levels of the rostro-caudal axis of the hippocampus corresponding to the following levels of the canary atlas: A 3.5, characterised by the first appearance of the septo-mesencephalic tract; A 1.6, corresponding to the appearance of the anterior commissure; and A 0.4, recognised by the first appearance of the cerebellum [30]. Approx. 2700 cells were counted per individual. The cell counting was done by using an image processing system (Image 1.40) to display the viewing field on the screen of a Macintosh II computer. The neuron counting method used here is a conventional one, used in many previous studies where neuron number and density has been assessed. We recognise, however, that as recent and more sophisticated methods have shown these conventional techniques produce approximate results, the neuron counts obtained here are only estimates and subtle differences may not be detectable [12,31,32]. The total number of hippocampal neurons in each individual was obtained by multiplying the average density of neurons from each bird by its hippocampal volume.

Neuron density was estimated in a second forebrain region, ectostriatum, using similar techniques, to examine possible changes other than in the hippocampus. Neuron density was estimated by counting cells in two ectostriatal fields in each of the 22 birds above. Fewer fields were measured than for hippocampus because ectostriatum is a more homogenous region.

3. Results

Means (± standard errors) of telencephalic and hippocampal volumes for each of the groups are shown in Table 2. Also shown are mean relative hippocampal volume, mean cell number and mean cell density.

3.1. Garden warblers: Relative hippocampal volume

There were significant differences among groups A–D in relative hippocampal volume ($F(3,47)=9.69$, $P=0.0001$). Specific comparisons between groups were done using post-hoc analyses.

Relative hippocampal volume was significantly larger in old, experienced than in young, naive birds (groups A and B ($P<0.01$), Fig. 2a), indicating that either age and/or experience is associated with an increase in this measure. The effect of age alone is demonstrated by the comparison between groups B and D ($P<0.05$; Fig. 2b): 15-month-old naive birds have a significantly larger relative hippocampus than do 3-month-old naive birds. The two groups of experienced birds (A and C), those

Fig. 1. A photomicrograph of a coronal section through the forebrain of a garden warbler shwoing lateral hippocampal boundaries. T, telencephalon; H, hippocampus; L, lateral boundary. Inset: A schematic view of the hippocampal region in coronal section. This illustrates the three sampling regions used for cell density and number estimates. The sets of three squares indicate the sampling regions, HA, hyperstriatum accessorium, HV, hyperstriatum ventrale, S, septum, V, lateral ventricle.

killed immediately after capture at age ≥15 months and those held for 12 months after capture, did not differ in relative hippocampal volume. This suggests that captivity per se, and age beyond 15 months, have no effect. Finally, the naive birds aged 15 months had a significantly smaller relative hippocampus than did the experienced birds aged ≥15 months (groups D and A, $P<0.05$; Fig. 2c). Given that captivity and age beyond 15 months have been shown to have no effect, this comparison indicates an effect of experience. To sum up, the post hoc comparisons show that relative hippocampal volume is influenced by both age (within the first year of life) and experience. It does not appear to be influenced by age beyond the first year or by holding the birds in captivity for 12 months.

3.2. *Garden warblers: Absolute hippocampal and telencephalon volumes*

The overall analysis showed no significant difference among groups in log hippocampal volume ($F(3,47)=1.66$, $P=0.19$); however, one of the four post hoc comparisons was significant, suggesting a possible effect. This was the comparison between groups A and B ($P<0.05$). Thus absolute hippocampal volume may be influenced by a combination of age and experience. None of the other comparisons showed significant effects, indicating that age alone (B vs. D), experience alone (A vs. D) and captivity (A vs. C) have no detectable effects.

For absolute telencephalon volume, the overall analysis showed a significant effect ($F(3,47)=6.74$, $P=0.0007$).

Table 2
Telencephalic, hippocampal, paleostriatal and ectostriatal volumes (all in mm^3), cell number and cell density for the four groups of Garden warblers and the two groups of Sardinian warblers

Experimental group	Telencephalon volume	Hippocampal volume	Paleostriatal volume	Ectostriatal volume	Cell number	Cell density
A	252.92±8.56	15.58±0.52	8.10±0.47	4.39±0.26	$4.10\times10^6\pm1.73\times10^5$	$2.66\times10^5\pm7.31\times10^3$
B	309.54±11.29	13.96±0.54	10.04±0.25	5.63±0.25	$3.26\times10^6\pm1.22\times10^5$	$2.41\times10^5\pm7.01\times10^3$
C	275.19±10.89	15.68±1.06	–	–		
D	281.55±6.03	14.77±0.32	–	–		
E	280.81±4.59	15.62±0.57	–	–		
F	369.13±7.90	15.28±0.29	–	–		

Values are means±standard errors.

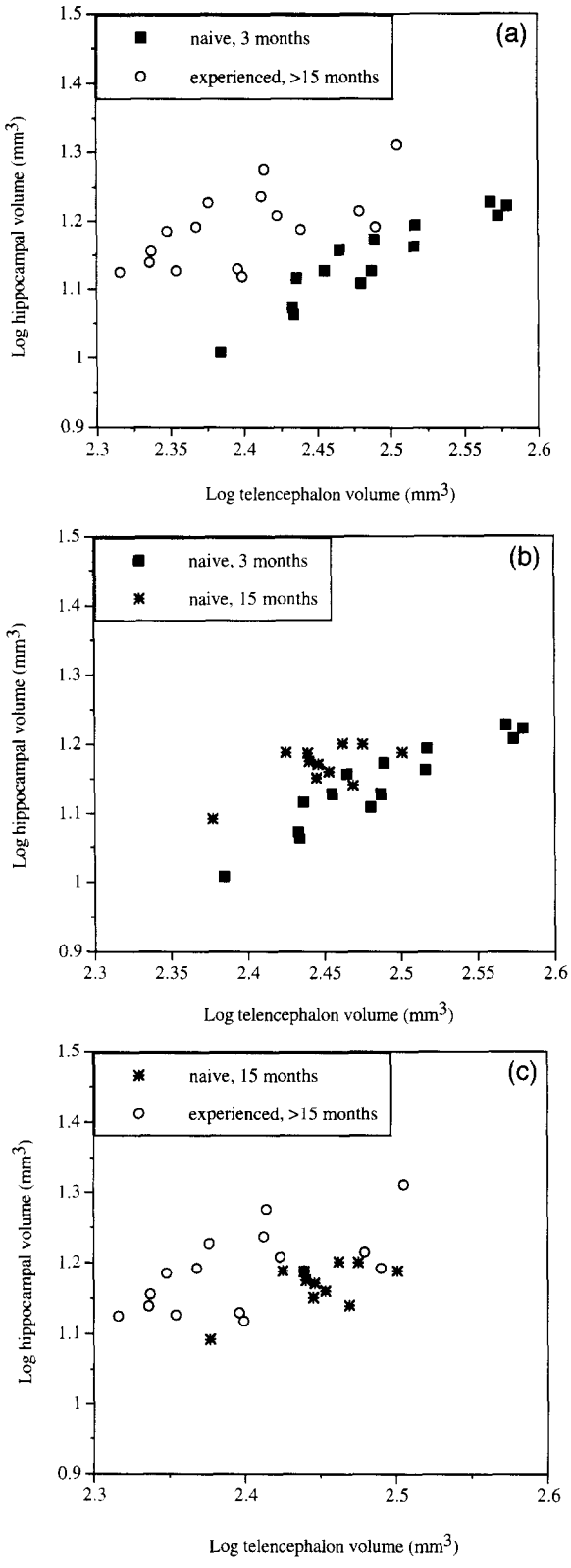

Fig. 2. (a) Hippocampal volume plotted against telencephalon volume for naive <3-month-old (group B; ■ squares) and >15-month-old experienced garden warblers (group A; ○). (b) Hippocampal volume plotted against telencephalon volume for naive <3-month-old (group B; ■) and naive, <15-month-old garden warblers (group D; *). (c) Hippocampal volume plotted against telencephalon volume for experienced >15 months (group A; ○) and naive 15-month-old birds (group D; *).

There were three significant post-hoc comparisons: between groups A and B, groups B and C, and groups A and C (P values of <0.05). In all of these cases the telencephalon volume was significantly smaller in the older birds than in the younger birds.

3.3. Sardinian warblers

Fig. 3 shows the comparison of the two groups of Sardinian warblers (E and F). Because there was no correlation between telencephalon and hippocampal volume it was not possible to calculate relative hippocampal volume by deviations from a regression. Older birds had a smaller telencephalon ($F(1,18)=71.48$, $P=0.0001$), but there was no difference in hippocampal volume ($F(1,18)=0.34$, $P=0.568$).

3.4. Hippocampal neuron size, number and density in garden warblers: comparison between ≥15-month-old, experienced birds and birds ≤3-month-old, no experience of migration (groups A and B)

Older, experienced birds had a greater total number and a higher density of hippocampal neurons than did younger, inexperienced birds (number: $F(1,20)=16.80$, $P=0.0006$; density: $F(1,20)=6.37$, $P=0.02$). There was no difference in neuron size ($F(1,10)=0.19$, $P=0.675$).

3.5. Ectostriatal and paleostriatal volumes

The volume of both ectostriatum and paleostriatum was larger in the younger, naive birds than it was in the older, experienced birds (ectostriatum: $F(1,20)=11.93$, $P=0.003$; paleostriatum: $F(1,20)=8.79$, $P=0.008$).

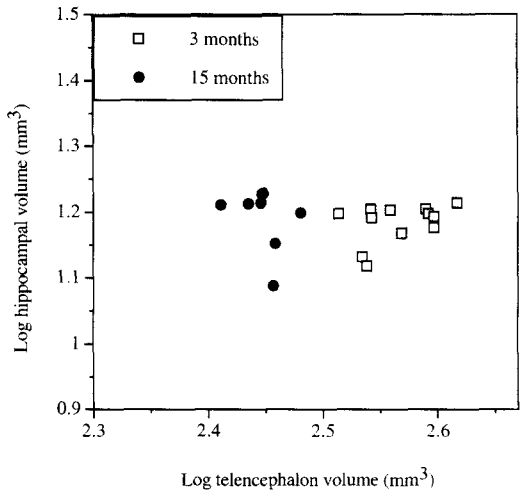

Fig. 3. Hippocampus volume plotted against telencephalon volume for <3-month-old (○) and at least 15-month-old Sardinian warblers (■).

3.6. Ectostriatal neuron number and density

The density of neurons in the ectostriatum was greater in older, experienced birds than it was in younger, naive birds ($F(1,20)=9.46$, $P=0.006$). There was no difference between the two groups in the number of neurons in the ectostriatum ($F(1,20)=1.13$, $P=0.29$).

3.7. Water content of telencephalic hemispheres and cerebellum: comparison of ≥15-month-old, experienced birds and birds ≤3-month- old, no experience of migration (groups G and H)

Both wet and dry telencephalic hemisphere mass of inexperienced birds was greater than that of experienced birds (wet mass (g): inexperienced birds: 0.389 ± 0.01 mean $\pm$ SE; experienced birds: 0.346 ± 0.007; $P=0.005$; dry mass (g): inexperienced birds: 0.083 ± 0.0023; experienced birds: 0.077 ± 0.002; $P=0.047$). These values imply that there is an 11% difference in wet mass between the two groups of which only 3.7% is due to a difference in water content. There were no differences between the groups in wet or dry mass of the cerebellum.

4. Discussion

The main results of this study are as follows. (1) In migratory garden warblers, relative hippocampal volume increases with age during the first year of life and with experience of migration. It does not appear to change as a result of holding birds in captivity or with age beyond the first year. (2) The change in relative hippocampal volume is a result both of a decrease in the absolute size of the telencephalon, excluding the hippocampus, and, possibly, an increase in the absolute size of the hippocampus. The greatest effect was seen in the decrease in telencephalon volume which was significantly smaller in old, experienced birds than in young, naive birds. (3) The possible increase in hippocampal volume with age/experience is accompanied by an increase in neuron density and number in this part of the brain. (4) Water loss plays only a minor role in the decrease in telencephalon volume. The decrease is accompanied by an increase in neuron density but no change in overall number, as indicated by sampling neurons in the ectostriatum. Both ectostriatum and paleostriatum are also smaller in older birds with migration experience than they are in the younger, inexperienced birds. (5) In a closely related, non-migratory species, the Sardinian warbler, older birds have a significantly smaller telencephalon volume than do younger birds, but there is no difference in hippocampal volume.

The results on garden warblers lend some support to the hypothesis that migratory experience is associated with changes in relative hippocampal volume (comparisons between old experienced and young naive birds, and between old naive and old experienced birds; Figs. 2a and c). However, there is also an effect of age (young vs. old naive birds, Fig. 2b), and the comparisons we have made do not reveal the relative importance of these two factors. The age effect shows up only in comparisons involving young birds (<3-month-old vs. older birds), which suggests that this effect is restricted to the first year. However, the absence of an age effect in older birds is based on the comparison of >15-month-old and >27-month-old experienced birds (groups A and C in Table 1). The latter group was held in captivity, and it has to be assumed that captivity itself does not cause a decrease in relative hippocampal volume. This assumption is supported by the comparison of naive birds, in which the 15-month-old group, after 12 months in captivity, had a larger relative hippocampus than did the 3-month-old birds.

The changes in relative hippocampal volume of garden warblers result from changes in the absolute value of both hippocampal and telencephalon volume. In three comparisons, between old, experienced and young naive birds, there was a significant difference in absolute telencephalon volume but there was only one comparison in which absolute hippocampus differed. Thus although both hippocampus and telencephalon changes appear to have contributed to the overall changes in relative hippocampal volume, it is the changes in absolute telencephalon which play the greater role.

In the groups for which data on neuron density and number were collected, age and experience were associated with a significant increase in both density and number of hippocampal neurons. This suggests that neurons are added to the hippocampus by immigration and/or neurogenesis. A parallel association of neuron addition with behavioural experience has been found in the hippocampus of food-storing birds [3,11] and the avian song system (e.g. [26]).

A change in telencephalon volume with age or experience, was not observed in previous studies in which young naive and old experienced food-storing birds were compared [18,19]. The garden warbler results do not allow us to discriminate between effects of age or migratory experience, but the fact that the non-migratory Sardinian warbler shows a similar effect argues against a specific association with migration. The decrease is not principally a result of water loss, which accounts for 3.7% of the difference between young, naive and old, experienced garden warblers, whilst the differences in mass and volume are 11% and 18%, respectively. This result, therefore, contrasts with seasonal changes in brain mass of certain small mammals where the effect is largely due to changes in water content (e.g., [27,33]). In order to assess whether the decrease in telencephalon was

associated with a change in neuron number or density, ectostriatum was chosen as a sampling area. These results showed that the decrease in telencephalon volume is associated with an increase in neuron density but no change in neuron number.

In conclusion, it appears that the two effects, hippocampal growth and telencephalon shrinkage, may involve different processes. Further analysis is required to find out where in the telencephalon shrinkage occurs and what accounts for the substantial loss in volume and mass. The fact that the cerebellum did not decrease in mass with age and experience shows that the effect is not general to all parts of the brain.

In a previous study involving interspecies comparisons of migratory behaviour and relative hippocampal volume, no association was found [20]. However, the age of birds in that study was unknown and it is still possible that with experienced, known-age birds a comparative study would demonstrate an association.

The results reported here raise a number of questions for the future. (a) Are the effects robust and general? (b) When in the first year do the two distinct changes in the hippocampus and telencephalon occur? (c) What are the underlying processes? (d) What is the relative importance of, or interaction between, age and experience? (e) What kinds of experience are involved?

Acknowledgement

We thank Jenny Corrigan and Martina Oltrogge for the preparation of the brains, M. Gahr, V. Bingman and S.J. Shettleworth for helpful comments on the manuscript and the Royal Society, SERC and the Nuffield Foundation for financial support. We are also grateful to Dr. U. Safriel, Jerusalem, for his help and advice when collecting Sardinian warblers.

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