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Research Report

Development of hippocampal specialisation in two species of tit (*Parus spp.*)

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Abstract

Food storing birds have been shown to have a larger hippocampus, relative to the rest of the telencephalon, than do non-storers. A previous study reported that this difference in relative hippocampal volume is not apparent in a comparison of nestling birds, but emerges after birds have fledged. This conclusion was based on a comparison of a storing and a non-storing species in the corvid family. The present study compared another storer/non-storer pair of species in order to test whether the results of the previous study can be replicated in another family of birds. The volumes of the hippocampal region and remainder of the telencephalon were measured and estimates of neuron size, density and total number in the hippocampal region were made for nestlings and adults of the food-storing marsh tit *Parus palustris* and non-storing blue tit *Parus caeruleus*. Relative hippocampal volume did not differ between nestlings of the two species, whilst the relative hippocampal volume of adult marsh tits was greater than that of blue tits. The difference between adults arose because in marsh tits but not blue tits, adults had a significantly larger relative hippocampal volume than did nestlings. Neuron density was significantly higher in both species in nestlings than in adults and adult blue tits had fewer neurons than did adult marsh tits. The results of this study are largely consistent with the earlier study comparing a storing and non-storing species of corvid, suggesting that the observed patterns may reflect a general difference between storers and non-storers in the development of the hippocampal region.

Key words: Food-storing; Avian; Hippocampus; Development; Neuron number

1. Introduction

The retrieval of stored food by certain species of passerine birds depends on spatial memory for the storage sites [2,24,27]. Lesion studies have shown that the hippocampal region (dorso-medial cortex of birds) plays an important role in memory for stored food and in other kinds of spatial memory [3,4,14,15,20,25]. Furthermore, food-storing species show an anatomical specialisation of the brain: the hippocampus is larger, relative to the rest of the telencephalon, in storing species than in closely related non-storing species [19,26]. The objective of the present study is to investigate the ontogeny of this brain specialisation and specifically to test the hypothesis, suggested by earlier work, that the difference between storers and non-storers in relative hippocampal volume arises at a late stage in development, after young birds have left the nest.

A previous study of two species of the family Corvidae, the food storing magpie (*Pica pica*) and non-storing jackdaw (*Corvus monedula*) [17], showed that the relative volume of the hippocampus did not differ between the species in prefledging birds, whilst in adults the magpie had a relatively larger hippocampus than did the jackdaw. Although neuron density declined with age in both species, the decline was more marked in jackdaws than in magpies. The total number of neurons in the hippocampal formation was greater in adult magpies than in adult jackdaws. These results suggest that certain changes in the hippocampus that result in differences between storers and non-storers arise post-fledging, after the birds have reached maturity in terms of growth. The time of onset of food-storing behaviour in magpies is not known precisely although it probably occurs within the first few months of life. The adult birds sampled were at least one year old and therefore had had experience of storing and retrieving food.

This raises the possibility that the changes in the hippocampus of storing species after leaving the nest are

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associated with the onset of food-storing. However, the difference between magpies and jackdaws in the growth of the hippocampus relative to the rest of the telencephalon could be correlated with food-storing by chance. The present study therefore tests the prediction generated by the previous work that the difference between storing and non-storing species observed in adult birds is not seen in prefledging juveniles. The two species compared are the food-storing marsh tit (*Parus palustris*) and the non-storing blue tit (*Parus caeruleus*).

2. Materials and methods

2.1. Preparation of materials

Fifteen marsh tits, nine adults (older than 1 year; average weight 10.8 g) and six prefledging (age range 7–17 days post-hatching; weight range 7.5–11.0 g) birds, and nineteen blue tits, eleven adults (older than 1 year, average weight 10.44 g) and eight prefledging (age range 2–17 days post-hatching; weight range 0.92–10.06 g) birds were sacrificed with an intraperitoneal overdose of sodium pentobarbitone and perfused transcardially with 0.75% saline followed by 10% formal saline. Following perfusion the brains were dissected out and postfixed for 7 days in 10% formal saline before being transferred to 30% sucrose formalin. The brains were subsequently cut as frozen sections of either 25 μm (prefledging birds) or 50 μm (adults) thickness in the coronal plane. Every tenth section (25 μm) or fifth section (50 μm) was stained with Cresyl violet. The prefledging birds were taken under NCC licence from the nest during May 1991.

2.2. Volumetric analysis

The boundaries of the hippocampus were defined as described in refs. 12 and 19. Hippocampal and telencephalic (excluding the hippocampus) volumes were measured by magnifying sections 10 × with a photographic enlarger, tracing the outlines and calculating the area with a digitising tablet. The formula for a truncated cone was used to compute the volume of both 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 line were then further analysed in an analysis of variance to assess the effects of two further variables: species (storer, non-storer) and age (nestling, adult).

2.3. Neuron size, density and number

Neuron size, density and number were estimated by taking measurements from a random subsample of 20 birds (five nestlings, aged 8–17 days post-hatching, and five adults of each species). Neuron size was estimated by measuring the diameter of 20 neurons 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. These measurements were then adjusted by applying a correction factor for split nucleoli, computed by the Abercrombie method [1,18]. 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 of ref. 12), the dorsomedial region (area 4 of ref. 12) and the dorsolateral region (area 6 of ref. 12). 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 [29]: 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. Approximately 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

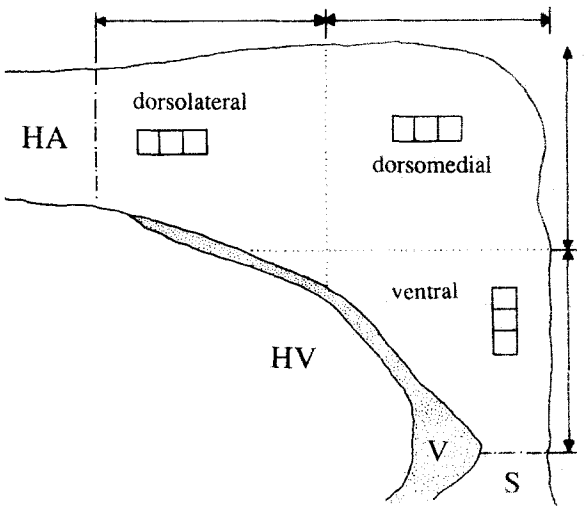

Fig. 1. A schematic view of the hippocampal region in coronal section. This illustrates the three sampling regions used for cell density and number estimates. The sets of three squares indicate the sampling regions. HA, hyperstriatum accessorium. HV, hyperstriatum ventrale. S, septum. V, lateral ventricle.

has been assessed. We note, however, that as recent and more sophisticated methods have shown these conventional techniques produce approximate results [11,31,32] the neuron counts obtained here are only estimates and subtle differences may not be detectable. 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.

3. Results

3.1. Volumetric analysis

A regression was used to assess the relationship between hippocampal and telencephalic volumes. A significant amount of variation in hippocampal volume was accounted for by telencephalon volume ($r^2 = 0.91$, $F_{1,33} = 345.52$, $P < 0.001$) so in the subsequent analysis the effect of telencephalon was first removed by taking the residuals generated from this regression (relative hippocampal volume). In a two-way analysis of variance (ANOVA) there was a significant effect on relative hippocampal volume of species ($F_{1,31} = 24.59$, p = 0.0001), of age ($F_{1,31} = 8.74$, $P = 0.006$) and there was a significant interaction between species and age ($F_{1,31} = 6.13$, $P = 0.02$). Marsh tits have larger relative hippocampal volumes than do blue tits and adults have larger relative hippocampal volumes than do juveniles. The significant interaction comes about because whilst adult marsh tits have a significantly larger relative hippocampus than juvenile marsh tits (one-way ANOVA: $F_{1,13} = 16.50$, $F = 0.001$), adult blue tits do not have a larger relative hippocampus than do juvenile blue tits ($F_{1,18} = 0.12$, $P = 0.74$). In addition, adult marsh tits have a significantly larger relative hippocampus than do adult blue tits (one-way ANOVA: $F_{1,19} = 30.67$, $p = 0.0001$) but relative hippocampal volume of juvenile marsh tits does not differ from that of juvenile blue tits ($F_{1,12} = 3.22$, $P = 0.10$) (see Figs. 2a and b).

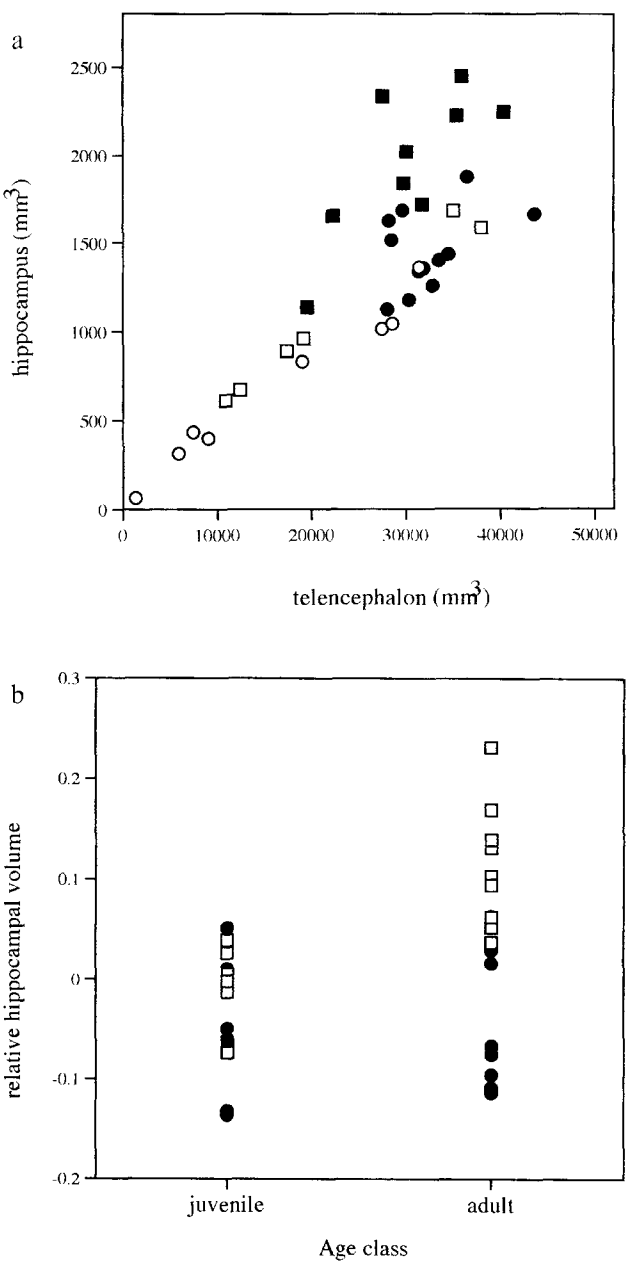

Fig. 2. a: hippocampal volume plotted against telencephalon volume (mm^3). Open squares: juvenile marsh tits (aged 7–17 days); solid squares: adult marsh tits; open circles: juvenile blue tits (aged 2–17 days); closed circles: adult blue tits. b: hippocampal volume (relative to the rest of the telencephalon) for the two species. The same data as plotted in Fig. 2a but expressed as relative hippocampal volume.

3.2. Neuron size, density and number

Neuron size was compared using a two-way ANOVA. There was no difference between the species nor between the two age groups in neuron size (species: $F_{1,16} = 0.18$, $P = 0.67$; age: $F_{1,16} = 0.41$, $P = 0.53$; species × age interaction: $F_{1,16} = 1.44$, $P = 0.25$).

Neuron densities (mm^3) for each of the four groups (marsh tits; adults and juveniles; blue tits: adults and juveniles) were compared in each of the three sampling areas and for the three rostro-caudal levels with two-way ANOVAs. In the analysis on the effect of sampling area there was no effect of species ($F_{1,16} = 0.57$, $P = 0.46$) or area ($F_{2,32} = 1.14$, $P = 0.33$) but there was a highly significant effect of age ($F_{1,16} = 131.96$, $P = 0.0001$). Neuron density was significantly higher in the juveniles than the adults (Fig. 3a). There were no significant interactive effects ($F_{2,32}$ 0.29–0.74, P0.75–0.49). In the analysis of rostro-caudal level there was no effect of species ($F_{1,16} = 0.03$, $P = 0.86$) nor of level ($F_{2,32} = 0.03$, $P = 0.86$). There was a highly significant effect of age ($F_{1,16} = 0.03$, $P = 0.86$). Juveniles had a greater density of neurons than adults at all three

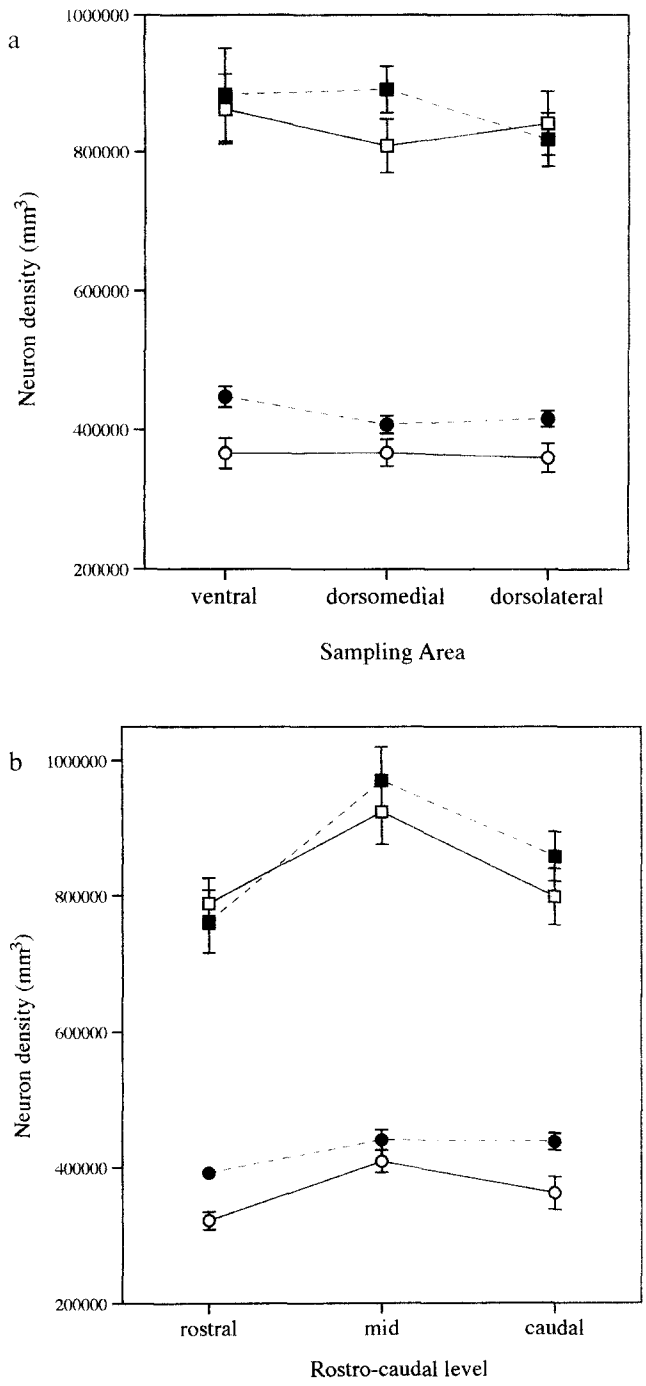

Fig. 3. a: neuron density (n/mm^3) (mean ± S.E.) in the hippocampus for the two age classes of the two species at each of the three sampling areas. Open squares: juvenile marsh tits (aged 7–17 days); solid squares: juvenile blue tits (aged 2–17 days); open circles: adult marsh tits; closed circles: adult blue tits. b: neuron density (n/mm^3) (mean ± S.E.) in the hippocampus for the two age classes of the two species at each of the three rostro-caudal regions.

rostro-caudal levels (Fig. 3b). There were no significant interactive effects ($F_{2,32}$ 0.013–0.96, P 0.98–0.39).

Total neuron number was estimated by multiplying the density of neurons by hippocampal volume (mm^3) for each of the twenty birds. A two-way ANOVA was used to test for differences between the four groups. There were no

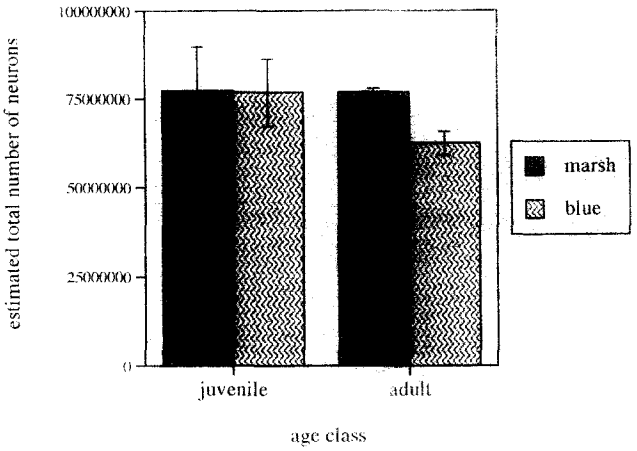

Fig. 4. Estimated total number of neurons (mean ± S.E.) in the hippocampus for the two age classes of the two species.

differences between the species ($F_{1,16} = 0.86$, $P = 0.34$), nor between the age classes ($F_{1,16} = 0.85$, $P = 0.37$) and there was no interaction between the main effects ($F_{1,16} = 0.74$, $P = 0.40$) (Fig. 4).

4. Discussion

The main results from this study are: (i) adult marsh tits have a larger relative hippocampus than do adult blue tits; (ii) prefledging marsh tits do not differ in relative hippocampal volume from prefledging blue tits; (iii) adult marsh tits have a larger relative hippocampus than do prefledging conspecifics whereas adult blue tits do not differ in relative hippocampal volume from prefledging conspecifics; (iv) neuron size does not differ between the two species nor between the two age classes; (v) neuron density is higher in prefledging birds of both species.

The results from this study support those of a previous study comparing hippocampal development in magpies, a storing species, and jackdaws, a non-storing species [17]. As with the previous study, this study demonstrates that adults of the food-storing species have a relatively larger hippocampus than adults of the non-storing species. This study also finds a similar lack of difference in hippocampal volume between the prefledging birds and between the prefledging and adult non-storing birds. Adult marsh tits, however, like adult magpies, have a relatively larger hippocampus than do prefledging conspecifics. In marsh tits, a storing species, prefledging birds have not yet begun to store food so this hippocampal difference must occur at, or after, the onset of storing. Unlike the corvids, the onset of food storing behaviour in tits has been documented: food storing in tits begins approximately five weeks after the young birds fledge and is fully developed within a few weeks [8,9,16] and therefore, the adult marsh tits in this

study, being at least a year old, are likely to have had considerable storing experience. Recent work on the dual ontogeny of food-storing and hippocampal structure has revealed by experimental comparison that much of the difference in relative hippocampal volume between the prefledging and adult marsh tits is caused by food storing experience [10]. If young postfledging marsh tits are deprived of the opportunity to store and retrieve food, their hippocampus is smaller than in similarly aged, but more experienced, conspecifics. It is not yet clear whether these affects on hippocampal volume of food storing experience are brought about by the same process as those which result in the interspecific difference in hippocampal volume between storers and non-storers. In addition to the volumetric difference, there are also cytoarchitectural differences between storers and non-storers e.g. presence of large cells containing calbindin in the hippocampus of storers but not in non-storers [21]. It is not yet known whether these differences are also experience-dependent.

The change in the size of the hippocampus in response to food storing has parallels with the passerine song system. In the spring the HVC (a telencephalic area involved in song production and/or memorisation) of male canaries, for example, significantly increases in size during which time the males are learning and singing new songs. The increase in HVC size is due to an increase in the number of neurons, without an increase in density, and this is thought to be a result of neurogenesis [23]. A decline in neuron number with increasing age has also been demonstrated in the song nucleus lMAN, an area of the passerine song system involved in early memorisation of songs. Male zebra finches retain cells in lMAN that are lost in females between days 20 and 55 after hatching, the sensitive phase for song learning [7]. During the same period two other song nuclei, HVC and Area X, both involved in song production and/or memorisation of songs increase in size in males, presumably due to neurogenesis [7,22,23]. Neuron addition and loss, however, are independent of auditory experience during song learning [7]. Whilst in male canaries these changes have been correlated with seasonal changes in acquisition or production in song, such seasonal changes in these nuclei have also been observed in species which do not exhibit seasonal variation in song production or memorisation. HVC volume in Gambel's white-crowned sparrows, for example, changes seasonally [28] yet the males produce song throughout the year and in rufous-sided towhees, males do not learn new songs as adults but there is seasonal fluctuation in HVC volume [6]. One suggestion is that the observed seasonal changes in the song system of the species studied so far, are a result of changing levels of circulating steroid hormones associated with reproduction rather than through a cause and effect relationship between song learning and anatomical changes in the brain [5]. The significance of the seasonal changes becomes even less clear when it is noted that such changes in HVC size are observed in Nissl stained material but not if estrogen receptor containing neurons are studied [14].

In the study on magpies and jackdaws the difference in relative hippocampal volume between the adults seemed to be associated with an increase in cell number without an increase in cell density: adult magpies had more neurons than adult jackdaws but the density of cells did not differ between the species. Whilst there is some suggestion that adult blue tits have fewer neurons than do adult marsh tits (Fig. 4) this effect is not significant. Two other effects seen in the corvid study were, however, seen in this study. Firstly, when the food storing adults (marsh tits) are compared with prefledging conspecifics there is no difference in cell number but a large difference in cell density: neurons are more densely packed in the hippocampus of the younger birds. The second effect similar to that seen in the corvid study was that the non-storing adult jackdaws had fewer cells in the hippocampus than conspecific prefledging birds and it was suggested that this result indicated that the difference in hippocampal volume between the adult magpies and jackdaws was due to cell loss in the non-storing species. In this study, whilst not a significant effect, it can be seen in Fig. 4 that there is tendency for the adult blue tits to have fewer cells than the prefledging birds. In addition, whilst there is no difference in hippocampal volume between the young and adult blue tits, neuron density is significantly higher in the younger birds. Cell loss in the hippocampus is at least in part a result of apoptosis or programmed cell death [10]. Programmed cell death is thought to be an important process in early development [30], although its role in later development (as in the present study) is not yet clear. In the song nucleus lMAN, cell death occurs at the same time as song learning (age 25–65 days) but does not seem to be causally related to song learning [7]. Further work is needed to clarify the roles of cell loss and recruitment in the morphometric changes in the hippocampus described in the present study.

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