Behavioural Brain Research, 53 (1993) 127–131

BBR 01402

Development of hippocampal specialisation in a food-storing bird

S.D. Healy and J.R. Krebs

Department of Zoology and Department of Pharmacology, Oxford University, Oxford (UK)

(Received 3 August 1992)
(Revised version received 5 October 1992)
(Accepted 20 October 1992)

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

Previous studies demonstrated that amongst food-storing passerine birds the hippocampal region (dorso-medial cortex) is enlarged relative to the rest of the telencephalon. It has been hypothesised that this hippocampal specialisation is related to the spatial memory requirements of retrieving large numbers of stored items. Here we compare the development of the hippocampus in a food-storing and a non-storing corvid, the adults of which differ in relative hippocampal volume. The volume, cell density and number of cells in the hippocampal region of nestling (5–25 days post hatching) and adult (>320 days old) magpies *Pica pica* (food-storing) and jackdaws *Corvus monedula* (non-storing) were measured. In both species the volume of the hippocampus increases with the volume of the rest of the telencephalon during the nestling growth phase. The relative volume of the hippocampus in 5- to 25-day-old nestlings of the two species does not differ significantly. In the food-storing magpie, the relative volume of the adult hippocampus is significantly larger than that of nestlings, whilst in the jackdaw, adults and nestlings do not differ. The density of neurons declines with increasing age and this effect is more marked in jackdaws than in magpies. Neuron number did not change significantly with age, but is significantly greater in adult magpies than in adult jackdaws. These results are discussed in relation to the possibility that changes in hippocampal volume and cell number are related to the use of spatial memory in retrieving stored food.

INTRODUCTION

Previous studies have shown that amongst food-storing passerine birds the dorsomedial cortex or hippocampal region is enlarged relative to the rest of the telencephalon in comparison with non-food-storing species[12,22]. This has occurred in at least three independent evolutionary lines (the titmice Paridae, corvids Corvidae and nuthatches Sittidae), suggesting an anatomical specialisation of the brain associated with storing behaviour. The hippocampal region plays a role in forming memories for stored food[14,21] and the enlargement of the hippocampus of food-storers may be an adaptation for processing the large amounts of information involved in storage and retrieval of food[11,13].

In the present study we examine how the difference between a storer and a non-storer in relative hippocampal volume arises during development. Although the ontogeny of food-storing behaviour has been documented in detail in only a few species[5,8] (for review see ref. 24), it is known that young birds do not store until after leaving the nest. In titmice, where the most detailed work has been done, birds start to store soon after independence from the parents, i.e. about 2 weeks after leaving the nest[5]. Thus by examining the relative hippocampal volume of storing and non-storing birds before and after leaving the nest, it is possible to ascertain whether or not the enlargement of the hippocampal region in storing species precedes the onset of storing behaviour. We examined the relative hippocampal volume, cell density and cell number of storing and non-storing species of corvid. The magpie *Pica pica* is known to store throughout the year and probably retrieves its cached food within a few hours to days of storing[2]. The jackdaw *Corvus monedula* rarely if ever stores food in the wild (review in ref. 9). Adults of the former have a significantly larger hippocampus than do adults of the latter[9].

MATERIALS AND METHODS

Preparation of material

Twenty-eight jackdaws (mean weight of adults 220 g) and 25 magpies (mean weight of adults 185 g) 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

Correspondence: S.D. Healy, Department of Zoology, Oxford University, South Parks Road, Oxford, OX1 3PS, UK.

7 days in 10% formal saline before being transferred to 30% sucrose formalin. The brains were subsequently cut as frozen sections of either 50 μm or 25 μm thickness in the coronal plane. Every fifth section (50 μm) or every tenth section (25 μm) was stained with Cresyl violet. Fourteen of the jackdaws and 10 of the magpies were taken under licence from the nest during April and May 1988, 1989 and 1990 and ranged in age from ca. 5 to ca. 25 days posthatching. The remaining 15 magpies and 14 jackdaws were adults, at least 320 days old, collected under licence at the same season and in the same years as the nestlings.

Volumetric analysis

Hippocampal and telencephalon (excluding the hippocampal region) volumes were measured by tracing outlines under 10 × magnification with a photographic enlarger and digitising the areas on each section to compute volumes using the formula for a truncated cone. The definition of the boundaries of the hippocampal region followed those described by refs. 7, 12, 15. The volume measurements were logarithmically transformed and analysed by stepwise multiple regression with hippocampal volume as the dependent variable and three independent variables: telencephalon volume, storer/non-storer (coded as a dummy variate) and age (nestling, adult) also coded as a dummy variate.

Neuron density and number

Neuron densities for a random subsample of 24 birds (six nestlings, aged 10–25 days posthatching, and six adults of each species) were estimated by counting profiles in a square graticule measuring 100 × 100 μm in a single plane of focus in the Nissl-stained sections and applying the standard correction factors to obtain an estimate of neuron density[1,10]. Neurons were identified by their pale nucleus and more darkly stained nucleolus: only cells with a visible nucleolus in the sample frame were included. The hippocampal formation is a heterogeneous structure in terms of its cell types and cell density[7]. We therefore sampled three different areas of the hippocampal region: the medial arm of the V-shaped structure of densely packed cells (area 2 of ref. 7); the dorsomedial region (area 4 of ref. 7) and the dorsolateral region (area 6 of ref. 7) (Fig. 1). These regions were sampled at three levels of the rostro-caudal axis of the hippocampal formation corresponding approximately to the following levels of the canary atlas[23]. 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. In each region at each level on the rostral-caudal axis,

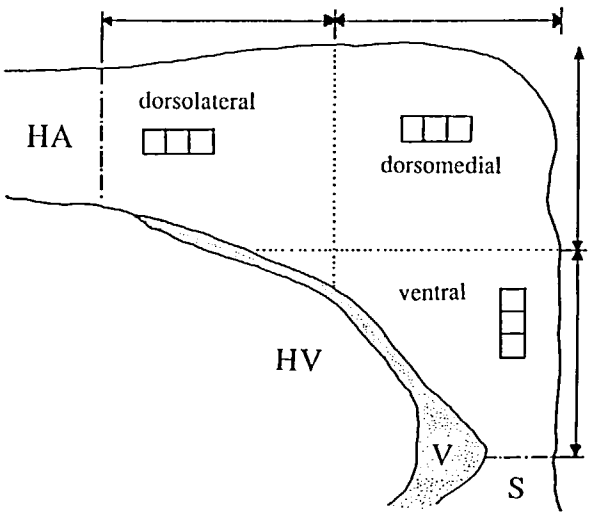

Fig. 1. A schematic of the hippocampal region in coronal section to illustrate the sampling regions used for cell density estimates. The set of three squares in a row (not to scale) indicate the sampling regions. HA, hyperstriatum accessorium; HV, hyperstriatum ventrale; V, lateral ventricle; S, septum.

three adjacent fields of cells in the left hippocampus were counted at 400 × magnification. Each field contained approximately 100 cells, thus the total number of cells counted per birds was about 900 per section (300 × 3 regions) or 2,700 in total. In a preliminary analysis of the data, the 3 sampling regions and the three rostro-caudal sections were considered separately, but no differences in the patterns reported below were seen between these subsamples, so all data were combined for the main analysis. The cell counting was done by using an image processing system (Image 1.40) to display the field on the screen of a Macintosh II computer. The images were enhanced to sharpen the nucleolus. The average density estimated from nine sampling areas in each bird was multiplied by hippocampal volume to obtain an estimate of the total number of neurones in the hippocampal formation for each individual. The method we used is similar to that used in most previous studies of neuron density and number, but recent developments of more sophisticated methods such as the optical dissector, have shown that conventional techniques are biased[6,26,28]. Thus the measurements provide only an approximate estimate of total cell number and therefore may not detect subtle differences.

RESULTS

Volumetric changes

In the multiple regression of hippocampal volume on telencephalon, species and age, there was a signifi-

cant effect of telencephalon volume ($F_{1,47} = 823.679$, $P = 0.0001$), age (cumulative, $F_{1,47} = 30.614$, $P = 0.0001$), a significant species effect ($F_{1,47} = 43.65$, $P = 0.0001$), and a significant interaction between species and age ($F_{1,47} = 12.418$, $P = 0.0001$). The results are displayed in Fig. 2A,B, which shows that during the late nestling stage the relative hippocampal volume of the storing and non-storing species is similar, whilst in the adults there is a striking difference: in the non-storing jackdaw, hippocampal volume relative to telencephalon volume remains the same as in the late nestling stage, whilst in the magpie, relative hippocampal volume increases (Fig. 2A). When the nestling and adult birds were analysed separately the difference between species

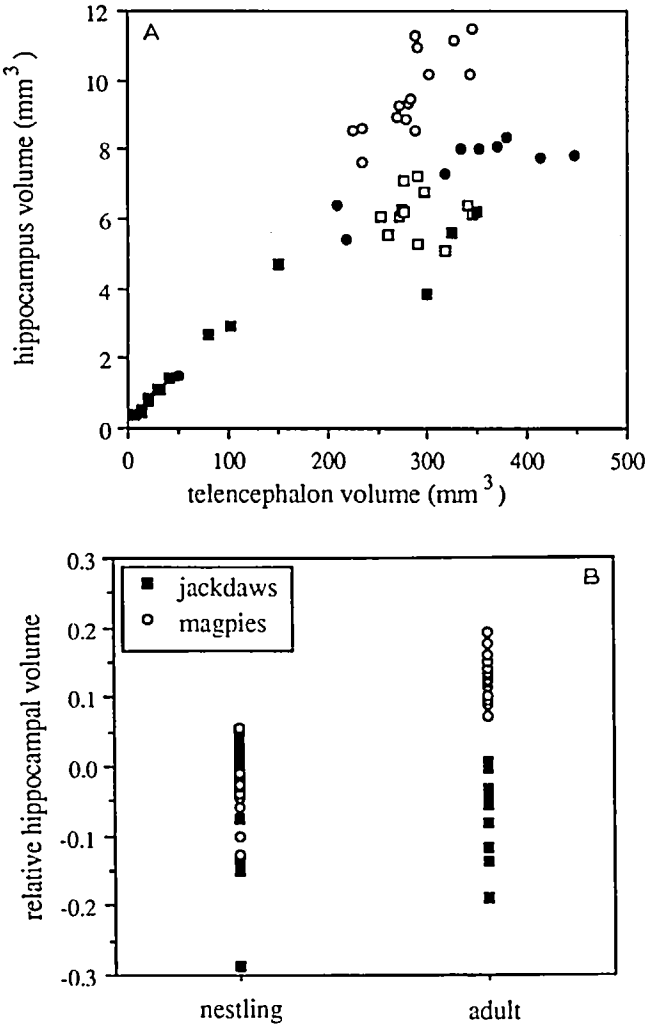

Fig. 2. A: a plot of hippocampal volume as a function of volume of the rest of the telencephalon (correlated with age) showing that in magpies but not in jackdaws there is an increase in relative hippocampal volume after 25 days. Solid squares: 5- to 25-day-old jackdaws; open squares: >320-day-old jackdaws, solid circles: 5- to 25-day-old magpies; open circles: >320-day-old magpies. B: hippocampal volume relative to telencephalon volume in nestlings and adults. The values are deviations from the regression of a log–log plot.

was not significant in the former, although there was a trend for magpies to have a larger relative hippocampus ($F_{2,21} = 3.318$, $P = 0.083$) and highly significant in the latter ($F_{2,25} = 133.67$, $P = 0.0001$) (Fig. 2B).

Neuron density and number

Fig. 3A,B shows the results of the estimates of density and number of neurons. Neuron density declined with age ($F_{1,20} = 7.24$, $P = 0.014$; two-way ANOVA), did not differ overall between species ($F_{1,20} = 1.29$, $P = 0.269$) and showed a weak, non-significant trend towards a greater decline with age in jackdaws than in magpies ($F_{1,20} = 2.036$; $P = 0.169$) (Fig. 3A). When the two species were analysed separately, jackdaws showed a significant decline with age ($F_{1,11} = 7.845$, $P = 0.019$), whilst magpies did not ($F_{1,11} = 1.484$, $P = 0.251$). Total cell number did not vary with age, but overall magpies had more cells than did jackdaws (Fig. 3B) (age: $F_{1,20} = 0.368$, $P = 0.551$; species: $F_{1,20} = 11.732$, $P = 0.003$; interaction: $F_{1,20} = 0.171$; $P = 0.683$). When

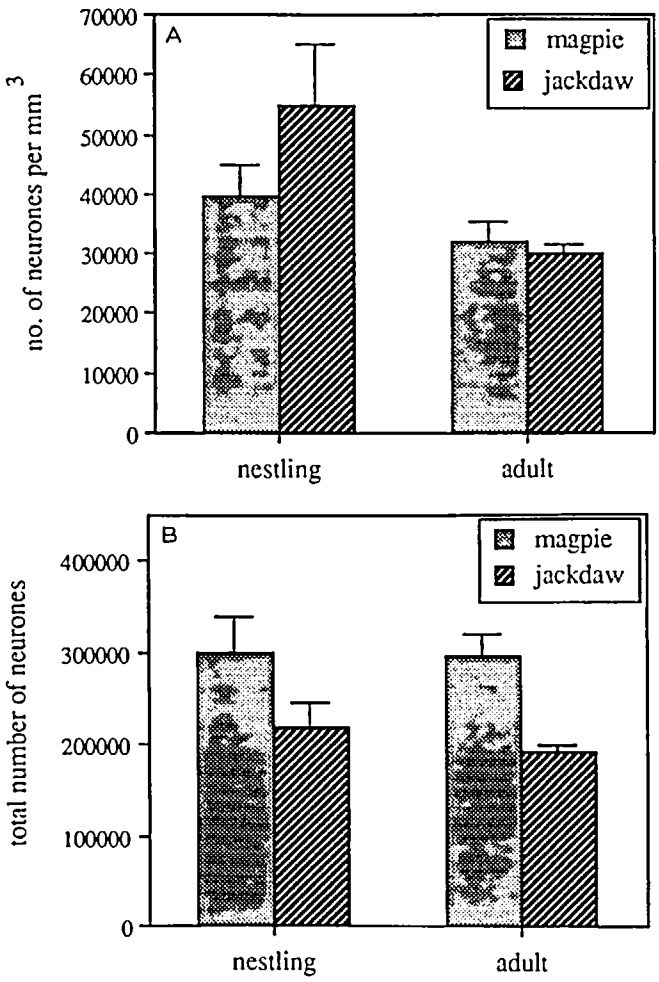

Fig. 3. A: cell density and (B) cell number in the hippocampal region of nestling (10–25 days) and adult (>320 days) magpies and jackdaws.

adults and nestlings were analysed separately, there was no significant difference between nestlings ($F_{1,11} = 3.039$) but adult magpies had significantly more cells than did jackdaws ($F_{1,11} = 14.50$, $P = 0.003$).

DISCUSSION

The main results of this study are as follows: (i) The difference between adults of the non-storing jackdaw and the food-storing magpie in relative hippocampal volume and neuron number arises partly between 5 and 25 days posthatching but largely as a result of divergence after fledging (between 35 and 365 days); (ii) in jackdaws, but not magpies, neuron density declines with age; (iii) adult magpies have a larger total number of neurons in the hippocampal region than do jackdaws.

Previous work has shown that differences between species of passerine birds in relative volume of the hippocampal region is associated with food-storing behaviour[9,12,22]. Here we show that, at least in one comparison between a storer and a non-storer, the difference arises at a relatively late stage of development, after the birds are fully grown and have left the nest. This raises the possibility that development of the hippocampal region proceeds in parallel with behavioural development and the use of spatial memory in storing and retrieving food. Although early workers pointed to possible correlations between neuron number and learning ability in amphibians[25] and rats[3] (for review see ref. 27), these studies had many confounding variables. The most detailed more recent studies of correlations between learning and changes in volume of brain nuclei and associated recruitment and loss of neurons are those of the passerine song control system. The male zebra finch learns its song in a sensitive phase between the ages of 20 (immediately postfledging) and 65 days. During this period, the volume of two song control nuclei, HVC and Area X, both of which are thought to play a role in memorisation and/or production of songs, increases rapidly in males but not in females[16]. This increase in volume is associated with a substantial increase in neuron number[16], with relatively little change in neuron density. The change in neuron number is presumed to reflect neurogenesis, which in one study accounted for about 20% of neurons in HVC[17]. In another song nucleus, LMAN, which from lesion studies plays a particular role in early memorisation of songs, the number of neurons and the size of the nucleus decrease in males (but not in females) during the sensitive phase[4,16]. The decrease of neurons in the male is due to cell death, perhaps, by analogy with other systems, under hormonal control[18]. Thus memorisation and development of motor production of songs in the young male zebra finch is accompanied by both neurogenesis and by cell death. It is still not clear how these changes are causally related to memorisation, although in the swamp sparrow, changes in volume and cell number of the song control nuclei occur during the memorisation phase (20–60 days of age) and not in the motor production phase, which in this species does not start until the age of 275 days[19]. A further complication is that neurogenesis also occurs in adult zebra finches of both sexes, well after the sensitive phase for learning song for motor production, but perhaps associated with learning to recognise songs[20].

At the moment we do not have sufficiently detailed data on the dual ontogeny of food-storing behaviour and brain structure to make detailed comparisons with the findings on the passerine song system. However, in broad terms, our results suggest that the emergence of a structural difference between two species in the brain is related to the emergence of a behavioural difference. At the level of volumetric measurement, the difference between jackdaws and magpies is that the magpie hippocampus appears to grow relative to the rest of the brain between 35 and 320 days, whilst the jackdaw hippocampus does not. This difference is not mirrored by a greater increase in neuron number in the magpie than in the jackdaw, but rather by a greater cell loss in the latter. Further experimental work, in which the opportunity to store and retrieve food during development is manipulated, will be necessary to further evaluate the relationship between food storing memory and hippocampal development.

ACKNOWLEDGEMENTS

This work was supported by SERC (S.D.H. and J.R.K.), St John's College Oxford (S.D.H.) and the Royal Society (J.R.K.). We thank the following for helpful comments and advice: Hugh Perry, Kevin Martin, Jonathan Newman, Andrea Székely and Nicky Clayton. Tim Birkhead, Philip Heeb and Frank Cezilly helped to provide birds.

REFERENCES

1 Abercrombie, M. Estimation of nuclear populations from microtome sections, *Anat. Rec.*, 94 (1946) 239–247.
2 Birkhead, T., *The magpies*, T. & A.D. Poyser, London, 1990.
3 Block, J.B. and Essman, W.B., Growth hormone administration during pregnancy: a behavioural difference in offspring rats, *Nature*, 207 (1965) 1136–1137.

4 Bottjer, S.W. and Sengelaub, D.R., Cell death during development of a forebrain nucleus involved with vocal learning in zebra finches, *J. Neurobiol.*, 20 (1989) 609–618.
5 Clayton, N.S., Development of food-storing behaviour in marsh tits, *Behaviour*, 120, in press.
6 Coggeshall, R.E., A consideration of neural counting methods, *Trends Neurosci.*, 15 (1992) 9–13.
7 Erichsen, J.T., Bingman, V.P. and Krebs, J.R., The distribution of neuropeptides in the dorsomedial telencephalon of the pigeon (*Columba livia*): a basis for regional subdivision, *J. Comp. Neurol.*, 314 (1991) 478–492.
8 Haftorn, S., Ontogeny of food storing in titmice *Parus* spp. *Ibis*, 134 (1992) 69–71.
9 Healy, S.D. and Krebs, J.R., Food storing and the hippocampus in corvids: amount and volume are correlated, *Proc. R. Soc. B.*, 248 (1992) 241–245.
10 Konigsmark, B.W., In W.J.H. Nauta and S.O.E. Ebbeson (Eds.) *Contemporary Research Methods in Neuroanatomy*, Springer, Berlin, 1970.
11 Krebs, J.R., Food-storing birds: adaptive specialisation in brain and behaviour?, *Phil. Trans. R. Soc. Lond. B.*, 329 (1990) 153–160.
12 Krebs, J.R., Sherry, D.F., Healy, S.D., Perry, V.H. and Vaccarino, A.L., Hippocampal specialisation of food-storing birds, *Proc. Natl. Acad. Sci. USA*, 86 (1989) 1388–1392.
13 Krebs, J.R., Hilton, S.C. and Healy, S.D., Memory in food-storing birds: adaptive specialisation in brain and behaviour? In G.M. Edelman, W.E. Gall and W.M. Cowan (Eds.) *Signal and Sense: Local and Global Order in Perceptual Maps*, Wiley–Liss, New York, 1990, pp. 475–500.
14 Krushinskaya, N.L., Some complex forms of feeding behaviour of nutcracker *Nucifraga caryocatactes*, after removal of old cortex, *Zh. Evol. Biochim. Fisiol.*, 11 (1966) 563–568.
15 Montagnese, C.M., Krebs, J.R., Székely, A.D. and Csillag, A., A sub-population of large calbindin-like immunopositive neurones is present in the hippocampal formation in food-storing but not in non-storing species of bird, *Brain Res.*, in press.
16 Nordeen, K.W. and Nordeen, E.J., Sex and regional differences in the incorporation of neurons born during song learning in zebra finches, *J. Neurosci.*, 8 (1988) 2869–2874.
17 Nordeen, K.W. and Nordeen, E.J., Projection neurons within a vocal motor pathway are born during song learning in zebra finches, *Nature*, 334 (1988) 149–151.
18 Nordeen, E.J., Nordeen, K.W., Sengelaub, D.R. and Arnold, A.P., Androgens prevent normally occurring cell death in a sexually dimorphic spinal nucleus, *Science*, 229 (1988) 671–673.
19 Nordeen, K.W., Marler, P.R. and Nordeen, E.J., Addition of song related neurons in swamp sparrows coincides with memorisation, not production, of learned song, *J. Neurobiol.*, 20 (1989) 651–661.
20 Nottebohm, F., Alvarez Buylla, Cynx, J., Kirn, J., Ling, C.-Y., Nottebohm, M., Suter, R., Tolles, A. and Williams, H., Song learning in birds: the relation between perception and production, *Phil. Trans. R. Soc. B.*, 329 (1990) 115–124.
21 Sherry, D.F. and Vaccarino, A.L., Hippocampus and memory for food caches in black-capped chickadees, *Behav. Neurosci.*, 103 (1989) 301–318.
22 Sherry, D.F., Vaccarino, A.L., Buckenham, K. and Herz, R.S., The hippocampal complex of food-storing birds, *Brain Behav. Evol.*, 34 (1989) 308–317.
23 Stokes, T.M., Leonard, C.M. and Nottebohm, F., The telencephalon, diencephalon and mesencephalon of the canary *Serinus canaria* in stereotaxic co-ordinates, *J. Comp. Neurol.*, 156 (1976) 337–374.
24 Vander Wall, S.B., *Food Hoarding in Animals*, University of Chicago Press, Chicago, 1990.
25 Vernon, J.A. and Butsch, J., Effect of tetraploidy on learning and retention in the salamander, *Science*, 125 (1957) 1033–1034.
26 West, M.J. and Gunderson, H.J.G., Unbiased stereological estimation of the number of neurons in the human hippocampus, *J. Comp. Neurol.*, 296 (1991) 1–22.
27 Williams, R.W. and Herrup, C., The control of neuron number, *Annu. Rev. Neurosci.*, 11 (1988) 423–453.
28 Williams, R.W. and Rakic, P., Three-dimensional counting: an accurate and direct method to estimate numbers of cells in sectioned material, *J. Comp. Neurol.*, 278 (1988) 344–352.