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Hippocampal specialization of food-storing birds

(passerines/memory)

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ABSTRACT **In a study of 52 individuals belonging to 35 species or subspecies of passerine birds it was shown that the volume of the hippocampal complex relative to brain and body size is significantly larger in species that store food than in species that do not. Retrieval of stored food relies on an accurate and long-lasting spatial memory, and hippocampal damage disrupts memory for storage sites. The results suggest, therefore, that food-storing species of passerines have an enlarged hippocampal complex as a specialization associated with the use of a specialized memory capacity. Other life-history variables were examined and found not to be correlated with hippocampal volume.**

Some species of birds store large numbers of food items, each in a separate place, and use an accurate, long-lasting spatial memory to retrieve their stores (1–5). We show here that the hippocampal complex (dorsomedial forebrain) (6) of food-storing passerines is larger relative to body and brain size than that of nonstorers. Thus, across a range of species, a relationship has been found between the structure of a specific brain area outside sensory and motor areas and a specific behavior.

METHODS

We measured the volume of the hippocampal complex and striatum of 52 individuals belonging to 35 species or subspecies distributed among 9 passerine families [taxonomy in this paper follows that of Sibley *et al.* (7) based on DNA·DNA hybridization]. We defined the hippocampal complex as including the closely interconnected hippocampal and parahippocampal areas (6). The evidence from both embryological and connectivity studies (8–10) suggests that these two structures as a whole are homologous to the mammalian hippocampal complex, although the homology of the different subdivisions is not known. The behavioral consequences of damage to the avian hippocampal complex show that it is broadly functionally equivalent to the mammalian hippocampus in playing an important role in certain memory tasks, including those involving spatial memory (11–18). The avian hippocampal complex is a paired structure located adjacent to the midline of the dorsal telencephalon (19). It extends from the caudal limit of the striatum along approximately two-thirds of the caudal–rostral extent of the striatum. In coronal section it is bounded medially by the midline and ventrally by the lateral horns of the ventricle and by the septum (Fig. 1). The region defined as the hippocampus by Karten and Hodos (19) is a V-shaped structure of densely packed cells lying ventrally and medially (Fig. 1). In the parahippocampal area large and small neurons are sparsely and nonuniformly distributed. The lateral boundary of the parahippocampal area is characterized by a change in the size distribution of neurons. Medial to the boundary the distribution is bimodal with peaks at cell areas of about 20 μm^2 and 130–150 μm^2, while lateral to the boundary the distribution is unimodal with a peak at about 20–30 μm^2 (Fig. 2): the boundary is often clearer in food-storers than in other species, but once recognized in the former group it can be identified in the latter. The cytoarchitectural characterization of the hippocampal complex is supported by immunocytochemical evidence, which revealed that the lateral boundary is coincident with the boundary of an area rich in substance P present along the whole rostro–caudal extent of the hippocampal complex. The hippocampal complex itself is characterized by populations of large somatostatin- and avian pancreatic polypeptide (APP)-immunoreactive cells (unpublished results).

The techniques of preparation and measurement were as follows. Birds were killed with an intraperitoneal lethal dose of sodium pentabarbitone and then perfused transcardially with physiological saline followed by formal/saline. The brains were dissected from the cranium after perfusion, weighed and measured, and post-fixed for 3–7 days in 10% Formalin before being transferred to 30% sucrose/Formalin. The material was cut into 25-μm frozen sections in the coronal plane and every tenth section was stained with cresyl violet. Care was taken to ensure that all procedures were standardized, but no corrections were made for shrinkage, which appeared to be very similar for all the brains.

The boundaries of hippocampal complex and of the rest of the telencephalon (19) [closely correlated with brain mass ($r = 0.96$)] in all sections were traced at $\times 10$ magnification with a photographic enlarger (where necessary, boundaries were confirmed by inspection at higher magnification) and the areas were digitized on a Summa-graphics tablet. Volumes were calculated by calculating the volume between successive sections by the formula for a truncated cone. The only potential source of observer error in defining the hippocampal boundaries is at the lateral boundary, where, as already described, the boundary depends on recognizing a change in cell sizes. To evaluate the magnitude of this error, two of us (J.R.K. and S.D.H.) independently located the boundary in sample sections from 12 randomly chosen species (8 nonstorers and 4 storers). The mean error (±SEM), expressed as a percentage of the total area of the hippocampal complex on the sections examined, was 3.73 ± 0.73%. The boundary was determined with less error for food-storers (2.75 ± 1.25%) than for nonstorers (4.2 ± 0.8%), but the overall level of these errors is very small in relation to the differences in volume of about 30% observed between storers and nonstorers. In species for which we sampled more than one individual the interindividual variation in the ratio of hippocampus volume to telencephalon volume was about 7%.

RESULTS

Initial inspection of the data showed that the hippocampus of food-storing species is considerably larger than that of closely related nonstorers. For example, the 11-g marsh tit (*Parus palustris*), which stores food, has a hippocampal volume of

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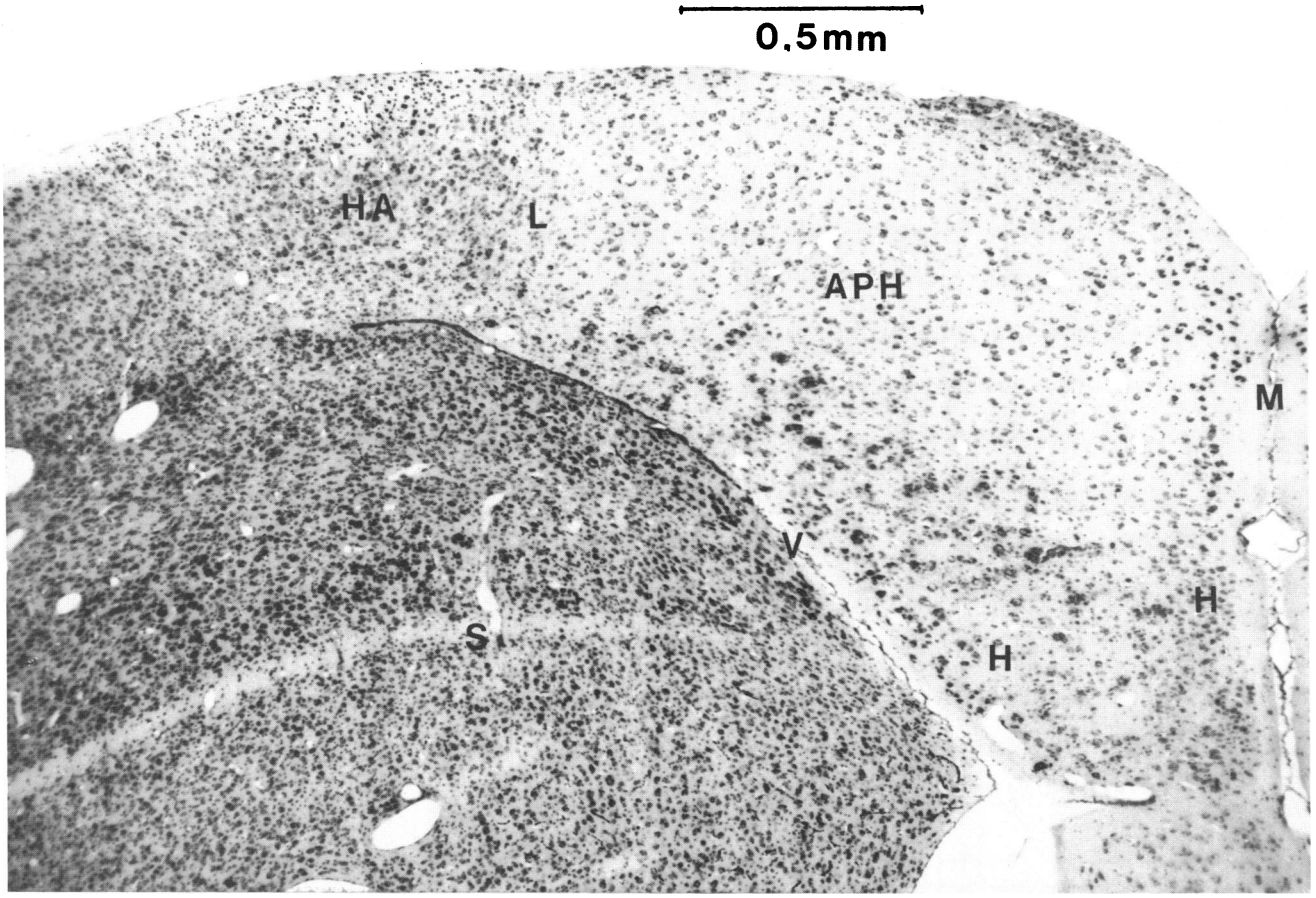

FIG. 1. A coronal section of the hippocampal complex and adjacent areas of the telencephalon of a food-storing tit, *Parus palustris*. The section is from a point 0.3 of the rostro–caudal extent of the hippocampus. Key is as follows [after Karten and Hodos (19): H, V-shaped area of dense cells; APH, parahippocampal area; L, lateral boundary, characterized by sharp change in cell density; V, ventricle; M, midline; HA, hyperstriatum accessorium; and S, striatum.

14.26 mm^3, while the 20-g great tit (*Parus major*), a nonstorer, has a hippocampal volume only 11.2 mm^3. The remainder of the telencephalon of the great tit is larger (325.94 mm^3) than that of the marsh tit (257.12 mm^3) as might be expected from its larger body size. Thus the hippocampus of the marsh tit is 31% larger than that of the great tit, although the remainder of its forebrain is 21% smaller. To test whether or not this striking association between hippocampus size and food storing holds in general, it is necessary to compare different species after partialling out the effects of body mass and brain size, since these two variables could confound any effects due to food storing. Furthermore it is important in comparative analyses to choose an appropriate taxonomic unit to be as sure as possible that the comparison is based on independent data points (20, 21). Species are clearly not independent units in our analysis (for example, four of the nine food-storing species we studied belong to one genus, *Parus*). We used families according to Sibley *et al.* (7) as independent data points, this being the highest taxonomic subdivision of the suborder, Oscinae, to which all our species belong [we also did the analysis at the level of families, using the older classification of passerines (22), and obtained similar results, although the levels of significance were slightly lower]. The mean value of each family was used in the analysis, and in the case of the Paridae and Corvidae, which contain nonstoring as well as storing members, we entered a mean value for each of these subsets, yielding 11 data points from nine families.

The results are summarized graphically in Fig. 3. Fig. 3 *A* and *B*, respectively, show regressions of logarithm of hippocampal volume on logarithm of body weight and logarithm of telencephalon volume on logarithm of body weight. The deviations of each data point from the regression lines represent the hippocampus and telencephalon size after the effects of body mass have been removed. In Fig. 3*C* the deviations from the hippocampal regression are plotted against the telencephalon deviations. Deviations from this regression indicate hippocampus size relative to the rest of the forebrain after any effects of body size have been removed. As the graph shows, food storers have a larger hippocampus relative to forebrain size than do nonstorers. The statistical analysis of the data shown in Fig. 3 was done by subjecting the log-transformed hippocampal volumes to stepwise multiple regression with three independent variables entered in the following order: logarithm of body mass, logarithm of telencephalon volume, and nonstorer/storer (this was treated as a dummy variate). Not surprisingly, body mass and telencephalon volume accounted for most (89%) of the variation in hippocampal volume. Of the remaining variance, more than 89% was accounted for by whether or not the birds store food. The effect of food storing was significant at the 0.001 level (partial correlation = 0.892).

An alternative way of expressing the results is to make pairwise comparisons between storing and nonstoring members of the same family. This is possible for the Corvidae and Paridae, and a third pair can be added by comparing the closely related Sittidae (storers) and Troglodytidae (nonstorers). When the deviations from the regression of Fig. 3*C* (relative hippocampal volume after removal of effects of body and brain size) are compared in this way with the three pairs by using a t test, storers have a significantly larger relative hippocampal volume ($P < 0.01$).

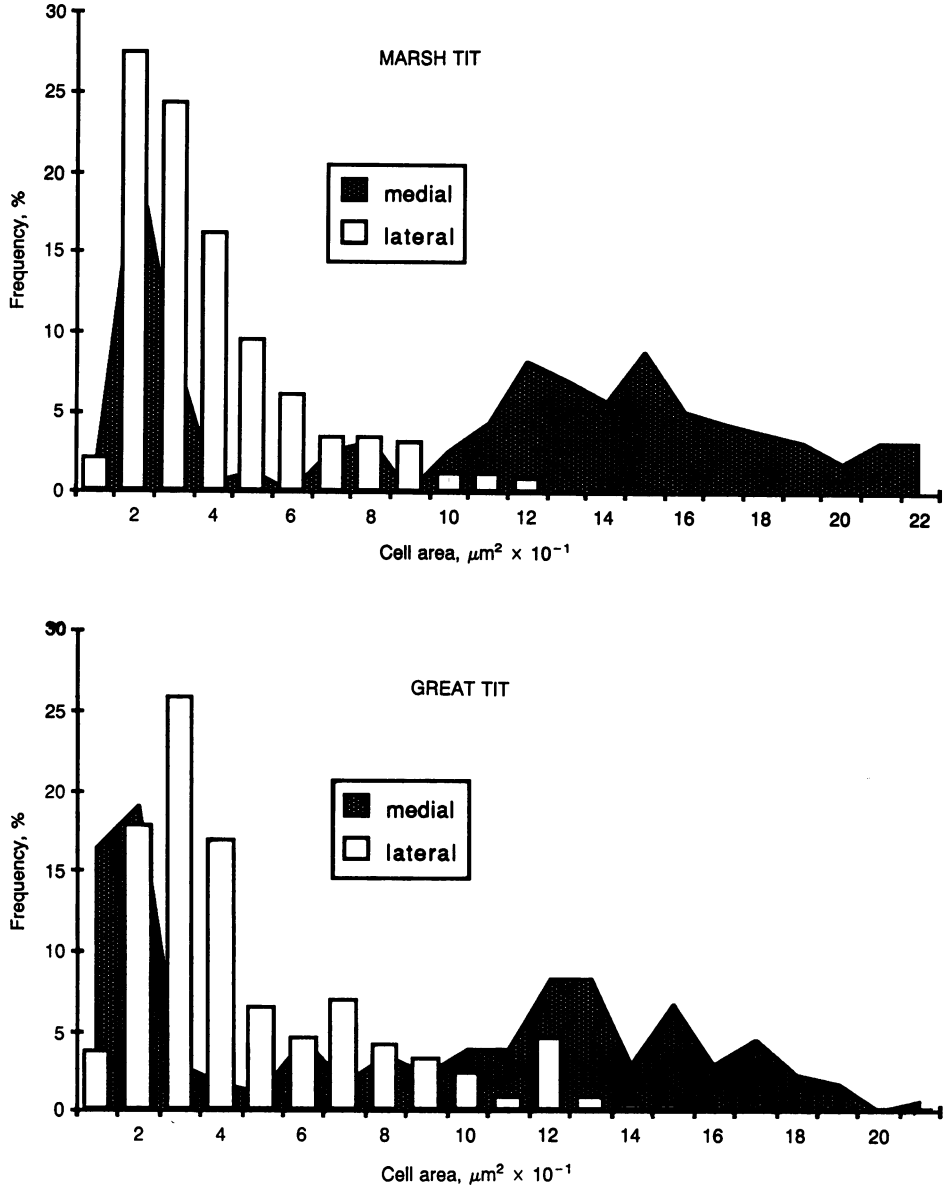

FIG. 2. Data from two species, a storer (marsh tit) and a nonstorer (great tit), to show the change of the size distribution of neurons at the lateral boundary of the hippocampal area. The data were obtained by tracing around all neurons in a square graticule with a camera lucida at ×1000 magnification and measuring the cell areas with a digitizing tablet. The samples were taken by moving the graticule along a transect running for 1 mm on either side of the lateral boundary, which was determined by inspection before starting the drawing of cells. Sample sizes of cells measured for storer and nonstorer, respectively, are as follows: medial to the boundary, 280 and 297; lateral to the boundary, 332 and 318.

Other life history and ecological variables were examined to see if they differed between storers and nonstorers in a systematic way that might confound our result, but on inspection no patterns were evident in the following variables: developmental mode, timing of activity, diet, habitat, mating system, and nesting dispersion. One variable that might be expected to correlate with hippocampal volume, migration, was examined in more detail. The rationale for suggesting a link with migration arises from the observation that the hippocampus plays a role in home area recognition (15) and the fact that migrants have to learn two home areas, the winter and summer quarters, while residents have to learn only one. However, when migrant/resident was included as a fourth independent variable in a stepwise multiple regression, it did not account for a significant proportion of the variation in hippocampal volume. The migrating species are indicated in the legend of Fig. 3. The wild-caught birds were all taken in autumn and winter and therefore we did not look for possible seasonal changes in hippocampal volume.

DISCUSSION

The association between a large hippocampus and food storing is likely to be related to the facts that food storing places special demands on spatial memory and that the hippocampus plays a role in spatial memory. Examination of the spatial memory of storing and nonstoring tits should allow us to find out whether food storers have special memory capacities. The correlation we have observed is more likely to be associated with memory than with other aspects of food storing, given the established role of the hippocampus in memory (11–18) and the fact that hippocampal damage does not disrupt other aspects of food storing behavior (18). In a study of 29 species of North American passerines a relationship between hippocampal volume and food-storing similar to that reported here has been observed (D.F.S., unpublished results).

Although other studies of vertebrates have revealed relationships between the size of specific brain areas and behav-

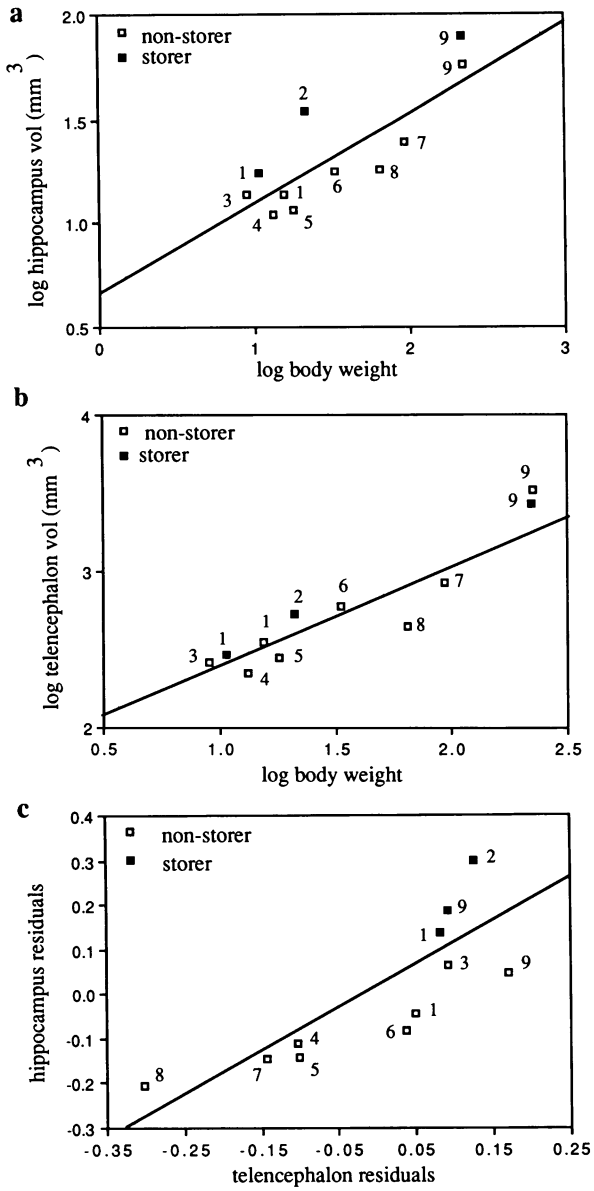

FIG. 3. Graphical representation of the results of the multiple regression analysis in which body size and brain size were partialled out. In all graphs the squares are families or subdivisions of families into storers and nonstorers (see text). The numbers refer to families as listed below. (*A*) Regression of logarithm hippocampal volume on logarithm of body mass in grams. (*B*) Regression plot of logarithm of telencephalon volume on body mass in grams. (*C*) Family deviations from graphs *A* and *B* plotted one against the other. The individual species and sample sizes are as follows (where more than one individual per species was examined the two individuals were usually of opposite sexes; s, storer; n, nonstorer; m, migrant). 1, Paridae: *Parus major* one (n), *Parus caeruleus* two (n), *Parus atricapillus* one (s), *Parus ater* one (s), *Parus palustris* one (s), *Parus montanus* one (s). 2, Sittidae: *Sitta europea* one (s). 3, Certhiidae: *Troglodytes troglodytes* two (n), *Certhia familiaris* one (n). 4, Sylviidae: *Sylvia borin* two (n, m), *Sylvia atricapilla* one (n, m), *Phylloscopus trochilus* two (n, m), *Phylloscopus collybitus* one (n, m). 5, Passeridae: *Poephila guttata* two (n), *Lonchura striata* two (n), *Vidua paradisea* two (n), *Prunella modularis* one (n). 6, Fringillidae: *Agelaius phoeniceus* one (n, m), *Fringilla coelebs* two (n), *Carduelis chloris* two (n), *Emberiza schoeniclus* one (n, m). 7, Sturnidae: *Sturnus vulgaris* three (n). 8, Muscicapidae: *Turdus merula* two (n), *Turdus philomelos* one (n), *Erithacus rubecula melophilus* two (n), *Erithacus rubecula rubecula* two (n, m), *Saxicola torquata rubicula* two (n, m), *Saxicola torquata axillaris* two (n), *Ficedula abicollis* one (n, m). 9, Corvidae: *Corvus monedula* two (n), *Corvus frugilegus* two (s), *Pica pica* two (s), *Garrulus glandarius* two (s), *Cissa erythrohyncha* one (s), *Pyrrhocorax graculus* one (s).

ioral capacities, most previous work has concentrated on sensory and motor areas of the brain (23, 24) rather than on areas concerned with higher levels of integration and cognitive capacities. Previous workers have attempted to relate differences in hippocampal structure to behavioral differences within a species by using inbred strains (25, 26), but we know of no other example of a consistent relationship across a wide taxonomic range between differences in the size of a particular brain region and a specific difference in a higher-order behavioral capacity. The closest parallel is the demonstration by Nottebohm and others (27–29) that there is a correlation within canaries (*Serinus canarius*) and between two populations of long-billed marshwrens (*Cistothoris palustris*) in the size of song repertoire and the nucleus Hvc.

We have reported only on volumetric differences, but preliminary work suggests that there may be differences between storers and nonstorers at a more detailed cytoarchitectural and neurochemical level.

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