

The ecology of the avian brain: food-storing memory and the hippocampus

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Some species of birds store food, often hoarding several hundreds of seeds over a period of just a few weeks. Field and laboratory studies have demonstrated that food-storing species have an impressive memory and an enlarged region of the brain, the hippocampal region. Lesion experiments have shown that the hippocampus is important in accurate retrieval of stored food. Taken together, these results have led to the hypothesis that the enlarged hippocampus is associated with the memory requirements of retrieving stored food. In this review, we discuss four areas of study: comparative studies of the brain, comparative studies of behaviour, developmental plasticity and seasonal changes in food storing and the hippocampus.

It is now well established that food-storing birds in the passerine families Corvidae and Paridae rely, at least in part, on memory for retrieval of their food hoards. Field observations suggest that cache retrieval may operate over long time periods, from weeks (in parid species) to months (in certain corvid species), and involves tens of thousands of items, often with each item stored in a separate place, spread over many hundreds of square metres (Swanberg 1951, Haftorn 1954, 1956a,b, Bossema 1979, Tomback 1980, Pravosudov 1985, review in Vander Wall 1990, A. Brodin, unpubl. PhD thesis, Stockholm University). Experimental field studies have shown that the same individual that stores seeds also retrieves them (Stevens & Krebs 1986). Meanwhile, laboratory studies have shown that memory-based retrieval can operate for periods of up to about 40 days in titmice (Hitchcock & Sherry 1990, A. Brodin, unpubl.) and for at least 270 days in corvids such as the Clark's Nutcracker *Nucifraga columbiana* (Balda & Kamil 1992, Kamil & Balda 1994). Although these studies do not conclusively demonstrate a role for memory of individual sites in the field, they are consistent with this idea. Food storers, in short, would appear to possess an impressive memory capacity, and in the past 8 years, much effort has been directed to exploring whether or not this assertion is correct (Shettleworth in press).

Food-storing birds also have an unusual feature of the brain: an extraordinarily enlarged dorsomedial cortex, or hippocampal region. In the laboratory, memory-based retrieval of caches in food-storing passerine birds has been impaired by lesions to the hippocampus (Krushinskaya 1966, Sherry & Vaccarino 1989, H.N. Rice, unpubl. MA thesis, University of Western Ontario), a forebrain nucleus thought to be involved in processing of spatial memory in birds (Sherry & Vaccarino 1989, Bingman 1993) as hypothesized for the mammalian hippocampus (O'Keefe & Nadel 1978). Two

parallel studies, in which phylogenetic analyses of the morphology of the brain of passerine species were carried out, have shown that food storers have a larger hippocampus, relative to the rest of the forebrain, than do nonstorers (Krebs *et al.* 1989, Sherry *et al.* 1989).

The observations that food storers have an apparently impressive memory and a special structure in the brain have led to the hypothesis of 'adaptive specialization of brain and memory' in food-storing birds. This is the idea that the ecological niche of utilizing scatter-hoarded food for winter survival has generated a selection pressure for certain special properties of memory, which in turn has led to the evolution of specialized brain structures associated with processing these memories. This hypothesis is, of course, an umbrella for more specific and directly testable ideas: by the accumulation of evidence for or against more specific ideas, the general hypothesis of adaptive specialization will eventually stand or fall. A major technique in these tests is comparison between species, one of the oldest, but nevertheless most effective methods for testing hypotheses about evolutionary adaptation. The methodology of comparative studies has evolved substantially in the past few years (e.g. Kamil 1994) as a result of the combination of the appearance of molecular phylogenies, which provide a more accurate picture of evolutionary relationships than was available from morphological data (Sibley *et al.* 1988, Sibley & Ahlquist 1990, Sibley & Monroe 1990), and the development of statistical methods to ensure that comparisons are made using truly independent data points (Harvey & Pagel 1991). In the old days (and in some studies today), species were taken as independent data points without regard to the fact that a trait (such as enlargement of the hippocampus) may have evolved only once in a particular lineage, which has since divided into many sublineages. The comparative studies of hippocampal

morphology referred to above based their statistical analyses on phylogenetically independent comparisons: enlargement of the hippocampus in food storers has occurred independently at least three times in the evolution of the passerines (in the Corvidae, Sittidae and Paridae). Similarly, assessment of the studies of behavioural differences between storing and nonstoring species, summarized in a later section, must include the recognition that in order to make the case that a particular memory trait is associated with food storing, it must be shown to have evolved independently along with food storing in more than one evolutionary lineage. These behavioural studies have focussed on the Paridae and Corvidae, so the evidence for independent evolution of the same behavioural traits in food storers relies principally on showing parallel trends in these two families. As more detailed phylogenies within each family become available, it may prove possible to establish whether or not food-storing behaviour has evolved more than once in each of these families, which would allow more than one independent comparison within each. Recent work by Gill *et al.* (1994) suggests that there may be a single seed-caching lineage of *Parus* which represents one evolutionary divergence from the present noncaching lineages (including the Great Tit *Parus major* and the Blue Tit *Parus caeruleus*) and that noncaching may be the ancestral state, although further analyses of sister taxa are needed to test this hypothesis.

Although studies of food-storing memory and the brain were aimed initially at testing the hypothesis of adaptive specialization in memory and brain using the comparative approach, in the past 2 years, two new lines of investigation have emerged. First, experiments on the development of food-storing behaviour, spatial memory and the brain have shown that, in food-storing parids, not only are there striking developmental transitions but also that these transitions are accompanied by dramatic changes in the brain (Clayton 1992, 1994, 1995, Clayton & Krebs 1994c). Furthermore, the current evidence suggests that the changes in the brain are at least partly caused by changes in behavioural experience, as we shall review below. Second, studies of seasonal changes in food storing have just begun to investigate whether or not changes in the intensity of storing (in both parids and corvids there is more storing in the late summer, autumn and winter than in the spring and early summer) are accompanied by changes in the brain. The results to date are equivocal, but more work needs to be done.

In this review, we focus on four areas of study, briefly summarizing the current state of knowledge as follows: (1) comparative studies of the brain, (2) comparative studies of behaviour, (3) developmental plasticity and (4) seasonal changes in food storing and the hippocampus.

COMPARATIVE STUDIES OF THE BRAIN

In describing the anatomical specialization of food-storing birds, we refer to the avian hippocampus. In accord with the nomenclature of classical avian neuroanatomy (Craigie

1935, Ariens Kappers *et al.* 1936, Showers 1982), we use the term 'hippocampus' (short for hippocampal formation or hippocampal region, to indicate that we include the hippocampus and related structures) to describe a small strip of tissue lying close to the midline on the dorsal surface of the telencephalon (Fig. 1). This strip, which is bounded ventrolaterally by the lateral ventricles, runs from the posterior limit of the telencephalon and extends to cover about two-thirds of the anterior-posterior axis. Although anatomists have agreed, on the basis of a variety of criteria including cell morphology (Molla *et al.* 1986), embryology (Rose 1914), connectivity (Casini *et al.* 1986) and neurochemistry (Erichsen *et al.* 1991), that this structure is part of the archicortex of birds and is homologous with the mammalian hippocampus, the exact boundaries of the hippocampal region in birds have only recently been investigated and are still subject to some dispute, in particular the lateral and anterior boundaries. We use the boundaries defined on the basis of cell morphology (Krebs *et al.* 1989, Montagnese *et al.* in press), connectivity (Székely & Krebs 1993b), immunocytochemical markers (Erichsen *et al.* 1991, Krebs *et al.* 1991, Montagnese *et al.* 1993b) and histochemical markers (Montagnese *et al.* 1993a, Sherry *et al.* 1993b). Krebs *et al.* (1989) used Nissl-stained material to show that there is a sharp transition in the frequency distribution of cell sizes at the lateral and anterior boundaries. This transition is clearly visible in most passerine species but is less clear or absent in the Pigeon *Columba livia*, on which the classical bird brain atlas is based (Karten & Hodos 1967). Montagnese *et al.* (in press) used a variety of Golgi techniques to show that in the Zebra Finch *Taeniopygia guttata* the hippocampal region, as defined here, is characterized by cell populations not observed elsewhere. Using a range of antibodies to neuropeptides, transmitters and transmitter-related enzymes has shown that the lateral and anterior boundaries can be clearly visualized (for Pigeons, Erichsen *et al.* 1991, Krebs *et al.* 1991; for parids and corvids, Montagnese *et al.* 1993b). For example, the neuropeptides, calbindin and substance P, have characteristic staining patterns at the lateral boundary, although there is dispute over whether the strongly immunoreactive substance P field at the lateral boundary is just inside or just outside the hippocampal region (Shimizu & Karten 1990, Erichsen *et al.* 1991). Székely and Krebs (1993b) revealed the pattern of intrinsic and extrinsic projections of the hippocampal region of the Zebra Finch using *Phaseolus lectin* injections and showed that the lateral boundary is the limit of a rich intrinsic projection field. Some other features of this study are summarized in Figure 2. Finally, Montagnese *et al.* (1993a) showed that the histochemical distribution of zinc in the Zebra Finch characteristically delineates the lateral boundary, and Sherry *et al.* (1993b, see also Clayton & Krebs 1994a) have used the distribution of acetylcholinesterase stained histochemically to map the lateral and anterior boundaries in passerines, although this technique does not produce clear boundaries in the Pigeon (E. Gwinner, S.D. Healy & J.R. Krebs, unpubl. obs.).

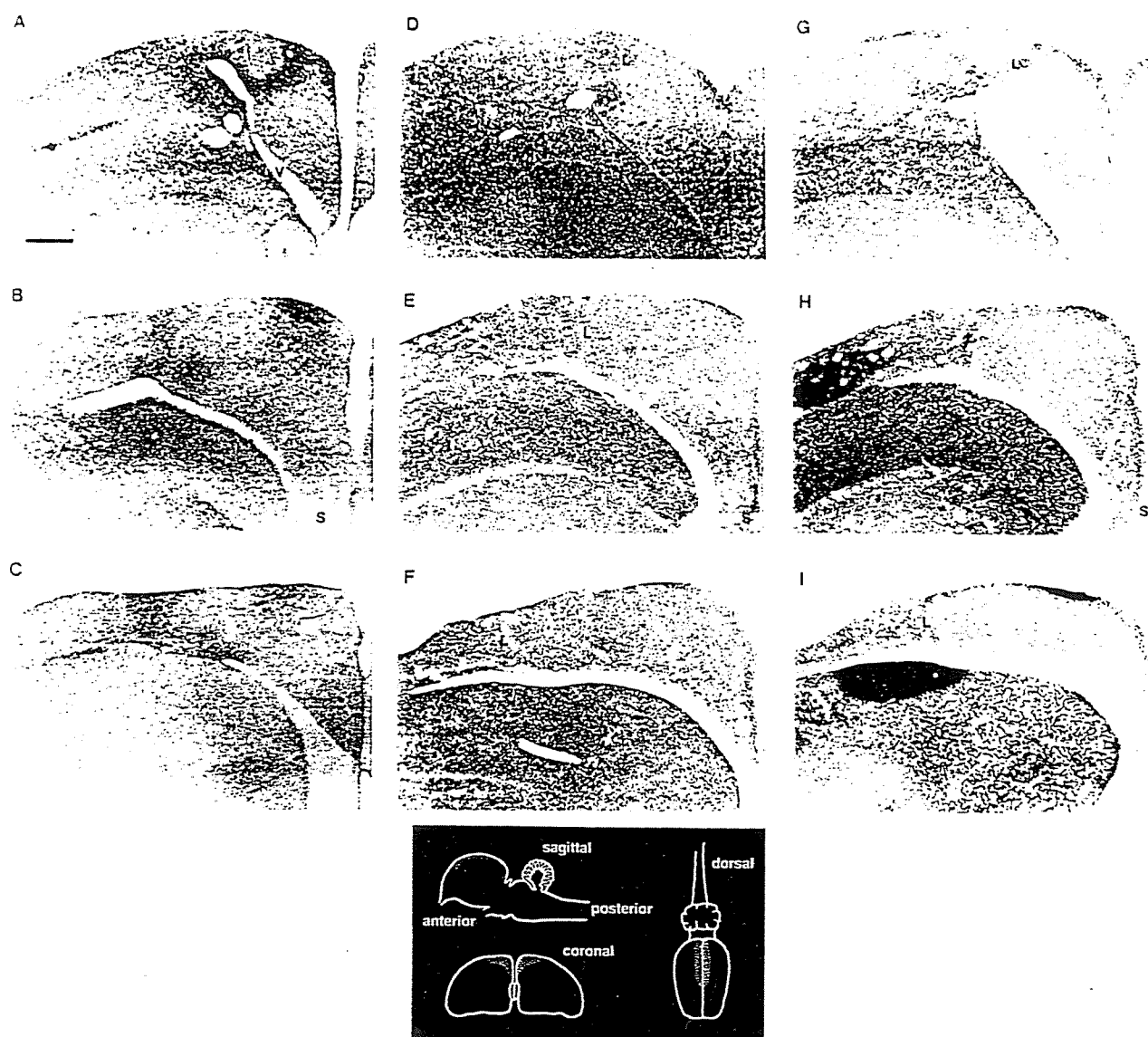


Figure 1. Photomicrographs to illustrate the boundaries of the hippocampal region in the Marsh Tit *Parus palustris* in three different coronal sections (rostral, mid- and caudal, from top to bottom of each column), using three different staining techniques (A–C, calbindin immunocytochemistry; D–F, cresyl violet; G–I, acetylcholinesterase). The middle and right-hand series are from the same bird and are from similar planes of section: the left-hand series is from a different individual, and the plane of section is not identical to that of the other two series. The hippocampal region lies on the dorsomedial surface of the telencephalon above the lateral ventricle (V) and is a paired structure. Here, only the left side is shown although in the top row part of the right side can be seen. The lateral boundary (L) is indicated in calbindin-stained sections by a band of densely stained neuropil, in cresyl violet by a change in cell density and in acetylcholinesterase-stained material by a densely stained band of neuropil. The septohippocampal boundary (S) is indicated by an abrupt change in the staining pattern in all three stains. Quantitative analysis of the volume of the hippocampus stained by the different methods shown here indicates that the three stains reveal the same boundaries. Scale bar = 500 μ m. After Clayton & Krebs (1994a). Inset below shows position of hippocampus (shaded) in sagittal, coronal and dorsal sections.

Comparative studies of the hippocampus have revealed morphological differences between storing and nonstoring species in terms of volume, neuron number and neurochemistry.

Volume

Food-storing species have been shown to have a larger hippocampus, relative to the rest of the telencephalon, in two

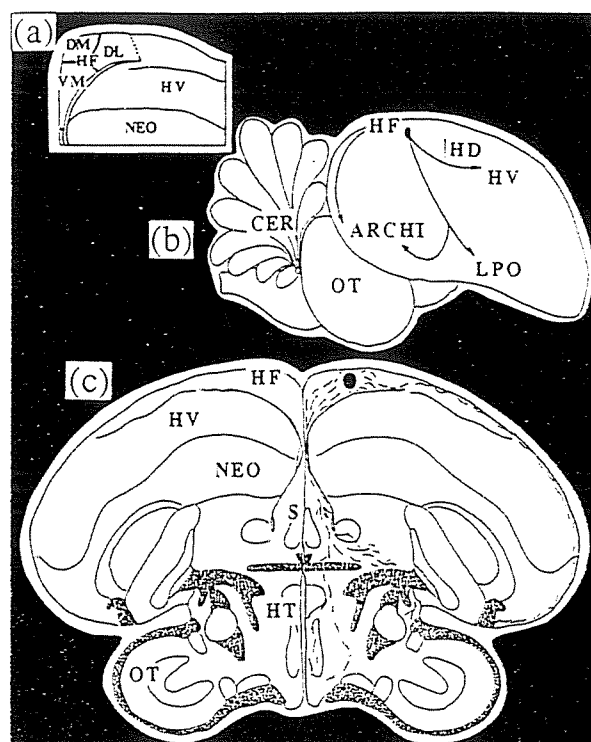


Figure 2. Efferent projections of the Zebra Finch hippocampal formation (HF) revealed by *Phaseolus vulgaris leucoagglutinin* injections. (a) Three subdivisions of HF. DM, dorsomedial injections visualized a local fibre system within the ipsilateral HF. DL, dorsolateral injections show extensive efferent projections to other limbic nuclei, hypothalamic regions and archistriatum. VM, ventral injections gave rise to local axons within ipsilateral HF and fibres passing through the septohippocampal junction and pallial commissure to the contralateral medial septum and ventral HF; (b) Schematic of connections visualized by dorsolateral injections; (c) An example of a dorsolateral injection. Abbreviations: ARCHI, posterior archistriatum; CER, cerebellum; HD, hyperstriatum dorsale; HV, hyperstriatum ventrale; HT, hypothalamus; LPO, lobus parolfactorius; NEO, neostriatum; OT, optic tectum; S, septum (Székely & Krebs 1993b).

studies which have compared species across a range of passerine families (Krebs *et al.* 1989, Sherry *et al.* 1989). As mentioned in the introduction, in these studies the statistical comparisons were based on phylogenetically independent units (families or subfamilies) rather than on individual species, so the key conclusion is that food storers in the parid, corvid and sittid families have a relatively larger hippocampus than do nonstorers in the same family or nonstorers in different families. The molecular phylogeny of Sibley *et al.* (1988) and Sibley and Alquist (1990) suggests that this reflects three independent evolutionary origins of food storing and of hippocampal specialization. These comparative studies also examined potential confounding variables (such as migration) but found no correlations with hippocampal volume. In the study of Krebs *et al.* (1989), food-storing behaviour, coded as a dummy variate in a multiple regression analysis, accounted for about 85% of the remaining variance

in hippocampal volume after effects of brain and body size had been removed. Comparisons within the Paridae and the Corvidae, in which there is interspecific variation in the extent of food-storing behaviour, have shown that there is a graded relationship between the amount of food-storing behaviour and the volume of the hippocampus relative to the rest of the telencephalon (Healy & Krebs 1992b, Basil *et al.* in press, Healy & Krebs in press, Hampton *et al.* 1995). For example, Healy and Krebs (1992b) ranked European corvids according to whether they stored large amounts over long time periods (Jay *Garrulus glandarius*), smaller amounts over shorter time periods (e.g. Magpie *Pica pica*) or stored little or not at all (e.g. Jackdaw *Corvus monedula*). The relative volume of the hippocampus correlates well with this ranking. Finally, comparisons of pairs of species from within the same family, using large sample sizes, have again confirmed that food-storing species have a larger relative hippocampus than do nonstorers (Healy & Krebs 1993, Healy *et al.* 1994).

Neurone number

Associated with the larger volume of the food-storing hippocampus, food storers have a larger number of neurones of the hippocampus than do nonstorers, although there are no differences in neurone density or size. This was shown first by Krebs *et al.* (1990) in comparisons of corvid, parid and sittid-troglodytid species and subsequently confirmed in further comparisons, based on larger sample sizes, of corvids (Healy & Krebs 1993) and parids (Healy *et al.* 1994).

Neurochemistry

Two studies have reported neurochemical differences between storers and nonstorers. Montagnese *et al.* (1993b) compared the pattern of immunoreactive staining of the calcium-binding protein calbindin in storing and nonstoring species of parids and corvids. They reported that the food-storing species studied in both families had a subpopulation of large calbindin immunoreactive cells in the dorsal and medial hippocampus that were not found in nonstorers. Székely and Krebs (1993a) found that two species of food-storing parid had higher levels of histochemically labelled diffuse neuropil containing nitric oxide synthase than that observed in two nonstoring species, as shown in Figure 3. In both of these studies, the link between the neurochemical markers studied and the putative role of the hippocampus in memory processing is only speculative. However, it is thought that calcium-binding proteins, perhaps including calbindin, play a role in second messenger systems. Gene knockout experiments have suggested that the role of a calcium-calmodulin-dependent protein kinase is important in memory formation in mice (Silva *et al.* 1992a,b). Nitric oxide has been proposed as a putative retrograde messenger involved in the maintenance of long-term potentiation (Vincent 1994), a possible cellular mechanism of memory formation.

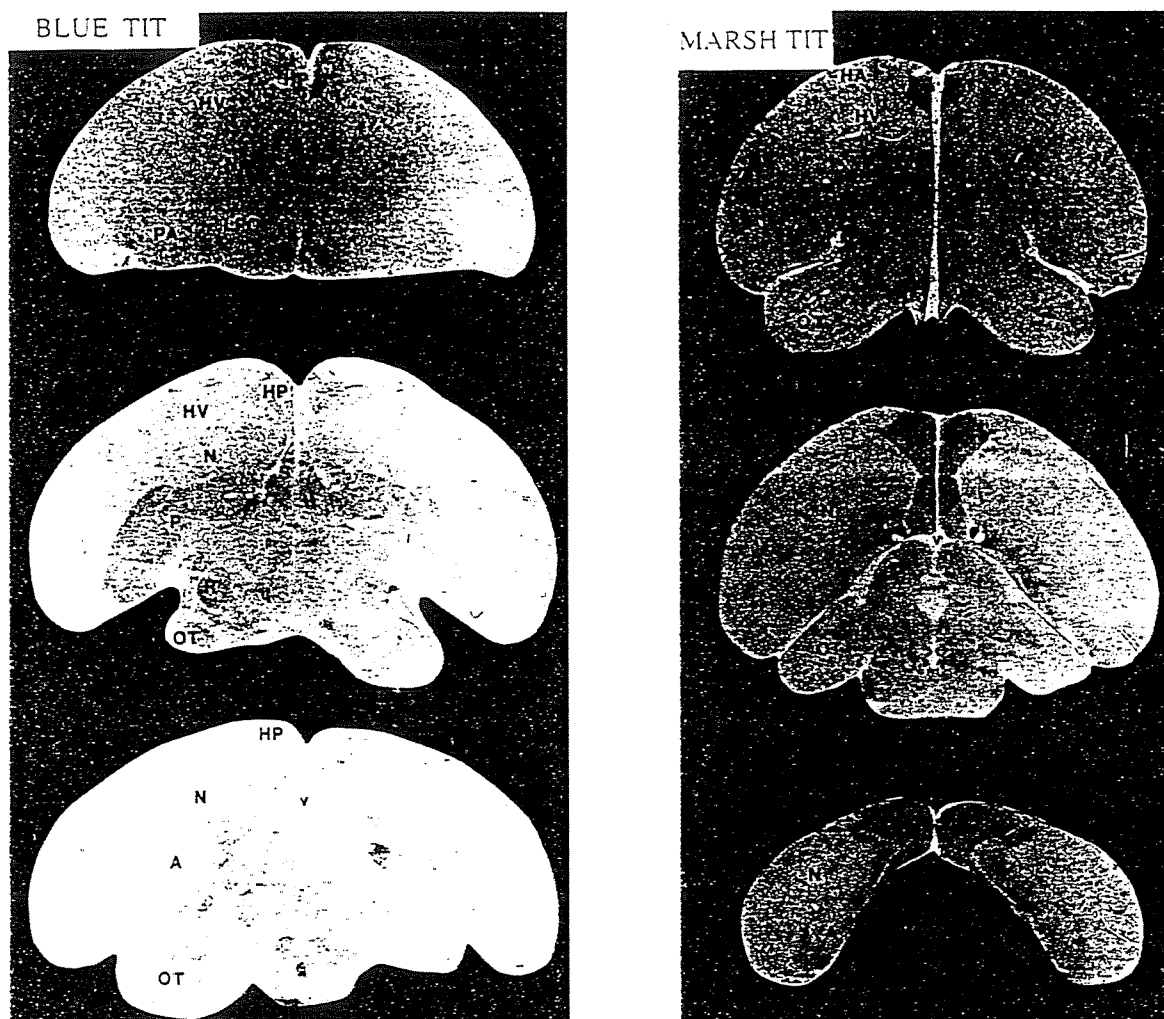


Figure 3. Distribution of NO-synthase in the brain of the Blue Tit (left), a nonstorer, and the Marsh Tit (right), a food storer. The food storer has a densely stained region of neuropil in the hippocampal region (HP), extending ventrally into the septum (S), which is absent in the nonstorer. The staining pattern is shown in three sections for each species. H.A., hyperstriatum accessorium; HV, hyperstriatum ventrale; V, ventricle; OT, optic tectum; N, neostriatum; A, archistriatum; P, paleostriatum; R, nucleus rotundus. Staining was by NADPH-diaphorase histochemistry. In addition to the stained neuropil, NOS⁺ neurones were observed in HP and in H.A. adjacent to the lateral border of HP (Székely & Krebs 1993a).

In conclusion, these comparative studies have shown unequivocally that the behaviour of storing and retrieving food is associated, in phylogenetic terms, both within and between families, with morphological enlargement of the hippocampus. The enlarged hippocampus of food storers has more neurones and possibly different kinds of neurochemicals.

COMPARATIVE STUDIES OF BEHAVIOUR

One-trial associative memory

The search for species differences in memory which might parallel the species differences in brain anatomy, and there-

fore reflect the hypothesized memory specialization of food-storing birds, has not yielded simple answers (see review by Shettleworth *in press*). However, in the last 2 years, a number of promising results have begun to emerge. An important set of methods in these studies is the procedures referred to variously as versions of "one-trial associative memory" (Brodbeck *et al.* 1992, Clayton & Krebs 1993) and the related "delayed matching (or nonmatching) to sample" (Olson 1991, Healy & Krebs 1992a) and 'one-trial recognition memory' (Gaffan 1974, Squire & Zola-Morgan 1988) procedures. The essential feature of all these related tasks is that the animal has to recognize a stimulus (often trial-unique) on the basis of a single exposure and use this information at a later stage to obtain a reward. In a one-trial associative memory task,

a trial consists of two phases. In the first phase, the bird is rewarded for choosing one of a number of available options ('the correct choice'), or it may be presented with a single 'correct' sample. In the second phase of the trial, the bird is offered the correct choice from phase 1 in the presence of alternatives (usually the same set of alternatives that were present in phase 1). The bird obtains a reward by returning to the alternative that was the correct choice in phase 1. Some studies have carried out the trials in a large room in which the bird flies to different feeders to search for rewards, whilst in other studies, the options have been presented as images on a touch screen. Sometimes the correct choice in phase 1 is rewarded, whilst in other studies, it is not. These differences do not appear to affect the results. In a true one-trial associative memory task, the objects or stimuli are used only once (i.e. they are trial-unique), although in many delayed matching or delayed nonmatching to sample experiments (in which the animal has to avoid the stimulus it has seen in phase 1), the same stimuli are used over and over again, rendering the task more difficult because information from one trial can interfere with that from another.

Food storers may be more resistant to interference in memory

One set of studies using this kind of procedure has shown that storing and nonstoring species differ in their ability to discriminate between alternatives presented in phase 1. The crucial experiments involved presenting a set of alternative feeding sites (small wooden blocks) in a large test arena (c. 4×4 m) in phase 1, one of which sites contained a food item hidden behind a cover in a small hole (Krebs *et al.* 1990, Clayton & Krebs 1994c). In phase 2, when the same set of feeders was presented with the same one containing hidden food, food-storing species selectively returned to the place where they found the hidden item in phase 1. Nonstoring species, however, returned to places they had visited in phase 1, whether or not they contained a food item (Fig. 4). As a result, the overall performance of food-storing species (measured by the number of 'incorrect choices' made before the correct choice in phase 2) was higher than that of nonstoring species. If, however, the procedure was modified so that in phase 1 the food reward was visible to the bird as soon as it entered the test arena, so that it visited only the rewarded site in this phase (i.e. the first choice was always the correct choice), the difference between food-storing and nonstoring species in phase 2 disappeared. The birds no longer had to make the discrimination between the rewarded site and the other sites that had been visited in phase 1, so the superior ability of food storers to perform this discrimination was not revealed. Thus, whether or not one observes a difference between storing and nonstoring species depends upon an important detail of the experimental procedure. Once this fact was recognized, it was possible to make sense of a number of earlier, apparently contradictory, results (reviewed in Clayton & Krebs 1994c). This difference in performance between storing and nonstoring species, which has been found

in comparisons of both parid and corvid species, could be interpreted in a number of ways. One suggestion is that food storers are less susceptible than are nonstoring species to interference between the memory of the visit to the correct site and the memory of visits to other sites; in other words, storers can successfully discriminate between a larger number of spatial events, which would be an appropriate memory skill for cache retrieval. This hypothesis links with one (of many) hypotheses about hippocampal function (see reviews by (O'Keefe & Nadel 1978, Cohen & Eichenbaum 1993, Squire 1992, 1993), namely, that it reduces interference between memories (Shapiro & Olton *in press*).

Food storers prefer spatial cues

A second series of experiments using the one-trial associative memory procedure has focussed on the question of the kinds of cues used by storing and nonstoring species to solve the memory problem. If the correct choice in phase 1 is a compound stimulus of spatial and nonspatial (e.g. colour or pattern) cues and in phase 2 the elements of this compound are dissociated (by placing the correct colour in the wrong place), food-storing species respond primarily to the spatial information whilst nonstoring species respond equally to spatial and nonspatial cues. This has been shown in studies of both parids and corvids (Brodbeck 1994, Clayton & Krebs 1994b). In both studies, there was no reward during the dissociation trial, so that it was possible to analyse not only the birds' first but also their subsequent choices because the birds continued to search in the feeders after their first unsuccessful choice. This analysis showed that storers preferentially go to the correct feeder on the second look, having chosen the correct spatial location on the first look, whilst nonstoring species choose both location and feeder equally on first and second visits.

A variety of mechanisms could explain this species difference. It could be a result of differences in attention to spatial and nonspatial cues, a difference in memory for these cues or a difference in response rule (preference), given that spatial and nonspatial memories are equally encoded. By presenting only one cue at a time (either spatial or nonspatial) and testing to see if the differences between storers and nonstoring species persist, it should be possible to gain some insight into the relative importance of these three processes.

Monocular occlusion

Clayton and Krebs (1993) used monocular occlusion to separate out presentation of spatial and nonspatial components of the cue. They found that when the right eye was covered during phase 1 of the trial, both storers and nonstoring species responded only to the spatial information in a dissociation, whilst with the left eye covered, both responded to only nonspatial cues. The most straightforward interpretation of these results is that the two eye systems (which input largely into the contralateral hemisphere) memorize different aspects of the stimulus and that in storers right eye information

(nonspatial) is normally dominated by left eye information (spatial), whilst in nonstorsers neither eye system dominates the other. In short, the results support the hypothesis that the difference between storers and nonstorers arises from a difference in the way that spatial and nonspatial information compete during encoding of memory.

Touch screens

Using touch screens, D.R. Brodbeck (unpubl. PhD thesis, University of Toronto) exposed birds to a compound stimulus (space plus colour) in phase 1, and then presented them with either the compound or space or colour cues on test trials in phase 2. Looking first at comparisons within a species, the food-storing Black-capped Chickadee *Parus atricapillus* performed as well with space alone as it did with the compound in phase 2, whilst with colour alone, performance was not above chance. In contrast, the nonstoring Dark-eyed Junco *Junco hyemalis* performed less well with space or with colour than with the compound, and performance with these two kinds of stimuli alone did not differ. Comparison between species in absolute level of performance showed only minor differences: the two species were similar with the compound in phase 2; storers were slightly better with space alone and worse with colour alone. Since the differential response to spatial cues persisted when only one cue at a time was presented in phase 2 of a trial, the difference cannot be explained purely as a response preference: it must have arisen from differences in attention and/or memory during encoding. This result is consistent with the conclusions of the monocular occlusion study.

Olson's (1991) results showed differences in performance (maximum retention interval) between the Clark's Nutcracker, which relies extensively on stored food and stores a large number of items, and the Scrub Jay *Aphelocoma coerulescens*, which is a less intensive storer, on a spatial delayed nonmatching to sample task. This result is consistent with the idea that memory, rather than attention, is involved, because in these experiments there was no competition between spatial and nonspatial cues, although it can always be argued that the Scrub Jays treated the experiment as though it were a compound spatial and nonspatial task. More recently, Kamil *et al.* (in press) reported that in a comparison of four corvids, Clark's Nutcracker, Pinyon Jay *Gymnorhinus cyanocephalus*, Scrub Jay and Mexican Jay *Aphelocoma ultramarina*, the nutcracker performed the spatial delayed nonmatching task at above chance with longer retention intervals than did the other three species, whilst in a colour version of the task there were no species differences. This again supports the idea that, at least in the corvids, the difference between species is in spatial memory.

Radial maze

The one-trial associative memory procedure is not the only technique that has been used for comparing storing and nonstoring species. Open field analogues of the radial-arm

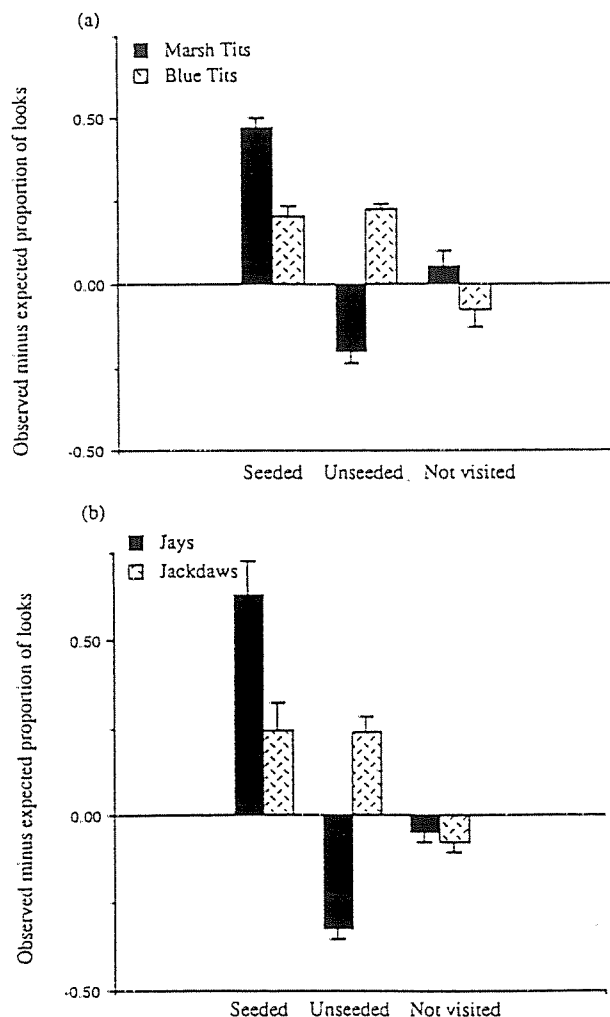


Figure 4. (a) Experiment 1 on Marsh Tits (food storer) and Blue Tit (nonstorer); (b) Experiment 2 on Jays (food storer) and Jackdaws (nonstorer). Mean and standard error of the observed minus expected proportion of visits in phase 2 to the three categories of site, based on behaviour in phase 1 of the "hidden" version of the task. See text for further information. After Clayton & Krebs (1994c).

maze have been used by Hilton and Krebs (1990) to compare the performance of storing parids (Coal Tit *Parus ater* and Marsh Tit *Parus palustris*) and nonstoring parids (Great Tit and Blue Tit) and by Kamil *et al.* (in press) to compare different species of corvids that rely on storing to different extents (Clark's Nutcracker, Pinyon Jay, Scrub Jay and Mexican Jay). The radial maze task tests an animal's ability to discriminate between sites it has already visited and sites that it has not yet visited within a trial. Both the parid and corvid studies suggest that, under certain conditions, storers perform better than nonstorers. In a typical radial maze study, the end of each arm of the maze is baited with a single reward. An animal with perfect memory for the sites it has visited will visit each arm in sequence, collecting all the

rewards without a single revisit. One procedure frequently used is to offer the animal the opportunity to visit half the baited arms, which are selected by the experimenter rather than by the animal, in order to eliminate simple response rules such as "turn left after each choice", and then, after a retention interval, to offer the animal a free choice of all the arms, including the previously visited ones. In this case, correct choices in the second part of the trial imply avoiding those arms that were visited in the first half. By varying the retention interval between the first and second half, it is possible to see how memory for already visited sites decays with time. The radial-arm maze was originally developed for study with rats, where the maze is constructed with wooden tunnels, typically eight, radiating out from a central start box like the spokes of a wheel. Because birds do not readily run along tunnels, an open field analogue of the maze is used in which the baited "arms" consist of feeding sites distributed along the circumference of the circle. In the studies of parids and of corvids, two measures of performance were used: initial acquisition of the task with free choices of all eight "arms", and performance as a function of retention interval in the procedure with forced choices followed by free choice of all arms after the retention interval. The corvid study suggested that the two species relying most intensively on storing acquired the task more rapidly and/or to a higher level of accuracy and that the difference between species diminished with increasing retention interval. The parid study showed no differences between storers and nonstorers in learning the task or in asymptotic performance, but a trend towards better performance by storers emerged with increasing retention (up to 24 h between the first and second half of the trial).

The radial maze, the one-trial associative memory task and the delayed matching/nonmatching to sample procedure all require the animal to form a memory on the basis of a single event (visit, reward, observation of a stimulus). This kind of task may be contrasted with one in which the animal is repeatedly exposed to the same stimulus reward pair over many trials and gradually improves its performance (a reference memory task). S.C. Hilton (unpubl. DPhil thesis, Oxford University) found that two food-storing species of parid actually performed less well in spatial reference memory task than did two nonstorers (i.e. they acquired the spatial discrimination more slowly and/or reached a lower asymptotic level of performance).

Summary

To summarize this section, although there is still a great deal of work to be done, the tentative conclusion can be drawn that food-storing species differ from nonstorers in a number of ways: (1) they have a preference for responding to spatial cues in one-trial associative memory tasks whilst nonstorers do not, (2) they are better able than are nonstorers to discriminate between sites where they have obtained a reward and sites they have merely visited, (3) under some conditions they are better able to avoid revisiting depleted sites in a

radial maze and (4) they may perform less well than nonstorers in spatial reference memory. Whether or not these results can be united under a single hypothesis (e.g. greater reliance on spatial cues) for a difference between storers and nonstorers is not yet clear. The results do indicate that the notion of storers having overall a "better memory" is too simplistic. In that they respond preferentially to spatial cues, storers could be said to have a less good memory than nonstorers for nonspatial cues. The current emphasis is to think in terms of differences rather than overall superiority in memory.

ONTOGENY

The first hint that the species differences in hippocampal volume, described in the introduction, may depend on early experience came from two comparative studies. Healy and Krebs (1993), studying Jackdaws and Magpies *Pica pica*, and Healy *et al.* (1994), studying Blue Tits and Marsh Tits, both observed that storing and nonstoring species did not differ in relative hippocampal volume as nestlings, whereas they did differ as adults. One interpretation of this was that the difference in the brain emerged only after the onset of food-storing behaviour. This hypothesis has been tested in detail by studying the development of both food-storing behaviour and hippocampal anatomy in hand-raised Marsh Tits (Clayton 1992, 1994, 1995, Clayton & Krebs, 1994a). The principal results of these extensive studies may be summarized in the following four points.

The onset of food storing is sudden

In hand-raised Marsh Tits, food storing started on day 44 post-fledging. The process of onset, which appears to be sudden, involved a suite of changes, including the ability to handle seeds in the correct orientation for storing, the frequency of storing food as opposed to inappropriate objects and the number of items stored (Clayton 1992). The onset appeared to be age dependent rather than experience dependent, as shown by comparing birds given the opportunity to store every day with those given exposure only every third day between nutritional independence (day 35) and day 44. If birds were deprived of the opportunity to store and retrieve food until beyond day 44, food-storing behaviour appeared rapidly after the opportunity was presented at a later stage. This suggests that there is no 'sensitive period' during which experience has to be obtained at least within the range tested, which was days 44, 59, 83 and 115 (see Appendix for experimental design).

Food-storing experience influences hippocampal growth

By depriving hand-raised Marsh Tits of the opportunity to store food until different ages and by measuring the volume of the hippocampus after different amounts of deprivation

and/or experience. Clayton and Krebs (1994a) showed that experience of storing and retrieving caused an increase in hippocampal volume and in neurone number, whilst absence of experience caused attrition of hippocampal volume accompanied by loss of cells (Fig. 5). The growth effect appeared to be a threshold: above a certain minimum amount of food-storing experience, cumulative experience had no further effect. In contrast, the attrition effect appeared to be cumulative: the longer the birds had gone without food-storing experience, the smaller the hippocampus became.

Hippocampal growth may occur in preparation for the increased memory demands

In a follow-up experiment, Clayton (in press a) tested whether hippocampal growth precedes or accompanies the changes in food-storing behaviour in hand-raised Marsh Tits. Experienced birds were provided with the opportunity to store and retrieve food every third day from day 35 post-hatch, and the hippocampal volumes of these birds were compared with those of age-matched controls at three different stages (days 41, 47 and 56 post-hatch). Experienced birds had larger absolute and relative hippocampal volumes than did controls at all stages of the experiment, even before the increase in food-storing intensity on day 44. These results suggest that the one or two seeds stored before day 44 may have been sufficient to stimulate the growth of the hippocampus and that growth of the hippocampus may occur in preparation for the increased memory demands associated with the sharp increase in food storing.

Hippocampal growth is also affected by other memory experience

In a further series of experiments, Clayton (1995) exposed both hand-raised Marsh Tits and Blue Tits to the one-trial associative memory task referred to earlier from day 35 to day 200. These experiments showed that in Marsh Tits experience of the one-trial associative memory task triggered hippocampal growth in exactly the same way as food-storing experience, whilst in Blue Tits there was no effect of experience on hippocampal growth, nor did hand-raised Blue Tits differ from wild-caught adult birds. This suggests two tentative conclusions. First, memory experiences other than those of storing and retrieving food are sufficient to cause changes in the Marsh Tit hippocampus. Second, the food-storing Marsh Tit may differ from the nonstoring Blue Tit in having the potential of the hippocampus to respond to this experience.

These remarkable results pose important questions about exactly how and what kinds of experience influence brain morphology. The effects are specific in the sense that they influence the hippocampus but not a 'control' area, the ectostriatum, or the telencephalon as a whole. They are also specific in the sense that the experience, whether of food storing or of the one-trial associative memory task, seems

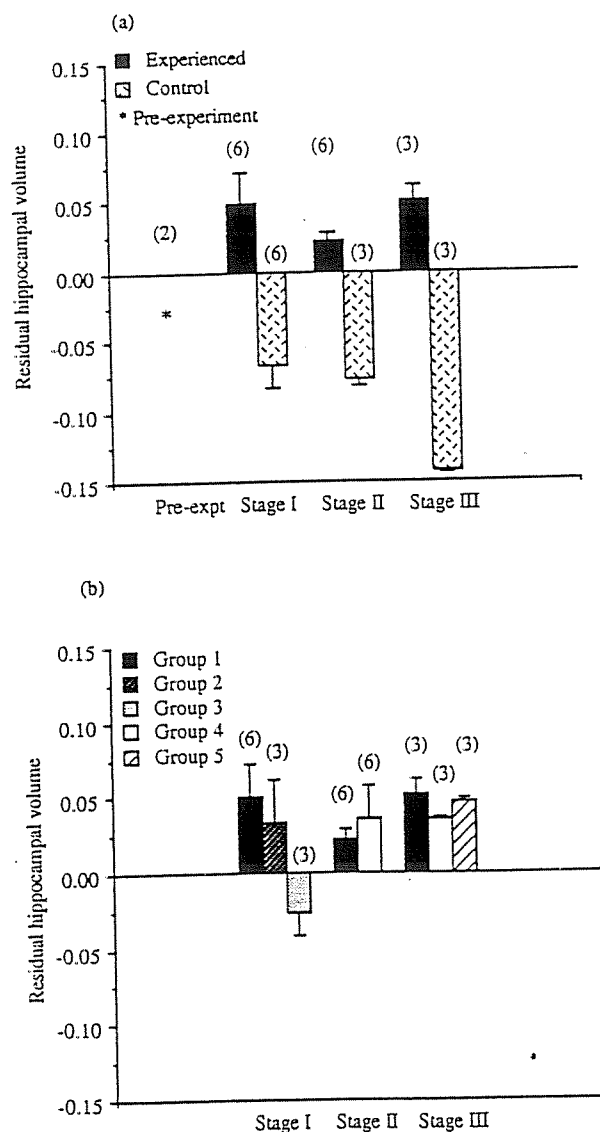


Figure 5. (a) Mean values (\pm s.e.) of relative hippocampal volume of experienced and control Marsh Tits at the three different stages of the experiment (see Appendix). The values are the residuals from a regression of hippocampal volume on telencephalon volume. Solid histograms represent birds that had food-storing/retrieval experience once every 3 days, and open histograms represent birds that had control experience every 3 days (i.e. the figure shows all 35 birds sacrificed from groups 1, 4, 5 and 6 in Appendix). The pre-experimental baseline birds are indicated by an asterisk. Sample sizes in each group are shown by numbers in brackets; (b) Mean values (\pm s.e.) of birds with different levels of experience (groups 1–5 in Appendix). Different shading patterns refer to the different treatments in Appendix. After Clayton & Krebs (1994a).

to be specific to memory. In terms of visual stimulation, motor experience and diet, the control and experienced birds were as nearly identical as possible: control birds were allowed to enter the same food-storing arena for the same

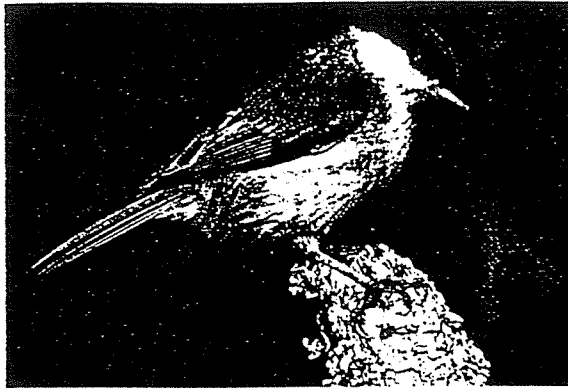


Figure 6. The Marsh Tit is a well-known food storer amongst the Paridae.

amount of time on each trial as the experienced group, but they were given powdered seed which they could not store. If, indeed, as present evidence suggests, this series of studies has shown that a specific kind of memory experience can stimulate growth of a specific area of the brain concerned with processing of this memory, this will be an important new effect in vertebrate brain-behaviour studies.

Recent work on the development of food storing and retrieval in a closely related species, the Coal Tit, showed that the development of food storing is very similar to that reported for Marsh Tits (A.R. Jolliffe, pers. comm.), and experiments are in progress to test whether or not the experience of storing and retrieving caches is associated with parallel changes in hippocampal volume (N.S. Clayton, pers. obs.).

The studies of Marsh Tits have shown that within the age range of 44–115 days, there is no "sensitive period" for either behavioural or brain development. However, recent work by Cristol (1994) suggests that much older birds may not respond to experience or deprivation of food-storing experience in the same way. Cristol showed that 4-year-old Willow Tits *Parus montanus* given intensive experience of food storing and retrieval over a 4-week period did not differ in relative hippocampal volume from a control group deprived of food-storing experience during the same period. At the moment, it is not clear whether this was because the Willow Tits were much older than the birds studied by Clayton or whether, having once had experience of storing and retrieving food (as all the Willow Tits in Cristol's experiment had done), the changes in the hippocampus were fixed and irreversible. Further work is needed to evaluate these alternatives.

SEASONAL CHANGES IN FOOD STORING AND THE HIPPOCAMPUS

Both field observations and laboratory studies suggest that food-storing behaviour in parids has an annual cycle: it is more marked in the autumn and winter than in the spring

and early summer (Hafthorn 1956, Ludescher 1980), although some storing behaviour persists throughout the year. In some corvid species which rely on seed crops to build up their stores, the seasonality may be even more marked (Bossemma 1979). Three studies have recently begun to investigate the question of whether or not these seasonal changes in food-storing behaviour are accompanied by changes in hippocampal anatomy. Such changes might be expected from extrapolation of the ontogenetic studies referred to in the last section, assuming that the effects are not only applicable to young birds, which the results of Cristol would argue against. A second reason for looking for seasonal effects is by analogy with the findings in song nuclei (Nottebohm 1981) in which seasonal volumetric changes were observed, although these changes probably were not directly linked to behavioural changes but may have resulted from changing levels of circulating steroid hormones associated with reproduction (Brenowitz 1992). On the other hand, the species that store food in the autumn (such as Jays) still use their stores the following spring and summer (Bossemma 1979), so that the circuitry involved in retrieval has remained intact even though the storing phase is seasonal. If the hippocampus is used in both storage and retrieval of memories, there would be less reason to predict seasonal changes in anatomy paralleling the changes in storing behaviour.

Shettleworth *et al.* (1995) used photoperiod manipulation to drive Black-capped Chickadees into 'autumn' or 'spring' conditions. The 'autumn' birds showed a substantially higher level of food storing than did the 'spring' birds. In a subsequent study, it was shown that these photoperiodically driven differences in food-storing behaviour were not accompanied by changes in relative hippocampal volume (J.R. Krebs, N.S. Clayton, R.R. Hampton & S.J. Shettleworth, unpubl. obs.). In parallel studies of hippocampal volume in wild-caught birds, however, Smulders *et al.* (in press) observed volumetric changes in the hippocampus, but not in the telencephalon as a whole, associated with season. The hippocampus was larger, relative to the rest of the brain, in October than at other times of year. Barnea and Nottebohm (1993) reported an increased level of neurogenesis in wild Black-capped Chickadees during October, when food-storing activity increased. Because this study did not include a control species, it is not clear whether the changes were linked to food storing or reflected a more general seasonal effect of, for example, changes in nutritional and/or hormonal status. At the moment, these studies of seasonal changes pose as many questions as they answer; clearly this is an area ripe for further study.

CONCLUSIONS

This brief review shows how the brain in food-storing species is providing a productive model system for studying a variety of problems of general conceptual interest.

(1) *The evolutionary specialization of the avian brain.* The phylogenetic analysis shows how an ecological selection

pressure can lead to changes in (or at least be associated with changes in) brain morphology.

(2) *Comparative cognition.* As many authors have pointed out in recent years (e.g. Sherry *et al.* 1993a, Squire 1993), the evolution of cognitive capacities in animals, including man, should not be viewed as a simple linear phylogenetic progression, but rather as a radiating suite of adaptive traits. In spite of this seemingly reasonable point of view, there are remarkably few cases in which it has been possible to show that a cognitive capacity such as memory has evolved as an adaptation to a particular ecological niche (MacPhail 1993). Food-storing memory may turn out to be the paradigmatic case in relation to ecological selection pressures.

(3) *Neural substrates of memory in birds.* If the programme of comparative brain and behaviour research described earlier is successful, it could provide a clear case in which it is possible to relate particular differences in neural substrates to differences in memory, hence providing a novel approach to the problem of how and where memory is encoded in the brain.

(4) *Plasticity of the brain in relation to experience.* The ontogenetic effects of experience on recruitment and loss of neurones may in the near future provide a remarkable system in which to study these processes, which are of general biological significance, at a whole range of levels from the behavioural to the molecular.

REFERENCES

- Ariens Kappers, C.U., Huber, G.C. & Crosby, E.C. 1936. The Comparative Anatomy of the Nervous System of Vertebrates Including Man, Vol. 2. London: Macmillan.
- Balda, R.P. & Kamil, A.C. 1992. Long-term spatial memory in Clark's Nutcracker, *Nucifraga columbiana*. *Anim. Behav.* 44: 761–769.
- Barnea, A. & Nottebohm, F. 1993. Seasonal patterns of neurogenesis in the hippocampus of adult food-caching birds and their possible relation to spatial learning. *Soc. Neurosci. Abstr.* 19: 1448.
- Basil, J.A., Kamil, A.C., Balda, R.P. & Fite, K.V. In press. Differences in hippocampal volume among food storing corvids. *Brain Behav. Evol.*
- Bingman, V.P. 1993. Vision, cognition, and the avian hippocampus. In Zeigler, H.P. & Bischof, H.-J. (eds) *Vision, Brain and Behavior in Birds*: 391–408. Cambridge, Mass.: MIT Press.
- Bossemma, I. 1979. Jays and oaks: An eco-ethological study of a symbiosis. *Behaviour* 70: 1–117.
- Brenowitz, E. 1992. Seasonal changes in avian song nuclei. *Proc. Int. Congr. Neuroethol. Abstr.* III: 58.
- Brodbeck, D.R. 1994. Memory for spatial and local cues: A comparison of a storing and a nonstoring species. *Anim. Learn. Behav.* 22: 119–133.
- Brodbeck, D.R., Burack, O.R. & Shettleworth, S.J. 1992. One-trial associative memory in Black-capped Chickadees. *J. Exp. Psychol.: Anim. Behav. Processes* 18: 335–340.
- Casini, G., Bingman, V.P. & Bagnoli, P. 1986. Connections of the Pigeon dorsomedial forebrain studied with WGA-HRP and ³H proline. *J. Comp. Neurol.* 245: 454–470.
- Clayton, N.S. 1992. The ontogeny of food storing and retrieval in Marsh Tits. *Behaviour* 122: 11–25.
- Clayton, N.S. 1994. The role of age and experience in the behavioural development of food-storing and retrieval in Marsh Tits, *Parus palustris*. *Anim. Behav.* 47: 1435–1444.
- Clayton, N.S. 1995. Development of memory and the hippocampus: Comparison of food-storing and non-storing birds on a one-trial associative memory task. *J. Neurosci.* 15: 2796–2807.
- Clayton, N.S. In press. Development of food-storing and the hippocampus in juvenile Marsh Tits (*Parus palustris*). *Behav. Brain Res.*
- Clayton, N.S. & Krebs, J.R. 1993. Lateralization in Paridae: Comparison of a storing and a non-storing species on a one-trial associative memory task. *J. Comp. Physiol. A* 171: 807–815.
- Clayton, N.S. & Krebs, J.R. 1994a. Hippocampal growth and attrition in birds affected by experience. *Proc. Natl. Acad. Sci.* 91: 7410–7414.
- Clayton, N.S. & Krebs, J.R. 1994b. Memory for spatial and object-specific cues in food-storing and non-storing birds. *J. Comp. Physiol. A* 174: 371–379.
- Clayton, N.S. & Krebs, J.R. 1994c. One-trial associative memory: Comparison of food-storing and non-storing species of birds. *Anim. Learn. Behav.* 22: 366–372.
- Cohen, N.J. & Eichenbaum, H. 1993. *Memory, amnesia and the hippocampal system*. Cambridge, Mass.: MIT Press.
- Craigie, E.H. 1935. The hippocampal and parahippocampal cortex of the Emu (*Dromiceius*). *J. Comp. Neurol.* 61: 563–592.
- Cristol, D.A. 1994. No effect of food-storing experience on hippocampal volume in adult Willow Tits. *Abstr. Int. Orn. Congr. XXI. J. Orn.* 135: 711.
- Erichsen, J.T., Bingman, V.P. & Krebs, J.R. 1991. The distribution of neuropeptides in the dorsomedial telencephalon of the Pigeon (*Columba livia*): A basis for regional subdivisions. *J. Comp. Neurol.* 314: 478–492.
- Gaffan, D. 1974. Recognition impaired and association intact in the memory of monkeys after transection of the fornix. *J. Comp. Physiol. Psychol.* 86: 1100–1109.
- Gill, F.B., Sheldon, F.H. & Slikas, B. 1994. DNA phylogenies of titmice (Paridae). *Abstr. Int. Orn. Congr. XXI. J. Orn.* 135: 576.
- Hafthorn, S. 1954. Contribution to the food biology of tits, especially about storing of surplus food. Part I. The Crested Tit (*Parus c. cristatus* L.). *K. Nor. Vidensk. Selsk. Skr.* 4: 1–122.
- Hafthorn, S. 1956a. Contribution to the food biology of tits especially about storing of surplus food. Part II. The Coal Tit (*Parus a. ater* L.). *K. Nor. Vidensk. Selsk. Skr.* 2: 1–52.
- Hafthorn, S. 1956b. Contribution to the food biology of tits especially about the storing of surplus food. Part III. The Willow Tit (*Parus atricapillus* L.). *K. Nor. Vidensk. Selsk. Skr.* 3: 1–79.
- Hampton, R.R., Sherry, D.F., Shettleworth, S.J., Khurgel, M. & Ivy, G. 1995. Hippocampal volume and food storing in three species of parid. *Brain Behav. Evol.* 45: 54–61.
- Harvey, P.H. & Pagel, M. 1991. *The Comparative Method in Evolutionary Biology*. Oxford: Oxford University Press.
- Healy, S.D. & Krebs, J.R. 1992a. Delayed-matching-to-sample by Marsh Tits and Great Tits. *Q. J. Exp. Psychol.* 45B: 33–47.
- Healy, S.D. & Krebs, J.R. 1992b. Food storing and the hippocampus in corvids: Amount and volume are correlated. *Proc. R. Soc. Lond. B* 248: 241–245.
- Healy, S.D. & Krebs, J.R. 1993. Development of hippocampal specialisation in a food-storing bird. *Behav. Brain Res.* 53: 127–131.
- Healy, S.D. & Krebs, J.R. In press. Food storing and the hippocampus in Paridae. *Brain Behav. Evol.*

- Healy, S.D., Clayton, N.S. & Krebs, J.R. 1994. Development of hippocampal specialisation in two species of tit (*Parus* spp.). *Behav. Brain Res.* 61: 23-28.
- Hilton, S.C. & Krebs, J.R. 1990. Spatial memory of four species of *Parus*: Performance in a radial maze. *Q. J. Exp. Psychol.* 42: 345-368.
- Hitchcock, C.L. & Sherry, D.F. 1990. Long-term memory for cache sites in the Black-capped Chickadee. *Anim. Behav.* 40: 701-712.
- Kamil, A.C. 1994. The evolution of animal intelligence: Avian studies. *Abstr. Int. Orn. Congr. XXI. J. Orn.* 135: 454.
- Kamil, A.C. & Balda, R.P. 1994. Spatial memory in seed-caching corvids. *Abstr. Int. Orn. Congr. XXI. J. Orn.* 135: 444.
- Kamil, A.C., Balda, R.P. & Olson, D.J. In press. Performance of four seed-caching corvid species in the radial-arm maze analog. *J. Exp. Psychol.*
- Karten, H.J. & Hodós, W. 1967. *A Stereotaxic Atlas of the Brain of the Pigeon (Columba livia)*. Baltimore, Md.: Johns Hopkins Press.
- Krebs, J.R., Erichsen, J.T. & Bingman, V.P. 1991. The distribution of neurotransmitters and neurotransmitter-related enzymes in the dorsomedial telencephalon of the Pigeon (*Columba livia*). *J. Comp. Neurol.* 314: 467-477.
- Krebs, J.R., Hilton, S.C. & Healy, S.D. 1990. Memory in food-storing birds: Adaptive specialisation in brain and behaviour. In Edelman, G.M., Gall, W.E. & Cowan, M.W. (eds) *Signal and Sense: Local and global order in perceptual maps*: 475-498. New York: Wiley-Liss.
- Krebs, J.R., Sherry, D.F., Healy, S.D., Perry, V.H. & Vaccarino, A.L. 1989. Hippocampal specialization of food-storing birds. *Proc. Natl. Acad. Sci. USA* 86: 1388-1392.
- Krushinskya, N.L. 1966. Some complex forms of feeding behaviour of Nutcracker, *Nucifraga caryocatactes*, after removal of old cortex. *Zh. Evol. Biochim. Fiziol.* 11: 563-568.
- Ludescher, F.-B. 1980. Fressen und Verstecken von Sämereien bei der Weidermeise *Parus montanus* im Jahreserlauf unter konstanten Ernährungsbedingungen. *Ökol. Vogel.* 2: 135-144.
- MacPhail, E. 1993. *The Neuroscience of Animal Intelligence. From seahorse to seahorse*. New York: Columbia University Press.
- Molla, R., Rodriguez, J., Calvet, S. & Garcia-Verdugo, J.M. 1986. Neuronal types of the cerebral cortex of the adult chicken (*Gallus gallus*): A Golgi study. *J. Hirnforsch.* 27: 381-390.
- Montagnese, C.M., Geneser, F.A. & Krebs, J.R. 1993a. Histochemical distribution of zinc in the brain of the Zebra Finch *Taenopygia guttata*. *Anat. Embryol.* 188: 173-187.
- Montagnese, C.M., Krebs, J.R. & Meyer, G. In press. The hippocampus and related structures of the Zebra Finch, *Taenopygia guttata*: A Golgi study. *Cell Tissue Res.*
- Montagnese, C.M., Krebs, J.R., Székely, A.D. & Csillag, A. 1993b. A subpopulation 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.* 614: 291-300.
- Nottebohm, F. 1981. A brain for all seasons: Cyclical anatomical changes in song-control nuclei of the canary brain. *Science* 214: 1368-1370.
- O'Keefe, J. & Nadel, L. 1978. *The Hippocampus as a Cognitive Map*. Oxford: Oxford University Press.
- Olson, D.J. 1991. Species differences in spatial memory among Clark's Nutcrackers, Scrubjays and Pigeons. *J. Exp. Psychol.: Anim. Behav. Proc.* 17: 1363-1376.
- Pravosudov, V. V. 1985. Search for and storage of food by *Parus cinctus labbonicus* and *P. montanus borealis*. *Zool. Zh.* 64: 1036-1043.
- Rose, M. 1914. Über die cytoarchitektonische Gliederung des Vorderhirns der Voegel. *J. Psychol. Neurol.* 21: 278-352.
- Shapiro, M.L. & Olton, D.S. In press. Hippocampal function and interference. In Schacter, D.L. & Tulvina, E. (eds) *Memory Systems*. Cambridge, Mass.: MIT Press.
- Sherry, D.F. & Vaccarino, A.L. 1989. Hippocampus and memory for food caches in Black-capped Chickadees. *Behav. Neurosci.* 103: 308-318.
- Sherry, D.F., Jacobs, L.F. & Gaulin, S.J.C. 1993a. Reply to Squire. *Trends Neurosci.* 16: 57.
- Sherry, D.F., Forbes, M.R.L., Khurgel, M. & Ivy, G.O. 1993b. Females have a larger hippocampus than males in the brood-parasitic Brown-headed Cowbird. *Proc. Natl. Acad. Sci.* 90: 7839-7843.
- Sherry, D.F., Vaccarino, A.L., Buckenham, K. & Herz, R.S. 1989. The hippocampal complex of food-storing birds. *Brain Behav. Evol.* 34: 308-317.
- Shettleworth, S.J. In press. Memory in Food Storing Birds: From the field to the skinner box. *NATO Maratea Conference Proceedings*.
- Shettleworth, S.J., Hampton, R.R. & Westwood, R.P. 1995. Effects of season and photoperiod on food-storing by Black-capped Chickadees *Parus atricapillus*. *Anim. Behav.* 49: 989-998.
- Shimizu, T. & Karten, H.J. 1990. Immunohistochemical analysis of the visual wulst of the Pigeon (*Columba livia*). *J. Comp. Neurol.* 300: 346-369.
- Showers, M.J.C. 1982. Telencephalon of birds. In Crosby, E.C. & Schnitzlein, H.N. (eds) *Comparative Correlative Neuroanatomy of the Vertebrate Telencephalon*: 218-246. New York: Macmillan.
- Sibley, G.C. & Ahlquist, J.E. 1990. *Phylogeny and Classification of Birds: A study in molecular evolution*. New Haven and London: Yale University Press.
- Sibley, G.C. & Monroe, B.L., Jr. 1990. *Distribution and Taxonomy of Birds of the World*. New Haven and London: Yale University Press.
- Sibley, G.C., Ahlquist, J.E. & Munroe, B.L., Jr. 1988. A classification of the living birds of the world based on DNA-DNA hybridization studies. *Auk* 105: 409-424.
- Silva, A.J., Stevens, C.F., Tanegawa, S. & Wang, Y. 1992a. Deficient hippocampal long-term potentiation in α -calcium-calmodulin kinase II mutant mice. *Science* 257: 201-206.
- Silva, A.J., Paylor, R., Wehner, J.M. & Tanegawa, S. 1992b. Impaired spatial learning in α -calcium-calmodulin kinase II mutant mice. *Science* 257: 206-211.
- Smulders, T.V., Sasson, A.D. & DeVoogd, T.J. In press. Seasonal changes in telencephalon volume in a food-storing Black-capped Chickadee. *J. Neurobiol.*
- Squire, L. 1992. Memory and the hippocampus: A synthesis from findings with rats, monkeys and humans. *Psychol. Rev.* 99: 195-231.
- Squire, L. 1993. The hippocampus and spatial memory. *Trends Neurosci.* 16: 56-57.
- Squire, L. & Zola-Morgan, S. 1988. Memory, brain systems and behaviour. *Trends Neurosci.* 11: 170-175.
- Stevens, T.A. & Krebs, J.R. 1986. Retrieval of stored seeds by Marsh Tits *Parus palustris* in the field. *Ibis* 128: 513-515.
- Swanberg, P.O. 1951. Food storage, territory and song in the Thick-billed Nutcracker. *Proc. Int. Orn. Congr.* X: 545-554.
- Székely, A.D. & Krebs, J.R. 1993a. More hippocampal nitric oxide synthase in food-storing than in non-storing birds. *Soc. Neurosci. Abstr.* 19: 1448.
- Székely, A.D. & Krebs, J.R. 1993b. Target structures of hippocam-

- pal projections in the Zebra Finch brain. Brain Res. Assoc. Abstr. 10: 21.
- Tomback, D.F. 1980. How nutcrackers find their stores. Condor 82: 10-19.
- Vander Wall, S.B. 1990. Food Hoarding in Animals. Chicago: University of Chicago Press.
- Vincent, S.R. 1994. Nitric oxide: A radical neurotransmitter in the central nervous system. Prog. Neurobiol. 42: 129-160.

APPENDIX

The experimental design

The purpose of the experiment was to expose Marsh Tits to their first experience of storing and retrieving food at different ages, whilst holding other aspects of experience constant (see text for a description of experienced and control procedures). The table shows the behavioural procedure to which each group was exposed at each stage and the number of birds sacrificed for brain measurement at the end of each stage. Each stage lasted for 24 days in order to allow the full development of storing and retrieving by birds exposed to storing for the first time. Note that groups 1, 2 and 3 in stage I all included birds that had experience from days 35-59.

These groups differed in the amount of experience: group 1 stored/retrieved *ad libitum* every third day; group 2 stored/retrieved *ad libitum* every day; group 3 stored/retrieved one seed per day. Stage II of the experiment followed immediately after stage I. However, there was a gap of 32 days between stages II and III, during which the birds were exposed to their appropriate behavioural trials once per week. The gap was included so that the third stage of the experiment was carried out at the time when wild birds would normally have been at their autumn peak of storing. The aim was to test whether birds that were prevented from storing until this late age would still develop normal storing and any associated changes in the brain.

APPENDIX

Stage of experiment (age, days post-hatch)

I (35-59 days)	II (60-83 days)	III (115-138 days)	
1 (n = 15)	Experienced (n = 6)	Experienced (n = 6)	Experienced (n = 3)
2 (n = 3)	Experienced (n = 3)	—	—
3 (n = 3)	Experienced (n = 3)	—	—
4 (n = 15)	Control (n = 6)	Experienced (n = 6)	Experienced (n = 3)
5 (n = 6)	Control	Control (n = 3)	Experienced (n = 3)
6 (n = 3)	Control	Control	Control (n = 3)