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Memory and the Hippocampus in Food-storing Birds

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Introduction

The relationship between memory and the hippocampus has been of great interest to neuroscientists for many years. Although the hippocampus has been shown to be involved with a number of types of learning, it is clear that this brain region is especially important for the formation of memories about the spatial aspects of the environment (O'Keefe and Nadel, 1978; Nadel, 1991). Much of this research has focused on the mammalian hippocampus. Since most of the animal experiments have used laboratory rodents or primates as models, it is unclear whether theories of hippocampal function derived from these experiments can be generalized to animals which have been tested in more naturalistic settings (see Jacobs, 1995, for an elaboration of this issue). Experiments performed under naturalistic or seminaturalistic conditions are important in establishing what role the hippocampus plays in memory, in the appropriate behavioral, evolutionary and ecological context. As is often the case in behavioral neuroscience, interdisciplinary studies of the brain and natural behavior of animals with highly specialized capacities, such as those of food-storing birds, may add to our general understanding of memory and its neural substrates for two reasons. First, birds are particularly suited for such studies because they show the most sophisticated and complex forms of memory-based spatial behavior

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including migration, homing and food-storing (Bingman, 1993). Second, the avian brain is remarkably plastic: learning episodes produce dramatic changes in the rates of cell birth and death, and in the overall size of specific brain regions in response to current memory demands.

Recent studies have capitalized on the relationship between spatial memory and the hippocampus by addressing questions about the concomitant development of both the brain and behavior of food-storing birds (Clayton, 1995a). An investigation of the development of the avian hippocampal formation (HF) and memory-based retrieval of caches in food-storing birds sheds considerable light on our understanding of neuronal plasticity and hippocampal function (Clayton and Krebs, 1995a). In this chapter, three basic questions concerning hippocampal function and neuronal plasticity will be addressed. First, how does the hippocampus change as a result of experiences during ontogeny? Second, what types of experiences are necessary to trigger changes in the hippocampus? And third, how do these ontogenetic changes relate to seasonal changes in hippocampal morphology? A brief introduction to avian/mammalian hippocampal homology will be followed by a discussion of each of these questions in turn.

As discussed in detail in the chapters by Balda and Kamil, and Shettleworth and Hampton, food-storing parids (chickadees and titmice), corvids (magpies, crows, nutcrackers and jays) and sittids (nuthatches) have evolved a remarkable feat of memory. Having hidden hundreds to thousands of food caches, each of which is typically hidden in a separate site and scattered throughout their territory, these birds use memory to retrieve their caches when they return hours to months later (e.g. Sherry *et al.*, 1981; Vander Wall and Balda, 1981; Shettleworth and Krebs, 1982; Balda and Kamil, 1989; Petersen and Sherry, 1998). This memory is remarkable in a number of respects: (1) it is based on a single brief visit during which the item was hidden; (2) the spatial location of a large number of items must be remembered and (3) the information must be retained over long periods of time. For example, food-storing birds show accurate retention of large numbers of locations over 285 days in Clark's Nutcrackers (Balda and Kamil, 1992) and 40 days in Willow Tits (Brodin and Eckman, 1994). Observations of food caching in the field allow one to estimate by extrapolation, that an individual bird probably stores somewhere between 10 000 and 100 000 items per year, rarely if ever reusing the same storage sites (Stevens and Krebs, 1986). In the wild, the time-scale over which caches are retrieved is in the range of a few days to a few weeks for members of the parid family (Brodin and Eckman, 1994), whilst in the crow family, some species, e.g. European Jay, *Garrulus glandarius*, retrieve their caches more than 6 months after storage (Bossema, 1979). Long-term storers such as jays and nutcrackers tend to harvest seeds in the autumn and store them for retrieval during the winter or the following spring, while the shorter-term storers in the tit family hoard and retrieve continually, albeit with seasonal peaks of activity.

Food-storing birds also possess an evolutionary specialization of the brain. A region of the dorsomedial cortex, referred to as HF to indicate that it includes both the hippocampus proper and the parahippocampus, tends to be enlarged in species which store and retrieve hidden food caches. This brain region is known to play a role in successful retrieval of stored food since birds with HF lesions continue to store, but cannot remember where they have stored (Krushinskya, 1966; Sherry and Vaccarino, 1989). Comparisons of diverse families of birds show that HF is not only relatively larger but also contains more neurons in species which store food than in those which do not (Krebs *et al.*, 1989; Sherry *et al.*, 1989). Among closely related food-storing species that engage in different amounts of food storing, relative hippocampal volume tends to be correlated positively with the number and/or length of time over which caches are left (Healy and Krebs, 1992; Hampton *et al.*, 1995; Basil *et al.*, 1996). One exception to this is the Pinyon Jay, which caches hundreds of items each autumn and winter, yet appears to possess a relatively small HF (Basil *et al.*, 1996; see Balda and Kamil, and Kamil, this volume).

A number of researchers have speculated that a large HF, relative to telencephalon and body size, is thought to reflect the increased demands on visuospatial cognition that may accompany scatter-hoarding of food (see Shettleworth and Hampton, this volume). This relationship is thought to be an example of a more general correlation between hippocampal volume and the importance of visuospatial cognition in the wild which occurs in several avian and mammalian species (Sherry *et al.*, 1992, 1993; Healy *et al.*, 1996; Reboreda *et al.*, 1996). To summarize, food-storing birds generally have an enlarged HF, and successful cache retrieval is a hippocampally dependent task which relies at least in part on an accurate, long-lasting memory for individual cache sites. These two findings have led to the use of the food-storing system as a model for investigating fundamental questions about the relationship between hippocampus and spatial memory in a naturalistic environment.

The avian hippocampal formation

In order to justify drawing comparisons between the avian HF and mammalian hippocampus, it is important to demonstrate that the two structures are homologous. Despite initial skepticism, there is now a substantial body of evidence to suggest that this is the case (Bingman, 1993). In terms of embryology, the avian and mammalian hippocampi emerge from the same portion of telencephalon (Källen, 1962). Another similarity is that both share the same cell types including pyramidal cells (Molla *et al.*, 1986). Connectivity to other regions of the brain such as the septum, hypothalamus, brainstem nuclei and sensory processing areas show similarities, although there are some differences (Cassini *et al.*, 1986). The presence of the same types of neurotransmitters and transmitter-related enzymes in both the mammalian and avian hippocampus also suggests homology (Erichsen *et al.*, 1991; Krebs *et al.*, 1991). Furthermore, both structures show long-term potentiation of synaptic responses (e.g. Bliss and Lomo, 1973, for mammals; Wieraszko and Ball, 1991, for birds). Finally, and perhaps the most striking similarity of all, both structures have maintained the ability for neurogenesis in adulthood (e.g. Kaplan and Hinds, 1977; Bayer, 1982; Kaplan and Bell, 1984; Kempermann *et al.*, 1997, for mammals; Alvarez-Buylla *et al.*, 1988, 1992; Nordeen and Nordeen, 1990; Patel *et al.*, 1997b, for birds). None the less, there are some marked differences between the avian HF and mammalian hippocampus. For example, the avian HF lacks distinct structures such as the dentate gyrus, Ammon's horn and mossy fibers (Bingman, 1993) and NMDA-dependent long-term potentiation has been found only on homing pigeons and not in nonhoming pigeons (Wieraszko and Ball, 1993; Shapiro and Wieraszko, 1996). Given the 250 million years in which birds and mammals have diverged and evolved independently, it is not surprising that there are some anatomical differences between the two. In summary, the evidence that the avian HF is homologous to the mammalian hippocampus is persuasive.

Although structural differences exist, there appear to be a number of functional similarities between the avian HF and mammalian hippocampus (Nadel, 1991), not least of which is the correlation between food-storing behavior and enlargement of the hippocampal formation. For example, relative hippocampal volume is larger in scatter-hoarding mammals such as the Mirriam's Kangaroo Rat than in larder-hoarders, such as the Bannertail Kangaroo Rat (Jacobs, 1995). Second, lesions of HF in Black-capped Chickadees (Sherry and Vaccarino, 1989; Hampton and Shettleworth, 1996) suggest that the avian HF is involved in spatial memory, but not memory for visual cues, namely the same types of learning shown to be dependent upon an intact hippocampus in rodents (O'Keefe and Nadel, 1978; Olton, 1983; but see Rawlins *et al.*, 1993). Furthermore, small ibotenic acid lesions of the Zebra Finch hippocampus impair spatial but not color one-trial associative learning (Patel *et al.*, 1997a). A third similarity with mammalian studies of hippocampal function is that avian HF seems likely to be involved in consolidation but is probably not the site of memory storage, since lesions impair the acquisition of new information as opposed to the consolidation of established memories, at least in homing pigeons (reviewed by Bingman, 1993). Recent studies on the Zebra Finch hippocampus also indicate that the acquisition of new spatial memories is impaired when birds are given the aromatase inhibitor, fadrozole, but there is no effect on spatial memories that were acquired prior to the drug treatment (Clayton *et al.*, 1997). Interestingly, fadrozole treatment also results in about a 30% shrinkage in hippocampal volume in Zebra Finches (Saldanha *et al.*, 1997). Fourth, studies of hippocampal function in humans and primates have suggested that the hippocampus is important in various forms of long-term memory (LTM), whereas short-term memory (STM) does not depend upon an intact hippocampus (reviewed by Squire *et al.*, 1993). To date, only one study has investigated the effects of lesions on LTM for cache sites in birds (Krushinskya, 1966): when long retention intervals separated the storage of seeds from the opportunity to retrieve them, Eurasian Nutcrackers with lesions of the hyperstriatum (including HF) were significantly impaired in their attempts to retrieve. These results suggest that avian and mammalian hippocampi share striking functional similarities. Since the anatomical organization of HF is markedly different from that of the mammalian hippocampus, this raises the question of whether or not the two do a similar job but in different ways (Clayton, 1995a). Furthermore, since both the mammalian and avian hippocampus undergo neurogenesis even in adulthood, the two key questions may be, first, how does this process differ between the two, if at all?, and second, what role, if any, does the birth of new hippocampal neurons play in memory formation?.

How does ontogeny affect hippocampal change?

Comparative studies of relative hippocampal volume in food-storing and nonstoring species of corvids (Healy and Krebs, 1993) and parids (Healy *et al.*, 1994) show that while adult food-storing and nonstoring species differ in relative hippocampal volume, the nestlings do not. Thus, the species difference in hippocampal volume arises at a relatively late stage in development, after the young birds have fledged from the nest. This result raises the possibility that relative hippocampal growth is associated with some aspect of the experience of food-storing behavior. The hypothesis has been tested in detail by studying the development of food-storing behavior, memory and hippocampal anatomy in hand-raised, postfledging, juvenile Marsh Tits. Food-storing behavior begins around the time of nutritional independence (day 35) and involves a number of behavioral changes which occur rapidly over a period of 10 days: the birds become more dexterous at handling seeds, they leave their caches for longer periods of time before retrieving them, and they become more efficient in how quickly they store seeds and how well they hide them (Clayton, 1992). There is a sudden increase in number of items stored at day 44 (Clayton, 1994). This sudden increase in food caching on day 44 has also been observed recently in two other species of parids, the Coal Tit, *Parus ater* (Jolliffe, 1996; Jolliffe and Clayton, in prep.) and the Mountain Chickadee, *Parus gambeli* (Clayton, 1998). This sudden increase in food caching appears to be age dependent rather than experience dependent (Clayton, 1994), whereas memory-based retrieval performance increases gradually after the onset in food storing. If birds are prevented from storing and retrieving caches until after day 44, the behavior develops rapidly when the opportunity is presented at a later stage.

This result suggests that there is no sensitive period during which experience has to be obtained, at least within the range tested between day 35 and 115, which represents about half the typical life span of a Marsh Tit (Clayton, 1994).

By giving hand-raised Marsh Tits the opportunity to store and retrieve food at different ages, and by measuring the volume of the hippocampal formation, Clayton and Krebs (1994a) showed that the experience of storing and retrieving food results in increased hippocampal volume and neuron number. Age-matched control birds that were prevented from storing and retrieving caches but identical in all other respects had much smaller hippocampal volumes. These control birds also had a higher percentage of apoptotic (dead) cells than did experienced birds. This suggests that one effect of experience is to trigger recruitment of neurons, while the lack of experience may cause cell loss. The experience-dependent hippocampal changes could be triggered rapidly over a period of 24 days in which the birds received only eight food-storing and retrieval tests, once every third day. This effect has also been replicated in Coal Tits (Clayton *et al.*, 1998) and Mountain Chickadees (Clayton, in press) which suggests that the effects of food-storing experience on hippocampal growth are not exclusive to the Marsh Tit.

It would be of great interest to test whether hippocampal growth is also associated with food-storing experience in members of the crow and nuthatch families because food caching has evolved independently in the parids, corvids and sittids. Preliminary results indicate that, while many aspects of the developmental sequence of food storing are similar in parids, corvids and sittids (Clayton, personal observation), the onset of food storing occurs much earlier in corvids than in the sittids and parids. The development of food storing and cache retrieval is being studied in three species of corvid: the Yellow-billed Magpie, *Pica nutalli* (Clayton and Lee, personal observation), the European Magpie, *Pica pica* (Clayton and Jolliffe, personal observation) and the Scrub Jay, *Aphelocoma coerulescens* (Clayton, personal observation). In all three of these corvid species, the young birds begin to cache around the time of fledging by hiding regurgitated food.

It is important to note that experienced birds in the parid developmental studies differed from controls in only two respects: (1) they were provided with whole seeds, not powdered seeds, to eat and store; and (2) they could store and retrieve. Experienced birds did not differ from controls in the number of visits to potential cache sites, nor were there any other obvious differences in motivation or hunger levels. Thus, it would appear that some aspect of the specific experience of storing and retrieving caches is associated with hippocampal growth, while the absence of this experience results in hippocampal attrition. To test whether hippocampal growth precedes or accompanies changes in food-storing behavior, Clayton (1996) sacrificed birds one trial before (day 41), and one trial after (day 47), the sudden increase in food storing and associated increased demands on memory-based retrieval of caches. Experienced birds had larger absolute and relative hippocampal volumes than did controls at all stages of this experiment. Thus, a difference in hippocampal growth between experienced and control birds can be detected by day 41, 3 days before the relatively sudden increase in food-storing intensity. It is not known, however, when this hippocampal growth first appears and how much experience is actually necessary to trigger the increase in volume. Since adult food-storing and nonstoring species differ in relative hippocampal volume, but nestlings do not (Healy and Krebs, 1993; Healy *et al.*, 1994), it would be of interest to determine at what age this difference becomes apparent and whether it corresponds to the actual initiation of storing and memory-based retrieval or whether a critical number of such experiences is needed. Furthermore, since storing in corvids appears before fledging (Clayton, personal observation), the temporal pattern of hippocampal growth and attrition may be very different from that of parids.

These results may be taken to support the hypothesis that hippocampal growth occurs in preparation for food storing, rather than accompanying or following changes in food storing. However, why is HF enlargement and increase in neuron number confined to birds in the experienced groups? Control birds received virtually the same visual stimulation, motor experience and diet as experienced birds, so it seems unlikely that the difference between the groups could be accounted for in terms of a general deprivation vs. enrichment effect. Perhaps the presence of whole seeds, or the opportunity to store and retrieve just one or two seeds, acts as a switch to trigger hippocampal growth. Either way, there appears to be an increase in relative hippocampal volume, and total neuron number, in preparation for the increased memory demands associated with the increase in food storing that does not occur unless some minimal experience with food storing and retrieving is obtained (Clayton, 1996).

These volumetric changes in HF are correlated with changes in total numbers of neurons, rather than with changes in cell size or density (Healy and Krebs, 1993; Healy *et al.*, 1994; Clayton and Krebs, 1994a). A critical question concerns the relative importance of cell birth and programmed cell death since the total number of neurons in HF is a function of the rate of programmed cell death and the rate of cell proliferation within the ventricular zone. Studies by Patel *et al.* (1997b) have tested whether or not the volumetric changes in HF are accompanied by changes in cell birth, and whether cell birth occurs before, after, or at the same time as changes in hippocampal volume. Patel *et al.* (1997b) investigated cell birth and cell death in the hippocampus of the experienced and control birds that were sacrificed either one trial before, or one trial after, the sudden increase in food storing. They found that there was a significantly higher rate of cell proliferation in the ventricular zone adjacent to HF in experienced birds. This effect of experience on rates of cell proliferation was not significant for the ventricular zone adjacent to neostriatum. This result suggests that the effects of experience were localized, rather than having a general effect throughout the brain. Furthermore, these differences between experienced and control birds are evident by day 41, the time at which volumetric changes are apparent. In fact, the effect of experience was greatest after the third trial, at day 41. It is important to determine where new cells migrate and whether they are neurons or glial cells, but these data provide a promising starting-point for future studies. Further work is required to provide a more detailed analysis of the time course of the neurological events and to ascertain: (1) the relative contributions of cell birth and programmed cell death to the morphological changes; (2) the types of experience which trigger these events; (3) whether memory formation is a necessary condition for overall hippocampal change and (4) whether food-storing experience stimulates the same processes of cell birth and cell death as season and photoperiod.

What types of experience trigger hippocampal change?

In another series of experiments, Clayton (1995b) trained both hand-raised Marsh Tits (food storer) and Blue Tits (nonstorer) from day 35 to day 200 on a one-trial associative memory task in which birds were rewarded for returning to the feeder where they had eaten part of a peanut 20 minutes earlier. As described in detail in the chapter by Shettleworth and Hampton, this one-trial associative learning or 'peanut shopping' task does not require the bird to store food, but captures some of the essential aspects of food-storing memory in that the bird has to: (1) learn an association between food and its unique location on the basis of one visit; (2) remember the site using visuospatial cues; and (3) revisit the site 2 hours later in order to obtain the reward. The one-trial associative memory task is therefore a useful tool to assay species differences in the development of memory and HF, and particularly for comparing differences between food-storing and nonstoring species. In Marsh Tits, experience of the one-trial associative memory task triggered hippocampal growth in the same way as food-storing experience (Fig. 4.1), but there was no effect of experience on HF volume in Blue Tits (Fig. 4.2).

These results suggest that memory experiences other than food storing may trigger hippocampal growth, at least in the food-storing species, and that the HF of food storers may differ from that of nonstorers in having the potential to respond to this experience. However, an alternative explanation would be that the species differ in the way they learn to solve the task. For example, Sherry and Vaccarino (1989) have found that the avian HF in Black-capped Chickadees is important for solving spatial tasks but not color-discrimination tasks. Comparative tests of memory in the laboratory suggest that food storers may use predominantly spatial cues whereas nonstorers also use color cues

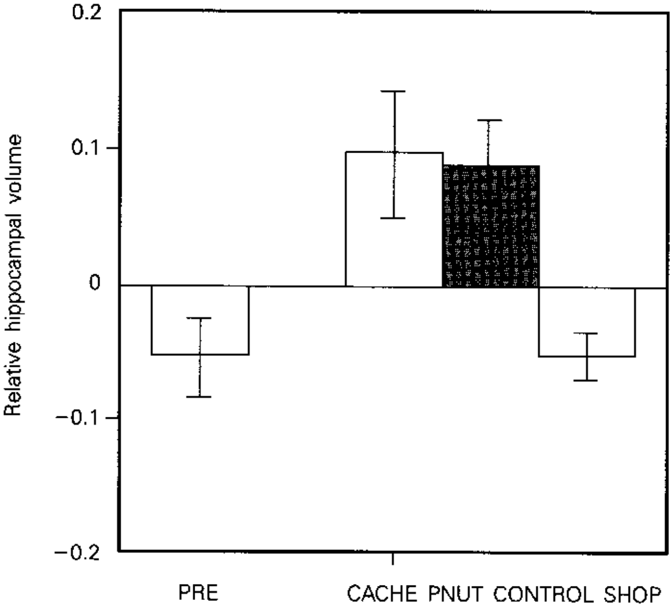

Fig. 4.1 The mean and standard error of the relative hippocampal volume of four groups of juvenile Marsh Tits that were either sacrificed prior to the start of the experiment at 35 days posthatch (PRE) or at 200 days posthatch (CACHE, PNUT SHOP and CONTROL birds) Both the CACHE and PNUT SHOP birds had been trained and tested on the one-trial associative memory task whereas CONTROL birds had not. CACHE birds were allowed to store and retrieve food whereas PNUT SHOP and CONTROL birds were prevented from caching and retrieving seeds by maintaining them on a diet of powdered food. The relative hippocampal volume per bird was calculated by plotting one linear regression of log hippocampal volume and log telencephalon volume, and calculating the deviations from the regression line.

(Brodbeck, 1994; Clayton and Krebs, 1994b). If the task is a spatial, hippocampally dependent one for the food-storing species but not for the nonstoring species, then this might explain why the HF of food storers responds to this experience, whereas the nonstoring HF does not.

What then of memory tasks that do not require the hippocampus? If it is memory formation, rather than nonmemory experiences related to food storing and retrieving, that triggers hippocampal growth, then logically it would follow that memory experiences which do not rely on the hippocampus should not stimulate its growth. It seems clear that experiences which involve the formation of memory for previously encountered food can trigger gross morphological changes in the brains of food-storing birds. To determine whether these changes occur as a result of memory or other nonmemory

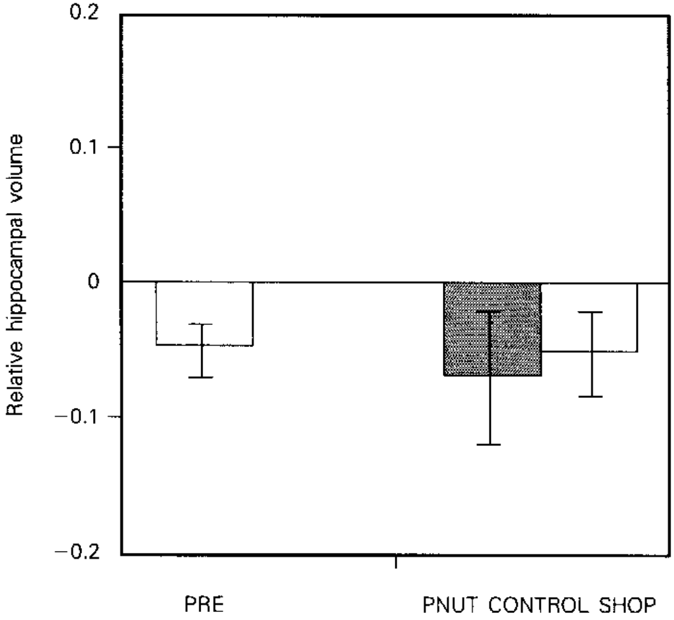

Fig. 4.2 The mean and standard error of the relative hippocampal volume of three groups of juvenile Blue Tits that were either sacrificed prior to the start of the experiment at 35 days posthatch (PRE) or at 200 days posthatch (PNUT SHOP and CONTROL birds). PNUT SHOP birds had been trained and tested on the one-trial associative memory task whereas CONTROL birds had not. For other details, see legend for Fig. 4.1.

factors related to finding food, at least three basic criteria must be met: (1) the morphological changes must be specific to the hippocampus and not involve structures unrelated to the memory task at hand; (2) the formation of these memories must depend upon hippocampal function and (3) memory formation not dependent upon an intact hippocampus should not stimulate growth of this area. One approach which may prove useful in this respect is to design studies which tease out the effects of hippocampally dependent vs. hippocampally independent memory formation on the subsequent growth or attrition of the developing hippocampus.

Previous studies have shown that the developing brain of both birds and mammals, including the mammalian hippocampal region, is plastic in response to specific kinds of sensory input or experience and to hormonal influences, but the results for food-storing birds are unique in having the following combination of features (Clayton and Krebs, 1994a). First, the effect of experience is independent of age within the range tested which represented about one third of their average life span. This is in contrast to effects of visual experience on the development of the visual cortex in mammals (see reviews in Raushecker and Marler, 1987). Second, the effect is specific, both in terms of experience because control and experienced birds did not differ in experience other than the specific task of storing and retrieving food, and in terms of the localization of the effect. It is not an effect following general kinds of environmental enrichment as that resulting in cortical growth in rats (e.g. Rosenzweig *et al.*, 1962; Rosenzweig, 1984; Cramer, 1988; Kempermann *et al.*, 1997). Third, growth of the hippocampus appears to be triggered by some aspect of memory for retrieving previously encountered food.

Specificity of the experience-dependent effects

The experience-dependent effects seem to be specific in that experience triggers growth of the hippocampus but not growth of another telencephalic brain region, the ectostriatum (ECTO), or the telencephalon as a whole (Clayton and Krebs, 1994a). This result suggests that specific kinds of memory experience can stimulate growth of a specific area of the brain. However, to ensure that any volumetric differences are really specific to the HF, other brain regions which do not have connections with HF but may be affected by such complex experience should be measured. Doupe (1994) has suggested that another potential candidate is the paleostriatum.

Work on Zebra Finches using the *Phaseolus vulgaris* leucoagglutin (PHAL) as an anterograde tracer (Székely and Krebs, 1993) has shown that HF has major connections with a number of other brain regions which have been implicated in learning and memory, including the hyperstriatum ventrale (HV), the archistriatum (ARCHI) and the lobus parolfactorius (LPO). Nuclei within these regions are thought to be involved in imprinting and/or passive avoidance learning (Horn, 1985; Rose, 1992). These brain regions also show up-regulation of c-*fos* expression following food storing in Marsh Tits (Székely *et al.*, 1993; Clayton and Krebs, 1995b), which suggests that they form part of a distributed memory circuit in the avian brain. In order to understand how the hippocampus responds to experience, it is crucial that growth and attrition in these other brain regions are investigated.

The extent of brain plasticity

The ontogenetic studies on hippocampal growth in Marsh Tits also raise the possibility that older experienced birds may respond to food-storing experience or the lack thereof in the same way as juveniles during their first food-storing opportunity. If birds are prevented from storing and retrieving caches until after the time period when storing would normally be first observed, the behavior develops rapidly when the opportunity is presented at a later stage (Clayton, 1994). To date only one study has addressed the possibility that adult birds may show similar behavioral and neurobiological plasticity: Cristol (cited in Krebs *et al.*, 1996) compared wild-caught adult Willow Tits which were given intensive experience of food storing and retrieval over a 4-week period with a control group that were prevented from food storing during the same period. The two groups did not differ in relative hippocampal volume. Unfortunately, the Willow Tits used in this study were very old (>4 years) and the sample size was small. The study needs to be repeated to test whether food-storing experience can stimulate hippocampal growth in adult birds or whether the prevention of storing could result in hippocampal regression and, if so, whether or not these changes are irreversible. It would seem fundamental to our understanding of brain plasticity to determine whether morphological changes found in juveniles also occur in adults and, if they do, whether such changes involve the same processes. Volumetric changes in the HF of food-storing birds are correlated with changes in total numbers of neurons, rather than with changes in cell size or density (Healy and Krebs, 1993; Healy *et al.*, 1994); and both cell birth and programmed cell death may be important, but the ratio of the two may vary depending on conditions. For example, volumetric changes in adults might result largely from changes in rates of cell birth, whereas the volumetric changes which occur during the first opportunity to store and retrieve food may result largely from differences in programmed cell death. A related question is whether food-storing experience triggers the same processes of cell birth and death as season and photoperiod.

Developmental vs. seasonal changes in hippocampal morphology

While some aspect of storing and retrieving food undoubtedly triggers hippocampal growth in juvenile birds, there is also some evidence that hippocampal growth and attrition may occur on a seasonal basis. Both field observations and laboratory studies suggest that food-storing behavior has an annual cycle: in parids, it is more marked in autumn and winter than in spring and early summer (Odum, 1942; Haftorn, 1956; Ludescher, 1980) Some corvids (e.g. European Jay) store more food, spend more time hiding caches, and leave them for longer periods in the autumn than in the spring (Bossema, 1979; Clayton *et al.*, 1996). There are also seasonal changes in HF. Smulders *et al.* (1995) found in Black-capped Chickadees that HF was larger, relative to the rest of the brain, in October than at other times of year. Barnea and Nottebohm (1994) reported an increased level of cell birth in wild Black-capped Chickadees during October, when food-storing activity increased. Unfortunately, these two studies did not include a nonstoring control species and, therefore, it is unclear whether the changes are linked to food storing or reflect a more general seasonal effect such as changes in territory use and reproductive status. Seasonal volumetric changes are observed in other brain areas, namely the avian song control nuclei (Nottebohm, 1981). In the case of the song control nuclei, the neuroanatomical alterations probably result from changing levels of circulating steroid hormones associated with reproduction rather than as a direct result of changes in singing behavior or song learning (Brenowitz, 1992). It is not yet clear whether or not the volumetric changes in HF are under the same hormonal control as the song control nuclei. Additional studies need to be performed to test whether or not seasonal changes in hippocampal volume are correlated specifically with the experience of food storing and the formation of new spatial memories, and whether or not they are to be found only in species which store.

In more controlled laboratory experiments, Shettleworth *et al.* (1995) and Krebs *et al.* (1996) manipulated what they termed 'photoperiod' (actually day length and temperature) to create 'autumn' or 'spring' conditions in Black-capped Chickadees. The autumn birds showed much higher levels of food storing than did the spring birds. As both day length and temperature were manipulated it is not clear which factors affected food-storing behavior. Since seasonal changes in food storing are accompanied by hippocampal changes in the wild (Barnea and Nottebohm, 1994; Smulders *et al.*, 1995), Krebs *et al.* (1996) tested whether photoperiodically driven differences in food-storing behavior were accompanied by changes in relative hippocampal volume. No such differences were found. However, birds were allowed to store and retrieve only in their home cages and seeds were visible at all times, so it seems likely that the birds were not using memory to retrieve their caches. Given that the hippocampus plays a role in memory for cache sites rather than in the motivation to store, it is not clear why such manipulations should induce volumetric changes in HF. The critical test would be to allow birds to store in an environment where they use memory to retrieve their caches. An obvious question to ask is if photoperiod-induced changes in food storing are associated with (1) changes in HF, and (2) changes in memory for hippocampally dependent tasks. If so, do these changes result directly from photoperiod manipulations or secondarily from changes in food-storing intensity?

Clayton and Cristol (1996) addressed these questions in adult Marsh Tits by manipulating photoperiod, independent of temperature. Birds were provided with the opportunity to store and retrieve seeds in both the home cage and in an observation chamber where caches were hidden so that they had to rely on memory-based retrieval. Food-storing intensity was recorded in the two conditions. Birds were also tested for their performance on a series of tests of memory for previously encountered food. These memory tests were designed to determine the accuracy of recall for spatial and nonspatial cues, the former being a hippocampally dependent task and the latter being a hippocampally independent task. The prediction was that changes in photoperiod would trigger changes in food-storing intensity and spatial memory but not changes in nonspatial memory. Birds were captured in July and housed individually indoors. After a period of about 2 weeks, during which they were maintained on a natural day length to adjust to captive conditions, they were randomly assigned to one of two rooms which were adjacent and identical in temperature but differed in light:dark cycle. One group received an accelerated autumn photoperiod (decreasing day length) in August, followed by a period of short days (simulated winter) in September and early October, and a sudden onset of long days (simulated summer) in mid-October. The other group was maintained on long days until mid-October, and then suddenly exposed to short days. The results indicate that differences in photoperiod caused differences between treatment groups in food-storing intensity in both the home cage and the observation chamber, and in spatial memory. Birds experiencing short days after an accelerated autumn were more intensive food storers and performed better on a spatial memory test than did those maintained on long days. When the photoperiod was reversed, the difference between groups disappeared. On a very similar test in which subjects could rely only on nonspatial cues (e.g. color), there were no differences between groups, irrespective of photoperiod, indicating that the effects of photoperiod seem to be specific to spatial memory.

There are only a handful of examples of seasonal shifts in behavior that are accompanied by cognitive changes. In some birds, the ability to discriminate between conspecific songs is greatest during the breeding season, the time when birds typically learn new songs (Cynx and Nottebohm, 1992; Calhoun *et al.*, 1993). In two rodent species, sex differences in spatial learning ability have been observed only during the breeding season, simultaneous with the greatest sex differences in spacing behavior (Galea *et al.*, 1994; Jacobs, 1995). In Black-capped Chickadees, food-storing intensity and hippocampal cell birth both increase in autumn, when day length is decreasing (Barnea and Nottebohm, 1994). The result that Marsh Tits on an autumn photoperiod have better recall for spatial cues is consistent with the idea that seasonal neural changes are related to retrieval of stored food and may be the first demonstration that photoperiod manipulations can affect this cognitive ability. The next step is to test if such changes are also accompanied by growth and attrition of HF and, if so, whether or not this occurs as a direct result of changes in photoperiod, or as a consequence of photoperiod-induced changes in food-storing behavior. Hippocampal development and plasticity may then be compared and contrasted to season-mediated hippocampal plasticity, and provide some intriguing answers to questions dealing with memory processes in both the young and old. Does seasonal neurogenesis occur at the same rate as ontogenetic neurogenesis or is it substantially reduced as the animal ages? Do rates of apoptosis differ seasonally as well as during development? Also, perhaps most importantly, can experience modulate the relative contributions of both regardless of age?

Summary

The overall objective of the research described in this chapter is to increase our understanding of the involvement of the hippocampus and other brain regions in memory by using a naturalistic model of memory, namely memory for cache sites in food-storing birds, in order to evaluate hippocampal function in the appropriate behavioral, evolutionary and ecological context. A review of the evidence provides a compelling case for the involvement of HF in memory in food-storing birds, and recent experiments clearly demonstrate that this is an excellent model for investigating how specific environmental influences trigger morphological changes in volume, programmed cell death, and cell birth of a specific region in the fully developed brain, both seasonally and during ontogeny. Investigating the relationship between memory, HF and the associated brain circuitry in food-storing birds may provide a rich framework with which to study the effects of experience on underlying neural mechanisms, brain plasticity and brain repair. As is the case in any program of research, far more questions are asked than are ever answered, and with every answer lie many, many more questions to ponder another day.

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