

BIRDS that store food have a relatively large hippocampus compared to non-storing species. The hippocampus shows seasonal differences in neurogenesis and volume in black-capped chickadees (*Parus atricapillus*) taken from the wild at different times of year. We compared hippocampal volumes in black-capped chickadees captured at the same time but differing in food-storing behaviour because of manipulations of photoperiod in the laboratory. Differences in food-storing behaviour were not accompanied by differences in the volume of the hippocampus. Hippocampal volumes also did not differ between two groups of a non-food-storing control species, house sparrows (*Passer domesticus*), exposed to the same conditions as the chickadees.

Effects of photoperiod on food-storing and the hippocampus in birds

J. R. Krebs,^{1CA} N. S. Clayton,^{1,2}
R. R. Hampton,³ and S. J. Shettleworth³

¹Edward Grey Institute of Field Ornithology, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK; ²Section of Neurobiology, Physiology & Behavior, Briggs Hall, University of California, Davis, CA 95616, USA; ³Department of Psychology, University of Toronto, Toronto, Ontario, M5S 1A5, Canada

Key words: Food-storing; Hippocampus; Photoperiod; Birds

^{CA}Corresponding Author

Introduction

Some species of bird store food in scattered locations and rely on an accurate, long lasting memory to retrieve their catches.¹ The avian hippocampus is involved in memory for the locations of stored items,² and food-storing species have a larger hippocampus, relative to telencephalon, than species that do not store.^{3,4} We use the term 'hippocampus' to refer to the dorsomedial cortex, including parahippocampus.⁵ Observations in the wild suggest that many food-storing species including the chickadees and titmice (*Parus* spp.), store most food in the autumn and early winter.^{6,7} However, in the wild, factors that influence food-storing such as food supply and energetic demands^{8,9} also vary seasonally. Seasonality in food-storing behaviour persists in captive willow tits (*P. montanus*) in outdoor aviaries,¹⁰ but in such experiments changes in temperature, day length and/or the abundance of wild food entering the aviary could directly affect the intensity of food-storing. However, recent studies on captive black-capped chickadees (*P. atricapillus*) show that seasonal changes in food-storing intensity are observed even when food supply and energetic demands are constant. When housed in the laboratory under identical conditions, birds caught in the autumn stored considerably more than those caught in the spring.¹¹ Furthermore, by manipulating photoperiod, Shettleworth *et al*¹¹ were able to trigger changes in food storing.

In some birds and mammals, seasonal changes in

behaviour are accompanied by changes in cognition and the brain. For example, sex differences in spatial learning that are related to social ecology are not observed outside the breeding season in deer mice (*Peromyscus maniculatus*)¹² or voles (*Microtus* spp).¹³ Investigations of possible seasonal changes in hippocampus have not yet been reported for these species, although the brains of small mammals, including hippocampus, do change seasonally.¹⁴ In birds, the ability to discriminate between conspecific songs is most acute during the breeding season, when the learning of songs occurs.¹⁵ Seasonal changes in the volume of song control nuclei accompany seasonal changes in song learning, although the relationship between the two is not entirely clear (reviewed in Ref. 16). Such findings raise the question of whether the hippocampus of food-storing birds changes seasonally along with intensity of food-storing behaviour.

Two groups of researchers have attempted to answer this question by examining the brains of black-capped chickadees taken from the wild at different times of year. Smulders *et al*,¹⁷ found that the volume of the hippocampus, relative to telencephalon and body size, is larger in October than at other times of the year; Barnea and Nottebohm¹⁸ used tritiated thymidine injections to examine seasonal variation in neurogenesis in free-ranging black-capped chickadees. They found a higher proportion of labelled neurones in the hippocampus in October than at other times of year, indicating a seasonal peak of neurogenesis. They also housed captive chickadees in outdoor aviaries in October and in May and found

the same pattern of results, although the proportion of labelled cells was less than in free-ranging birds. However, because neither of these studies included a control species which does not store food, it is not certain whether these effects are specific to food-storing species. Furthermore, neither group of researchers had data on the seasonal distribution of food-storing behaviour in their birds. Relevant data from other studies are scanty at best^{6,19}. In addition, groups of chickadees taken from the wild at different times of year could differ in a number of ways other than their food-storing behaviour. Individuals present in early spring are a different subset of the population from those present the preceding autumn because many birds do not survive the winter.¹⁹ Average nutritional status also varies seasonally, and the daylength, temperature, food and water availability to which the animals are exposed just before capture will differ. Finally, the brains of individuals captured in different seasons cannot be processed at the same time with identical materials.

In the present experiment we tested whether changes in food-storing behaviour are accompanied by changes in the volume of the hippocampus in two groups of chickadees that had been captured at the same time but differed in food-storing because their photoperiods had been manipulated in the laboratory following the methods described by Shettleworth *et al.*¹¹ The birds were all kept in the same room on the same feeding regimen and daylength for the last few weeks before they were sacrificed, so factors other than the bird's seasonal state that could have influenced the outcome of previous studies were equated. A further difference between our study and previous studies is that in order to test whether any effects found were specific to a food-storing species, house sparrows (*Passer domesticus*, a non-storing bird) were included as a control.

Materials and Methods

The subjects were 12 black-capped chickadees and 10 house sparrows. The chickadees (six males, six females) and sparrows (three males, seven females) were caught in and around Toronto, Canada, between March 30 and April 15, 1993. Birds were housed individually in wire mesh cages and maintained as described by Shettleworth *et al.*¹¹ Each cage was furnished with a 46×9×4 cm thick strip of wood containing holes in which the chickadees could store food.

The birds were initially housed in the same room at 18.33±1.11°C and an 11:13 h photoperiod with lights on at 07.00 h local time, a schedule approximating natural daylength at the time of capture. Three times each week the number of sunflower seeds stored in the wood blocks in 90 min was recorded; moulting

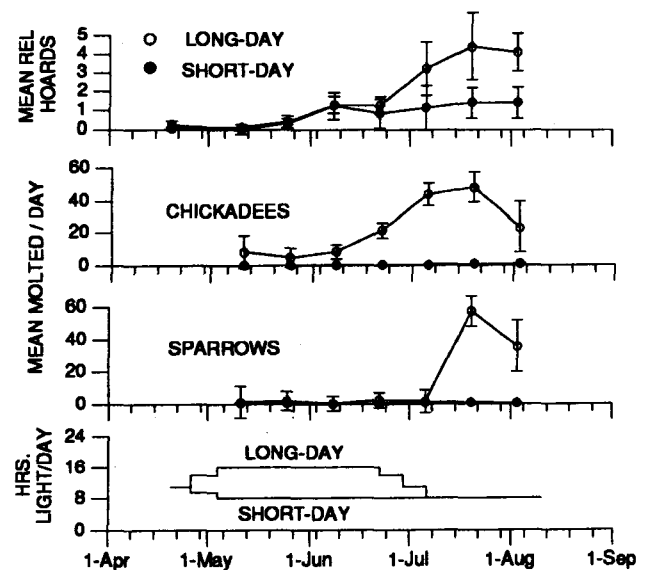


FIG. 1. Top panel: the mean (\pm s.e.) relative hoards (as defined in the text) made by the chickadees in two-week blocks. The first points are for the baseline tests and therefore constrained to be zero. Middle panels: mean moulting per day (\pm s.e.) in 2-week blocks for chickadees and sparrows. Bottom panel: hours of light per day throughout the experiment. The changes in photoperiod were introduced stepwise, to provide an approximation to natural changes in daylength.

was rated weekly as described by Shettleworth *et al.*¹¹ On April 26, 1993 the birds of both species were divided into two equal groups. The chickadee groups were matched as closely as possible for mean items stored up to that time. Sparrows and chickadees in the short-day group remained in the original housing room while birds in the long-day group were moved into a different room, similar in size and arrangement but kept at 22.22±1.11°C and on a different photoperiod. To control for disturbance, the cages in the short-day group were moved to different positions within their room. The bottom panel of Figure 1 shows the photoperiods experienced by the long-day and short-day groups of both species throughout the experiment. Whenever photoperiod was altered, the light period was changed by an equal amount in the morning and evening.

On July 6, 1993 all the birds were housed again in the same room at 16.67±1.11°C. Cages were positioned and labelled in such a way that the food-storing data could be recorded blind with respect to group. Three days before the first birds were sacrificed, an equipment failure was discovered which could have resulted in continuous light for as much as the previous 2 days. The birds were moved into another room where the lights were functioning properly. Although the chickadees received one sunflower seed storing test after this move, these data were not used.

At the end of the experiment, birds were given a lethal i.p. overdose of sodium pentobarbitone and

perfused transcardially with 0.75% heparinized physiological saline followed by Zamboni's solution. Following perfusion, the birds were sexed by examination of their gonads, brains were extracted and post-fixed in Zamboni's solution for seven days. The brains were cut into 25 μm frozen coronal sections and treated in an identical way to control for shrinkage. Every tenth section was stained with cresyl violet. The volume of the hippocampal formation and the remainder of the telencephalon were traced from the sections using a 10 \times photographic enlarger. The traced outlines were digitized using a HIPAD tablet to calculate the hippocampal and telencephalic area of each section. The volumes of the hippocampus and telencephalon (minus hippocampus) were computed using the formula for a truncated cone, as described in previous studies.⁵ All measurements were made blind: the slides were coded by number and the codes were not interpreted until all the measurements had been completed. The estimated observer error in tracing hippocampal boundaries is less than 4%.³

Results

The chickadees stored very little at the beginning of the experiment (long-day group mean = 0.095 ± 0.06 items per test; short-day group mean 0.238 ± 0.211). Since individual chickadees differed in their initial tendency to hoard, we assessed the effect of photoperiod by calculating each bird's increase in mean number of items stored per test in successive blocks of 2 weeks over the mean number stored per test during baseline days (Fig. 1 top panel, 'relative hoards'). After a few weeks in the summer photoperiod and relatively warm temperatures, chickadees in the long-day group began to moult. The increase in moulting was accompanied by an increase in the relative number of seeds stored by chickadees in the long-day group. The sparrows in the long-day group also moulted, but much later than the chickadees. Neither short-day group moulted (Fig. 1, middle panel). Since chickadees normally moult only at the end of the breeding season,¹⁹ these data confirm that the photoperiodic regime had functioned as an abbreviated summer for the long-day group. Both long-day and short-day groups were kept in the same room from early July onwards, so differences between groups in storing and moulting could not have reflected differences in immediate energetic demands but rather their different photoperiod treatments.

The effect of photoperiod on storing was tested using relative hoards from the final phase of the experiment, when the chickadees were in identical conditions. Data were normalised by a log (x+1) transformation, and a *t*-test was used. There was a significant effect of treatment ($t=2.335$, $df=5$, $p<0.042$), with the long-day group making a greater

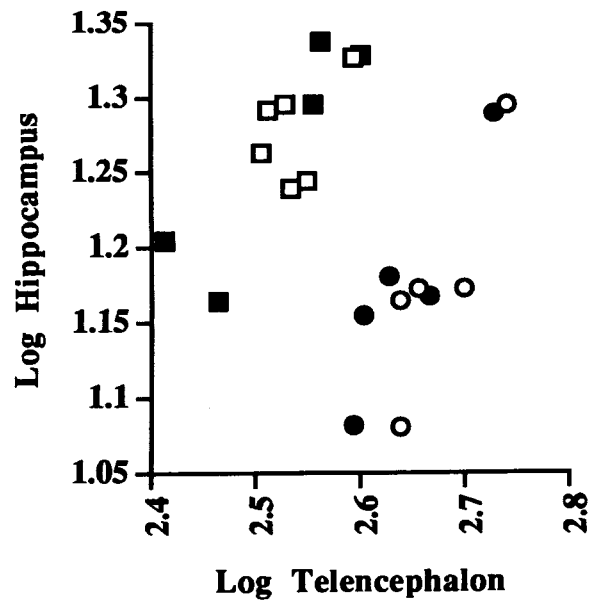


FIG. 2. Hippocampal volume plotted against telencephalon volume (mm^3), expressed as log values, for chickadees and sparrows in the short-day and long-day groups. One chickadee brain from the short-day group was damaged and could not be used. \blacksquare : chickadee short-day group, \square : chickadee long-day group, \bullet : sparrow short-day group, \circ : sparrow long-day group.

total number of relative hoards than the short-day group.

In both chickadees and sparrows, log hippocampal volume was correlated with log telencephalon volume (chickadees, $F=17.204$, $df=1,9$, $p<0.003$; sparrows, $F=19.011$, $df=1,8$, $p<0.002$; see Fig. 2). However, chickadees had a larger hippocampus relative to telencephalon than did sparrows ($t=2.75$, $df=19$, $p<0.0123$; comparison of deviation from the overall regression of both species), as would be expected from previous comparisons of food-storing and non-storing species within the passerine birds.^{3,4} Comparison of the deviations from species regression of log telencephalon on log hippocampus for long-day and short-day groups within each species showed that there was no significant effect of treatment group for either species (chickadees, $t=-0.227$, $df=9$, $p<0.826$; sparrows, $t=-1.499$, $df=8$, $p<0.172$). Furthermore, within the chickadees treated as a single group, there was no significant correlation between relative hippocampal volume and food-storing intensity ($F=1.203$, $df=1,9$, $p<0.301$).

Discussion and Conclusion

In this experiment, a photoperiodically induced increase in food-storing behaviour was not accompanied by an increase in the volume of the hippocampus. This finding appears to conflict with that of Smulders *et al.*¹⁷ on free-ranging chickadees. Furthermore, if the increased neurogenesis in autumn

observed by Barnea and Nottebohm¹⁸ is associated with volumetric changes (not measured by those authors), our results may also conflict with theirs. Previous studies of other systems have also reported seasonal changes in areas of the brain including the avian song system¹⁶ and the hippocampus of small mammals.¹⁴

Recent work on the ontogeny of food-storing and retrieval in juvenile hand-reared marsh tits²⁰ has shown that some aspect of food-storing experience during development results in an increase in relative hippocampal volume while absence of this experience causes a progressive decrease in hippocampal volume.²¹ These volumetric changes are accompanied by increases and decreases respectively in cell number, suggesting that one effect of experience is to trigger recruitment of neurones, while the lack of experience may cause cell loss.²¹ In view of these results, we might have expected a direct effect of the increased food-storing activity of long-day birds on the volume of the hippocampus. However, relatively small amounts of food-storing experience are sufficient to trigger hippocampal growth in juveniles,²¹ and all of our birds may have exceeded this threshold. Furthermore, it is not yet known whether experience later in life has similar effects nor whether early effects on hippocampal growth are permanent. Cristol (personal communication) found no difference in relative hippocampal volume between groups of adult wild-caught willow tits (*P. montanus*) that received either food-storing and retrieval or control experience in the laboratory. Because all the chickadees in the present experiment were caught in early spring, they must have had extensive food-storing experience for at least one winter.

If the aspect of food-storing experience important for hippocampal enlargement is not the amount of food storing *per se* but the associated formation, processing and/or retrieval of memory, then the present experiments may not have provided the correct experience to trigger hippocampal growth. The technique of providing the birds with storing blocks in their home cage in which to store may not demand sufficient memorisation and/or retrieval of locations to affect hippocampal volume. If this interpretation is correct, then differences in relative hippocampal volume of long-day and short-day birds might be found if the birds were allowed to store and retrieve food in a spatially more complex environment in which memory-based retrieval was crucial.

Although the absence of seasonal changes in relative hippocampal volume reported here seems to conflict with the results of Smulders *et al.*,¹⁷ effects found in free-ranging birds may be due to some of the extraneous variables, such as seasonal population or nutritional differences, that were controlled for in this experiment. Furthermore, as Barnea and Notte-

bohm¹⁸ noted, free-ranging chickadees encounter a number of new cognitive demands in autumn in addition to the need to remember locations of stored food. They move into new territories and form new social groups, and the nature of their food supply changes. Contributions of such variables could be assessed by including a control species that does not store food in future studies.

There are several reasons why photoperiodically-induced changes in food-storing behaviour in the laboratory might not be accompanied by the same changes in hippocampus seen in birds taken directly from the wild at different times of year,¹⁷ when they are assumed to be engaging in different amounts of food-storing. (1) Seasonal changes in hippocampus may require relevant experience, i.e. storage and memory-based retrieval of food in a spatially complex environment. (2) Changes in hippocampus in free ranging birds may reflect, at least in part, seasonal changes in cognitive demands other than those imposed by food-storing. (3) Hippocampal differences among groups caught at different times of year may reflect nutritional, population, or other non-cognitive variables. Experiments to distinguish these possibilities could profitably combine the complementary approaches of taking birds from the wild at different seasons and manipulating factors in the laboratory that are correlated with season in nature.

References

- Shettleworth SJ. *Phil Trans Roy Soc (Lond) B* 329, 143–151 (1990).
- Sherry DF and Vaccarino AL. *Behav Neurosci* 103, 308–18 (1989).
- Krebs JR, Sherry DF, Healy SD *et al.* *Proc Natl Acad Sci USA* 86, 1388–92 (1989).
- Sherry DF, Vaccarino AL, Buckenham K *et al.* *Brain Behav Evol* 34, 308–17 (1989).
- Bingman VP. Vision, cognition, and the avian hippocampus. In: Ziegler HP and Bischof H-J, eds. *Vision, Brain and Behaviour in Birds*. Cambridge, MA: MIT Press, 1993: 391–408.
- Odum EP. *Auk* 59, 499–531 (1942).
- Haftorn S. *K Norske Vidensk Selsk Skr* 3, 1–79 (1956).
- Lucas JR, Peterson LJ and Boudinier RL. *Anim Behav* 45, 639–58 (1993).
- Hurly TA. *Behav Ecol* 3, 181–8 (1992).
- Ludescher FB. *Okol Vogel* 2, 135–44 (1980).
- Shettleworth SJ, Hampton RR and Westwood, RP. *Anim Behav* 49, 989–98 (1995).
- Galea LAM, Kavaliers M, Ossenkopp KP *et al.* *Brain Res* 635, 18–26 (1994).
- Jacobs LF and Gaulin SJC. Meetings of the Animal Behaviour Society (1993).
- Yaskin, VA. Seasonal changes in brain morphology in small mammals. In: Merritt JF, ed. *Winter Ecology of Small Mammals*. Pittsburgh, PA: Carnegie Museum of Natural History, 1984: 183–91.
- Cynx J and Nottebohm F. *Proc Natl Acad Sci USA* 89, 1368–1371 (1992).
- DeVoogd TJ. The neural basis for the acquisition and production and bird song. In: Hogan JA & Bolhuis JJB, eds. *Causal Mechanisms in Behavioural Development*. Cambridge: Cambridge University Press, 1994: 49–81.
- Smulders TV, Sasson AD and DeVoogd TJ. *J Neurobiol*, In press.
- Barnea A and Nottebohm F. *Proc Natl Acad Sci USA* 91, 11217–21 (1993).
- Smith SM. *The Black-capped Chickadee*. Ithaca, NY: Cornell University Press, 1991.
- Clayton NS. *Behav Brain Res*, 70, 95–101 (1995).
- Clayton NS, Krebs JR. *Proc Natl Acad Sci USA* 91, 7410–4 (1994).

ACKNOWLEDGEMENTS: Supported by the Agricultural and Food Research Council, the Royal Society, the Science & Engineering Council, the Natural Sciences and Engineering Research Council of Canada and NATO. Chickadees were held under CWS Scientific Capture Permit EC 0316. We thank Bill Brown, Rick Westwood, Mike Child, Andrew Gristock and Jenny Corrigan for help, and Dan Cristol, Sue Healy and an anonymous reviewer for comments.

Received 19 April 1995;
accepted 13 May 1995