Seasonal Changes in Neuron Numbers in the Hippocampal Formation of a Food-Hoarding Bird: The Black-Capped Chickadee

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ABSTRACT: The volume of the hippocampal formation (HF) in black-capped chickadees (*Poecile atricapillus*) varies across the seasons, in parallel with the seasonal cycle in food hoarding. In this study, we estimate cell density and total cell number in the HF across seasons in both juveniles and adults. We find that the seasonal variation in volume is due to an increase in the number of small and large cells (principally neurons) in the fall. Adults also have lower neuron densities than juveniles. Both juveniles and adults show an increase in cell density in the rostral part of the HF in August and a subsequent decrease toward October. This suggests that the net cell addition to the HF may already start in August. We discuss the implications of this early start with respect to the possibility that the seasonal change in HF volume is driven by the experience of food hoarding. We also speculate on the functional significance of the addition of neurons to the HF in the fall. J Neurobiol 44: 414–422, 2000

Keywords: **food caching; spatial memory; hippocampus; cell number; cell density; development**

The hippocampal formation (HF) in both mammals and birds is involved in spatial cognition. This has been shown extensively in laboratory studies using pigeons, rats, and monkeys (Good and Macphail, 1994; Nadel, 1991; O'Keefe and Nadel, 1978; Robertson et al., 1998). The role of the HF in spatial navigation has also been demonstrated in natural real-world models, such as in homing pigeons (Bingman et al., 1984, 1985, 1987, 1988a,b; Rehkämper et al., 1988), brood-parasitic birds (Sherry et al., 1993; Reboreda et al., 1996; Clayton et al., 1997), and London taxi drivers (Maguire, 1997; Maguire et al., 1997, 2000). Scatterhoarding birds and mammals rely on spatial memory to relocate their many small caches (Sherry et al., 1981; Shettleworth and Krebs, 1982; Jacobs and Liman, 1991; Jacobs, 1992). Associated with this reliance on spatial memory, these species have a larger HF (with more neurons; Healy and Krebs, 1993; Healy et al., 1994) than non–scatterhoarding-related species [e.g., desert rodents, Jacobs and Spencer (1994); woodpeckers, Volman et al. (1997); and songbirds, Krebs et al. (1989) and Sherry et al. (1989)]. When comparing different scatterhoarding species to each other, the ones that rely more on hoarded food have a larger relative HF volume than those that do less so (Healy and Krebs, 1992, 1996; Hampton et al., 1995; Basil et al., 1996).

The HF volume of black-capped chickadees (*Poecile atricapillus*), a scatterhoarding bird, varies seasonally. Chickadees have a larger HF relative to telencephalon size in October, at the peak of their food-hoarding behavior, than they do at other times of the year (Smulders et al., 1995). This change in volume could be caused by different underlying physical factors. First, the density of neurons could be lower in October, resulting in a higher mean distance between neuronal cell bodies. This change in neuronal density

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Table 1 Sample Sizes for the Different Groups, Subdivided by Age Category

Group Dates	April 03/28–04/09	June 05/18–07/10	August 08/13–08/18	October 10/30–11/05	December 12/17–12/18
Adults	6	5	5	4	6
Juveniles	—	5	10	3	—

could be due either to larger dendritic arborization of the neurons or to an addition of glial cells. Alternatively (or in addition), there could be more neurons in October. Barnea and Nottebohm (1994) found that more newly generated neurons are incorporated in the HF in October than at other times of the year (although they did not report a change in total neuron number). Both an addition of cells and an expansion of the dendritic arborization would result in an enlarged network to cope with the increased demand for spatial processing. Both mechanisms have been documented in the seasonal variability of the song control system in songbirds (DeVoogd et al., 1985; Brenowitz et al., 1991; Smith et al., 1997a,b; Tramontin et al., 2000). This study investigates whether the seasonal variation found in the volume of the HF in black-capped chickadees is due to neuronal addition or to dispersion of the existing neurons.

MATERIALS AND METHODS

Subjects

Subjects were black-capped chickadees, caught near Ithaca, New York under State and Federal permits. A total of 44 birds were caught at 5 different time points of the year (Table 1). These were the same individuals that were used in a previous study (Smulders et al., 1995). In the winter months, birds were caught with Potter's traps baited with food, and in the summer with song playback and mistnets. All birds were taken into the laboratory on the day they were caught; age and sex were determined after perfusion. The birds were divided into two age classes, based on the separation of the two skull layers. Skulls of adult songbirds are made up of two layers of bone. Juvenile birds develop this separation over the first few months of life. In chickadees, this process is completed by the first fall (October/November) (Smith, 1991). Therefore, all the birds classified as juveniles had hatched in the same year they were caught, and we were only able to accurately age birds in our June, August, and October samples. We assumed the birds to be adults for the other two time points. Our June juveniles were caught in late June and early July, so they were likely very recently fledged.

Histology

Birds were perfused transcardially with 0.8% saline and 10% formalin in 0.8% saline. The heads were then postfixed in 10% formalin/0.8% saline for at least 1 day, after which the brain was removed from the skull, weighed, and allowed to postfix for at least another day in formalin/saline. The brain was then transferred to 10% formalin in 30% sucrose, until it sank (2–3 days). It was weighed again and embedded in 10% gelatin/30% sucrose, which was hardened in 10% formalin/30% sucrose. The weight change during immersion in sucrose was used as a crude measure of shrinkage. The brains were then sliced on a freezing microtome at 40 μm and transferred to microscope slides. Alternate sections were stained with Cresyl-violet stain and coverslipped with Permount (Fisher, Pittsburgh, PA) or Eukitt (EMS, Fort Washington, PA).

Volume Measurements and Cell Counts

Volumes of HF and nucleus Rotundus (Rt) used to estimate total cell numbers are the same volumes calculated by Smulders et al. (1995). For most sampling regions, cells were counted with a 20× objective and a 15× ocular containing a counting grid (for the exception to this rule, see below). At this magnification, the counting grid covers an area 0.2 × 0.2 mm, subdivided into a 10 × 10 grid. A cell was included in the count if at least one nucleolus was visible within its nucleus. This nucleolus had to be inside the grid. If the nucleolus was positioned on the outer edge of the grid, it was counted if this was the right or bottom edge, but not if it was the left or top edge. We focused through the section and counted all cells that fulfilled our criteria. The HF is an anatomically heterogeneous structure. Therefore, rather than counting in randomly placed grids, we decided to count cells in well-defined areas that could be reproduced on the basis of anatomical landmarks in each brain. Because the size of the particles counted (nucleoli) was small relative to section thickness, our method yields similar results to those obtained with stereological techniques such as the optical dissector (DeVoogd et al., 1991; Tramontin et al., 1998). Based on their size, the cells could easily be separated into two categories in our Nissl-stained tissue. Large cells had a soma size larger than one quarter of a grid unit or 100 μm^2. They had a clear nucleoplasm and were most likely neurons (Barnea and Nottebohm, 1994; Patel et al., 1997). Small cells (soma size smaller than a quarter of a grid unit) could be either neurons or glia. We counted the two cell classes separately.

Cell densities were determined at three rostrocaudal levels in HF and at one level in Rt. We counted in equivalent positions in both the left and the right hemispheres. The rostral-most level in HF was the section in which the lateral boundary of the HF coincided with the lateral corner of the ventricle. We placed the counting grid in the middle of the

dorsal half of HF (demarcated by the surface of the brain, the medial edge of the brain, the lateral edge of HF, and the line determined by the dorsal-most part of the ventricle). A second counting grid was positioned against the medial edge of the HF, with its ventral edge on the same line defined by the top of the ventricle [Fig. 1(A)]. The caudal-most level was the first section (going from rostral to caudal) at which the two hemispheres separated at the dorsal surface to make space for the cerebellum. In this section, one counting grid was placed in the dorsomedial corner of the HF, and another one in the very center of the HF at this level [Fig. 1(C)].

The third level was determined as the section that was exactly halfway between the rostral and the caudal level, as defined in the previous paragraph. At this level, one grid was placed at the lateral boundary, halfway between the surface of the brain and the ventricle, another one at the same dorsoventral level, but at the medial edge, and a third one midway between those two. A fourth grid was positioned in the ventral HF, against the medial edge of the brain, halfway between the dorsal and ventral boundaries of the medial arm of the "V"-shaped zone of high cell density (Krebs et al., 1989, 1991; Erichsen et al., 1991) [Fig. 1(B)]. In the lateral arm of the "V," cell density is even higher than in the medial arm, and this could significantly affect our estimates of total cell numbers. To obtain reliable counts in this region, we used a 100× oil-immersion objective and counted cells in three counting grids (representing 0.04 × 0.04 mm each) in the right hemisphere at each of the three rostrocaudal levels. One counting grid was placed at the intersection of the lateral and medial arms of the "V." Another grid was placed at the lateral-most extent of the lateral arm. A third grid was placed equidistant between the other two counting grids (Fig. 1). We used the same criteria of inclusion as described earlier, and we only included large cells. Cell counts in Rt were done in the center of the cross section of the nucleus, four sections (approximately 320 μm) from the rostral end of the nucleus, using the 20× objective.

We calculated the average cell density at each level separately for the cell-dense area (i.e., the lateral arm of the "V") and for the cell-sparse area (the rest of the HF), by dividing the number of cells counted by the total volume in which they were counted. To obtain an estimate of total cell density at each level, we then calculated a weighted average of these two densities, based on the proportion of the HF taken up by the lateral arm of the "V." This proportion was measured at the middle level by outlining the cell-dense area and measuring its surface area as well as the surface area of the entire HF at this level. Finally, to estimate the total cell number in the HF, we took the average cell density of the three rostrocaudal levels and multiplied this by the total volume of HF, as calculated in the previous study (Smulders et al., 1995). Cell numbers in Rt were calculated in the same way. We calculated cell density and number for the large and small cells separately, as well as for the two classes combined. The large cells are neurons, based on their morphology. The small cells can be either neurons or glia.

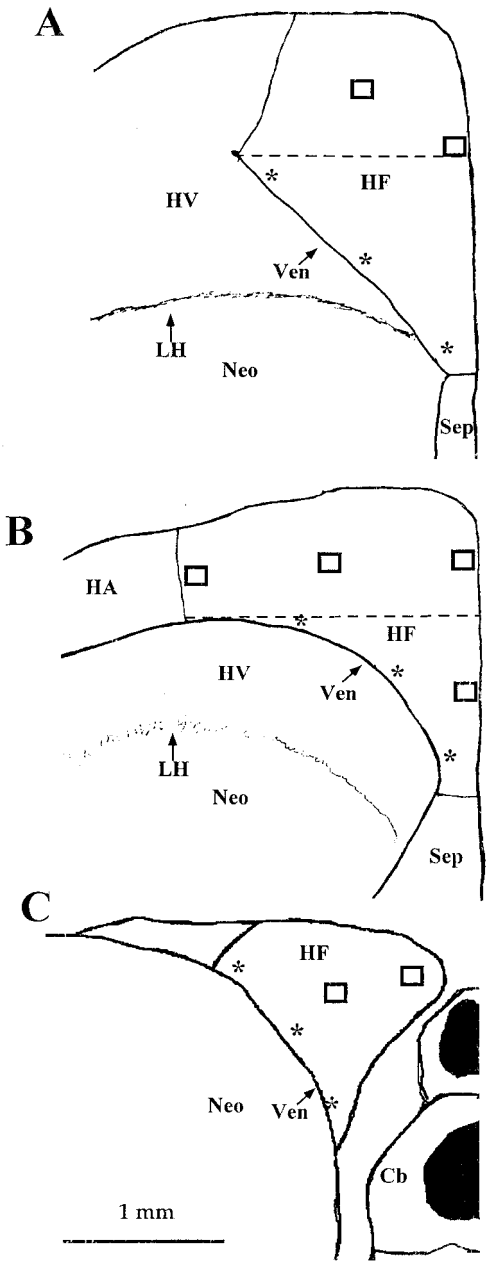

Figure 1 Drawings of the three levels at which cells were counted in the HF (A: rostral; B: middle; C: caudal). The rectangles represent the positions of the counting grid for counts performed at 20×, and the asterisks the equivalent positions of those counted at 100× (in reality, these counts were performed in the right hemisphere). The horizontal dashed line in the HF represents the separation between the dorsal and ventral portions. Cb, cerebellum; HA, hyperstriatum accessorium; HF, hippocampal formation; HV, hyperstriatum ventrale; LH, lamina hyperstriatica; Neo, neostriatum; Sep, septum; Ven, ventricle.

Statistical Analyses

All statistical analyses were done using the package Systat 5.2.1 on an Apple Macintosh LC III. The main statistical

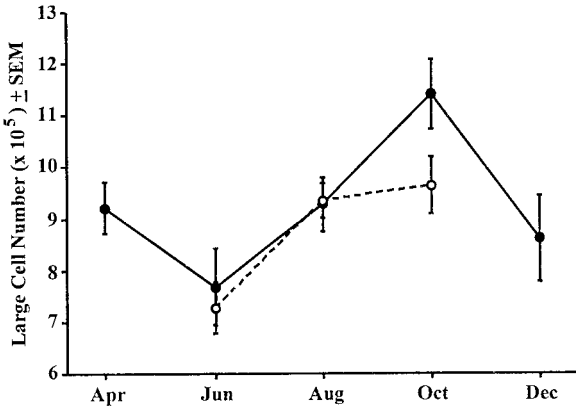

Figure 2 Estimated large cell (neuron) number in the HF is higher in October than at other time points. The June sample has the fewest large cells in the HF. Juveniles (open symbols) have similar large-cell numbers to adults (closed symbols) throughout the year.

technique used was the General Linear Model. This technique allows us to explain the variation in a continuous dependent variable by a linear combination of independent variables, which can be continuous (like a cell count) or discrete (like a grouping variable, e.g., age category). Such a model tests for effects of each of the independent variables, while keeping the other independent variables constant. When we mention effects of several independent variables on a dependent variable, they are always the result of one such model, unless mentioned otherwise. Results are considered statistically significant for $p < .05$. A concern when analyzing cell densities is the possibility that differential shrinkage may have occurred among the different groups during histology. To control for this, we used the percentage of weight change during immersion in sucrose as an additional independent variable in the statistics. Because shrinkage has its effect on both volume (smaller) and cell density (higher), the two cancel out when calculating total cell numbers. To control for brain size, we used the total number of neurons (i.e., large cells) in Rt as an independent variable when analyzing HF cell numbers.

RESULTS

Cell Numbers

There is a significant difference in the total number of large cells (neurons) in the HF of birds obtained at different times of the year [$F(4,31) = 6.096$; $p = .001$]. A Fisher LSD post hoc test shows that October birds have significantly more large cells than those at any other times of the year, with the possible exception of the August birds ($p = .0523$ for the pairwise comparison between October and August). June birds have the smallest number of neurons (Fig. 2). The total number of large cells does not differ between sexes or age classes, nor is there a significant interaction between age and season. This means the seasonal pattern is similar for juveniles and adults. October birds have approximately 1,065,000 ± 55,000 (SEM) large cells in their HF, whereas the mean for other times of the year is about 862,000 ± 26,000 (SEM). There are 3.3 times more large cells than small cells in the HF (repeated measures GLM: $F(1,32) = 27.631$; $p < .001$). Analysis on the small cells by themselves reveals the same pattern as for the large cells: October birds having the most cells (374,000 ± 48,000 (SEM), and June birds having fewest (178,000 ± 15,000, SEM; $F(4,32) = 7.199$, $p < .001$).

In n. Rotundus, there is no seasonal difference in the number of large cells making up the nucleus. There are also no differences between the sexes or age classes. The number of small cells does change across different times of year [$F(4,37) = 3.100$; $p = .027$]. A Fisher LSD post hoc test showed that there are significantly more small cells in June than at other times. In addition, females have more small cells than males [$F(1,37) = 7.138$; $p = .011$] and adults have more than juveniles [$F(1,37) = 5.666$; $p = .023$]. There are approximately twice as many small cells (127,000 ± 6,460, SEM) in Rt than there are large cells (55,500 ± 2,050, SEM) [repeated measures GLM: $F(1,37) = 81.762$; $p < .001$].

Overall Cell Densities

The average density of large cells in HF shows no significant seasonal variation. Juveniles have higher large-cell densities than do adults [$F(1,31) = 7.269$; $p = .011$]. There is no difference between males and females. There are no significant differences between any groups in the densities of small cells. In n. Rotundus, small cell-density is higher in females than in males [$F(1,37) = 9.716$; $p = .004$]. There are no other significant differences in cell densities (large or small) in Rt.

Cell Densities at the Different Levels of the HF

In all birds, large cells are packed more densely at the caudal end of the HF than at the rostral or middle levels [repeated measures GLM, $F(2,62) = 12.682$; $p < .0001$]. At the rostral-most level, juveniles' large cell densities are 32% higher than those of adults [$F(1,32) = 13.072$; $p = .001$] and there is a significant change in large-cell density across the different times of the year [$F(4,32) = 10.938$; $p < .001$]. A Fisher LSD post hoc test shows that this seasonal effect is due to a higher large-cell density in

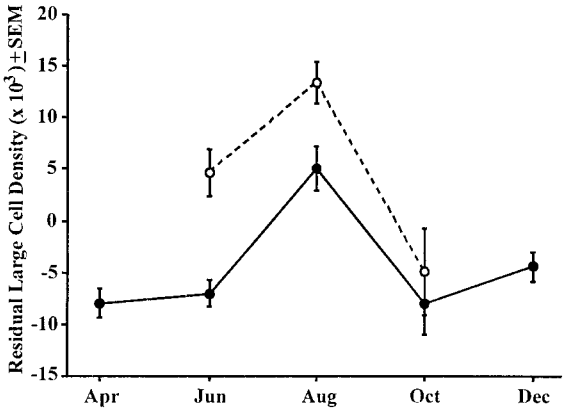

Figure 3 Estimated density of large cells (large cells per mm^3) in the rostral part of the HF is higher in August than at other times of year. Juveniles (open symbols) have a higher large cell density than adults (closed symbols). We plot the residuals from the regression that controls for the effects of shrinkage on cell densities (see Materials and Methods).

the rostral area in the August group (Fig. 3). This effect is not picked up in the analysis of overall cell densities because it is averaged out by the cell densities at the other two sampling levels. At the middle level, there are no seasonal changes, but juveniles have a 24% higher large-cell density than adults [$F(1,35) = 7.705$; $p = .009$]. There are no significant differences across any groups in the density of large cells at the caudal-most level. There are also no differences in small-cell densities at any level.

DISCUSSION

The seasonal change in HF volume in black-capped chickadees is due to an increase in total cell number during the hoarding peak in October. This increase takes place in both large cells (neurons) and small cells (possibly both neurons and glia). Large-cell number in the control nucleus (n. Rotundus) shows no seasonal changes. Juvenile chickadees (from fledging to their first autumn) have similar numbers of large cells (neurons) in the HF as adults at the same times of year, but these are packed more densely, resulting in a smaller volume.

The Mechanism of Cell Addition

Barnea and Nottebohm (1994, 1996) found that there are more neurons being added to the HF of adult birds in the fall than at other times of the year. They did not find any differences in overall neuron number across the season. From this lack of seasonal change in cell number, they concluded that neuronal turnover, rather than neuronal addition was crucial to deal with the increased spatial information processing requirements. They suggested that storing memories permanently alters neurons in such a way that they become useless for future processing. In order to process new information, these neurons would then need to be replaced (Barnea and Nottebohm, 1996). At first sight, their results seem to differ dramatically from ours. How can we explain this discrepancy? The population they studied was supplemented with food year-round and lived slightly further south than did ours. But even though most of our birds came from populations that did not have access to human-supplied food, some did, and the effects persisted even for those birds. A second possibility is that the different histological techniques used lead to varying amounts of shrinkage, but this should not affect estimates of total cell number.

The differences between our studies may be an artifact due to the timing of when the birds were collected. Both studies have birds that are called the "October group." In the Barnea and Nottebohm (1994) study, these were birds injected with ^{3}H-thymidine during the month of October, but not killed until at least 6 weeks later. Assuming birds were injected around mid-October, that would put the perfusion date in late November or maybe even early December, after the peak time for hoarding (and close to the time of our "December" sample). In contrast, our October group was perfused at the end of October. Our December sample (which was taken in mid-December) also had lower cell numbers than our October sample. This could explain why Barnea and Nottebohm (1994) did not find more cells in their October group. If this proposed scenario of a net cell loss at the end of the hoarding peak is true, we would expect a large peak in apoptosis to occur around that time. This prediction remains to be tested.

Are There Changes in any Subdivisions of HF?

We did not find an objective and reliable method, based on morphological landmarks, to split the HF into different rostrocaudal regions *a priori* so we could calculate the changes in cell numbers or volumes in different subdivisions. We were, however, able to compare cell densities at various rostrocaudal, mediolateral, and dorsoventral sites. Juveniles have higher overall cell densities than adults. We observed an interesting rostrocaudal gradient in these comparisons: the differences in density were higher at the rostral level than at the middle level, and they were not significant at the caudal-most level. This pattern

suggests that most developmental changes may be taking place in the more rostral parts of the HF. The seasonal changes may also mainly take place in the rostral part of the HF. The density of neurons at this level was higher in August birds than in any other group. This could represent the start of the seasonal increase in cell number. Barnea and Nottebohm (1994) found that most neurogenesis takes place at the rostral end of the HF. They showed a start of increasing neurogenesis in the birds that were injected as early as August and September. If this increase in neurogenesis starts in August in the rostral part of the HF, it could be expected that for a while, neuron density would be higher, before the new cells completely differentiate and grow out their dendritic arbors. When this eventually happens, the HF expands in volume, as we have found in the October birds, and rostral cell density returns to its original value.

Is the Increase in Neuron Number Experience Dependent?

During development, HF size and neuron number of food-hoarding birds is dependent on their experience with hoarding and retrieving (Clayton and Krebs, 1994; Clayton, 1995, 1996; Patel et al., 1997). It is not clear whether this is the case for the seasonal changes in adult birds as well. Our data suggest that neurons start being added to the HF around mid-August. At that time, flock formation has hardly started (Smith, 1991), and the large hoardable crops are not yet available, so the birds have not yet experienced much hoarding. During development in captivity (and presumably in the field as well), even limited experience with hoarding can initiate increased cell proliferation (Patel et al., 1997). However, much more substantial hoarding and retrieval experience in captivity by adults had no effect on HF size (Cristol, 1996). This would suggest to us that hoarding experience is not involved in initiating the process of seasonal cell addition. If hoarding behavior itself does not initiate the increase in HF size in adults, then what would? Decreasing photoperiod would be a logical candidate. Decreasing the daylength triggered food-hoarding behavior in the laboratory (Shettleworth et al., 1995), but it was not able to trigger an associated change in HF volume (Krebs et al., 1995). Perhaps, neurons were being added at a higher rate (triggered by decreasing daylength), but they were not retained long enough to result in an increase in HF volume. Even though the birds were storing food, they did not need to use memory to retrieve the caches, as they were storing in their home cages (Krebs et al., 1995). It is possible that it is the use of memory during storing and retrieving that maintains the newly generated neurons throughout the hoarding season. This could also account for the decrease in neuron number in winter, as large supplies of food are no longer available for caching. Alternatively, it may be that Krebs et al. (1995) did not observe an effect of photoperiod on HF volume because they worked with captive birds. Barnea and Nottebohm (1994) also had a group of captive chickadees, and they found that neurogenesis was decreased significantly in captive birds. In a recent study on dark-eyed juncos (*Junco hyemalis*) Smulders et al. (2000) also found that captive birds had a smaller relative HF size than free-flying birds. These explanations are not necessarily independent: the observed effect of captivity could be a consequence of a lack of experience with spatial information processing.

Age Differences

We find that juvenile birds have a higher packing density of neurons than do adults, but they do not seem to differ in the total number of neurons contained within the HF. This is in agreement with Healy and colleagues (Healy and Krebs, 1993; Healy et al., 1994) who found that nestling marsh tits (*P. palustris*), as well as nestling magpies (*Pica pica*), had the same number of neurons in the HF as their adult conspecifics, but the neurons were packed more densely in the juveniles. This suggests that posthatching development of the avian HF consists mainly of an expansion of existing neurons, rather than an addition of new ones. Our results also suggest that juvenile birds add neurons from summer to fall, paralleling the adult seasonal changes in cell number. By the end of the summer, there are no significant differences between adults and juveniles in either packing density or cell number. Barnea and Nottebohm (1996) counted cells from birds killed at the end of September and found that juveniles have a larger (or at least similar sized) HF than do adults and have a higher packing density of neurons. They concluded from this (but did not do the actual calculations individual by individual) that juveniles at 4.5 months of age have more cells in the HF than do adults. They also found a higher proportion of newly generated cells in juveniles than in adults. They suggested an overproduction of neurons in the first few months of life, which allows the birds to cope with the wide range of changes taking place in their environment (including dispersal and joining a new flock). We do not see such an effect in our data. As we have pointed out before, however, we only have data from mid-August and from the end of October. Therefore, the discrepancy could again be due to a different time of sampling. Possibly, juveniles start increasing their cell numbers

slightly earlier than adults in the fall, a head-start picked up on by Barnea and Nottebohm (1996).

Changes in Small Cells Number in Nucleus Rotundus

The volume of Rt shows some seasonal variation, being larger in the summer in adults (Smulders et al., 1995). The current study shows that this increase is largely due to an increase in the number of small cells (most likely glia) in June. Large cells do not show this pattern. We also found that females have more small cells than males. They do not have a larger Rt, however, since the increase in cell number is accompanied by an increase in cell density. Adults have more small cells than do juveniles. The overall pattern suggests the involvement of gonadal steroids. Goldman and colleagues (Goldman and Nottebohm, 1983; Hidalgo et al., 1995) found that steroid treatment increases both the number of glia and endothelial cells in HVC, a song control nucleus, in female canaries. It is possible that similar effects on nonneuronal cell populations take place in other parts of the brain as well.

Functional Significance

What is gained by having more neurons in the HF during the hoarding season? We would like to propose the following hypothesis. Recent results looking at the expression of immediate early genes (IEGs) in black-capped chickadees during hoarding and retrieval (Smulders and DeVoogd, 2000) suggest that when more items are recalled more accurately, more cells in the HF are activated to process the information. Logically extending this result, one could argue that if a really large amount of information needed to be processed, a larger neuronal network would be needed. The present study shows that during the fall (but not during winter), black-capped chickadees have such a larger neuronal network. Why would they need to process such large amounts of spatial information during the fall? The available evidence suggests that parid species cannot remember their cache locations for more than 4–5 weeks (Hitchcock and Sherry, 1990; Brodin, 1994a). This is not long enough to remember the locations from the hoarding peak in the fall all throughout winter. However, parids do use their caches all winter long (Haftorn, 1954; Haftorn, 1956a,b; Higuchi, 1977; Pravosudov, 1985; Brodin, 1993a,b; Brodin and Clark, 1997).

How do they manage to retrieve their hoards after so many months without remembering them? It has been suggested that during winter, they encounter their caches while regularly foraging, relying on the fact that each bird has distributed its caches throughout its own individual winter foraging niche (Pravosudov, 1986; Brodin, 1994b; Lens et al., 1994; Brodin and Clark, 1997; Smulders, 1998). By distributing caches as uniformly as possible in the fall, loss to pilferers is minimized (Sherry et al., 1982). Such a distribution can only be achieved if a bird is able to remember all existing cache sites, but it only needs to do this for the duration of the hoarding season (Smulders and Dhondt, 1997; Smulders, 1998). We argue that this is why the increase in neuron number takes place during the hoarding season only. Birds continue to store and retrieve seeds throughout winter, but since these are smaller amounts (maybe for use as short-term emergency food supplies; Brodin and Clark, 1997), a large memory capacity (and presumably a larger number of neurons) is not needed to maintain this behavior.

CONCLUSIONS

In the fall, black-capped chickadees add new cells (neurons and glia) to their hippocampal formation. The increase in neuron number provides a larger neural network with which to process information about a large number of cache locations. This may be necessary to more efficiently distribute food items, which will be available in the coming winter months. The increase in neuron number is probably not experience dependent, but driven by seasonal cues that predict the upcoming fall. Maintenance of the larger number of neurons in the HF during the hoarding season, on the other hand, could be experience dependent, and cell numbers decrease at the start of winter, when hoarding behavior is decreased. Fledgling chickadees have numbers of neurons similar to adults, but they are packed together at a higher density. During late summer, these neurons presumably expand their dendritic arbors and take on adult densities.

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