Greater Hippocampal Neuronal Recruitment in Food-Storing Than in Non-Food-Storing Birds

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ABSTRACT: **Previous research has shown heightened recruitment of new neurons to the chickadee hippocampus in the fall. The present study was conducted to determine whether heightened fall recruitment is associated with the seasonal onset of food-storing by comparing neurogenesis in chickadees and a non-food-storing species, the house sparrow. Chickadees and house sparrows were captured in the wild in fall and spring and received multiple injections of the cell birth marker bromodeoxyuridine (BrdU). Birds were held in captivity and the level of hippocampal neuron recruitment was assessed after 6 weeks. Chickadees showed significantly more hippocampal neuronal recruitment than house sparrows. We found no seasonal differences in hippocampal neuronal recruitment in either species. In chickadees and in house sparrows, one-third of new cells labeled for BrdU also expressed the mature neuronal protein, NeuN. In a region adjacent to the hippocampus, the hyperpallium apicale, we observed no significant differences in neuronal recruitment between species or between seasons. Hippocampal volume and total neuron number both were greater in spring than in fall in chickadees, but no seasonal differences were observed in house sparrows. Enhanced neuronal recruitment in the hippocampus of food-storing chickadees suggests a degree of neurogenic specialization that may be associated with the spatial memory requirements of food-storing behavior.** © 2007 Wiley Periodicals, Inc. Develop Neurobiol 67: 406–414, 2007

Keywords: **food-storing; hippocampus; neurogenesis; neuronal recruitment**

INTRODUCTION

It has previously been reported that more new neurons are recruited into the hippocampus of adult black-capped chickadees (*Poecile atricapillus*) in the fall than at other times of year (Barnea and Nottebohm, 1994). Chickadees undergo a number of behavioral and ecological changes in fall that are correlated with this peak in hippocampal neurogenesis. In fall, the proportion of seeds in the diet of chickadees increases and the proportion of insects decreases (Smith, 1991). The chickadee social system changes from breeding pairs to winter flocks and this transition results in a threefold increase in home range size. The structure and appearance of the habitat also changes as deciduous trees lose their leaves and snowfall occurs. Finally, food-storing by chickadees increases markedly in fall. To determine which of these correlated changes best predicts the occurrence of seasonal neurogenesis in the passerine hippocampus, we compared the frequency of neuron recruitment in fall and spring in two species of north temperate zone winter residents: black-capped chickadees and house sparrows (*Passer domesticus*).

Chickadees and house sparrows undergo comparable changes in diet, social system, home range, and habitat in fall. Insects make up a larger component of the diet of chickadees than house sparrows, but the relative size of the insect component decreases for both species. The proportion insects in the chickadee diet decreases from 80 to 90% in summer to about

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50% in fall as insects become less available (Smith, 1991). Insects constitute up to 10% of the house sparrow diet in summer, but this also decreases in fall with decreasing availability of insects (Lowther and Clink, 1992). Both species form dominance-structured winter flocks (Smith, 1991; Lowther and Clink, 1992). Neither chickadees nor house sparrows are migratory, though both experience an increase in the size of their winter home range. Chickadees and house sparrows are common in fall and winter in eastern North America and both experience the same seasonal changes in the appearance of their habitat. Only chickadees, however, store food. If the onset of food-storing activity is associated with the fall increase in recruitment of new neurons to the hippocampus, then seasonal recruitment should be more pronounced in chickadees than in house sparrows.

The present study was conducted to examine hippocampal neuron recruitment in chickadees and house sparrows during fall and spring. Birds were captured in the wild, injected with the cell birth marker BrdU (5-bromo-2′-deoxyuridine), and sacrificed 6 weeks later. The development of newly recruited hippocampal cells was assessed by colabeling for BrdU and NeuN (neuronal nuclei specific protein), a protein expressed specifically by mature neurons (Mullen et al., 1992). By staining for NeuN we were also able to investigate seasonal fluctuations in hippocampal volume and neuron number.

METHODS

Subjects

Fifteen black-capped chickadees (BCCH, mean body weight 10.55 g) and nine house sparrows (HS, mean body weight 27.44 g) were captured between October 2003 and November 2004 from field locations within a 20 km radius of London, Ontario, Canada (43.0° N 81.2° W). Birds were captured in two rounds through the year. Birds caught between October 15th and November 25th were treated as one group of birds (Fall group, caught between fall equinox and winter solstice) and birds caught between February 28th and April 11th were treated as a second group of birds (Spring group, caught both before and after spring equinox). Birds had not previously been banded, so it was not possible to unequivocally determine whether birds were in their year of hatch or older (Smith, 1991). Birds were group housed in a large outdoor aviary and experienced natural photoperiod and temperature while in captivity. The aviary contained nest boxes and evergreen boughs for cover, tree branches, and small wooden blocks in which holes had been drilled to provide additional caching sites. All birds were provided with water and powdered Mazuri mix small bird formulation *ad libitum*. Chickadees received hulled and whole sunflower seeds and house sparrows received millet and cracked corn *ad libitum*.

BrdU Administration and Immunohistochemistry

All birds received injections of BrdU (Sigma, St. Louis, MO) over 2 days, with injections commencing the day following capture. Birds received three injections of BrdU per day delivered 2-h apart for a total of six BrdU injections. All injections were administered to the pectoral muscle at a dose of 75 μg/g in 0.1 *M* phosphate-buffered saline (PBS; pH = 7.4; 0.005 mL/g body weight of BrdU solution prepared as 0.015 g/mL). Birds were sacrificed 6 weeks following the final BrdU injection. This interval between BrdU administration and sacrifice was selected to allow comparison with Barnea and Nottebohm's (1994) seasonal study of hippocampal neuron recruitment in chickadees. At the time of sacrifice, birds were anesthetized with a 0.03–0.05-mL injection of a 1:1 mixture of ketamine (100 mg/mL) and xylazine (20 mg/mL) and transcardially perfused with 0.1 *M* PBS followed with 4% paraformaldehyde in PBS. Brains were removed and immersed in a 4% paraformaldehyde solution in PBS for $\sim$20 h, at which time the brains were placed in a vial filled with a 30% sucrose solution for cryoprotection. When the brains sank to the bottom of the vial (2–3 days after immersion), they were frozen in isopentane at -40°C.

Brains were sectioned on a cryostat in the coronal plane at 30-μm thickness. Brains were cut and processed in batches of four over a 2-month period and as often as possible a brain from each of the four groups was included in each batch processed. Four sets of sections were collected from each brain (every 12th section through the brain was collected per set) with sections in each set spaced 360 μm apart. All processing was carried out on free-floating sections. For each brain one set of sections was stained to detect BrdU-immunoreactivity and a second set was stained to visualize NeuN-immunoreactivity using DAB. The third and fourth sets of sections were immersed in a cryoprotectant solution for subsequent processing. NeuN is a DNA-binding protein expressed specifically by mature neurons (Mullen et al., 1992). In characteristic staining for NeuN protein in mice, the highest levels of expression are typically observed in the nucleus of the neuron, though some expression of NeuN protein may be observed, to a much lesser degree, in the cytoplasm and no expression is detected in the nucleolus (Mullen et al., 1992; Lind et al., 2005). Relative intensities of nuclear and cytoplasmic NeuN staining can show substantial variability. Lind et al. (2005) reported differential cytoplasmic staining in different cell types of the mouse hippocampus. In NeuN labeling of the dentate gyrus of rats and humans, some neurons show substantially darker staining of the ctyoplasm while in other neurons nuclear staining may be absent (Kuhn et al., 1996; Eriksson et al., 1998). In the current study, NeuN protein was labeled to permit estimation of total neuron numbers, to define the boundaries of the hippocampus

for volume estimation, and to assess the neuronal identity of hippocampal BrdU-labeled cells. Use of a neuron-specific antigen permits clear delineation of the boundaries of the hippocampus and other structures in the avian brain (Tramontin and Brenowitz, 1999; Hoshooley and Sherry, 2004). Staining for BrdU and NeuN with DAB immunohistochemistry, using mouse anti-BrdU, 1:35 (BD Biosciences, Cat. No. 347580) and mouse anti-NeuN, 1:500 (Chemicon, Cat. No. MAB377) was carried out using the protocol described in Hoshooley and Sherry (2004).

Some brains were also processed using immunofluoresence to detect colocalization of BrdU and NeuN to estimate the proportion of new cells that had assumed a mature neuronal phenotype 6 weeks following cell birth marker administration. Alternate sections were taken from the third set of sections for eight chickadee brains and six house sparrow brains showing highest levels of hippocampal neuron recruitment in BrdU DAB stained sections, and were processed to detect coimmunoreactivity for BrdU and NeuN. Sections were transferred out of cryoprotectant solution and washed thoroughly in PBS. All steps were carried out at room temperature unless otherwise stated. Following washing, sections were immersed in 2 *N* HCl for 40 min. Sections were next washed in a solution of 0.1 *M* sodium borate (with 0.5% HCl) for 10 min and then washed in PBS. Sections were then treated with 10% normal horse serum for 30 min. Subsequently, sections were immersed in the first primary antibody for 20 h (mouse anti-BrdU, 1:35, BD Biosciences). On the second day of processing, sections were first washed with PBS and were then treated with a Cy3-conjugated donkey anti-mouse IgG (1:100, BioCan Cat No. 715-165-150) for 1 h. Following this, sections were washed in PBS and then immersed in 10% normal mouse serum for 30 min. Sections were then treated with a second primary antibody for 70 h at 4°C (biotin-conjugated mouse anti-NeuN, 1:250, Chemicon, Cat. No. MAB377B). Following immersion in the second primary antibody, sections were thoroughly washed and then treated with FITC-conjugated streptavidin (10 μg/mL, Chemicon, Cat. No. SA103) for 1 h. Sections were then washed and mounted on 1% gelatin-coated slides. When slides had dried, mounting medium was applied (Vectashield Mounting Medium, Vector Labs, Cat. No. H-1000) and slides were coverslipped. Slides were stored in the dark at 4°C. Negative controls, which were processed by omitting both primary antibodies, showed neither cytoplasmic nor nuclear staining.

Morphology and Cell Counting

To obtain estimates of hippocampal volume, we captured images of the NeuN labeled DAB-stained sections from each brain (mean of 13 sections per bird) through a 5× objective lens using a SPOT Insight Color video camera. SPOT Advanced imaging software (Diagnostic Instruments, Version 2.4.5 for Windows) was used to make bilateral area measurements of the hippocampus (hippocampus and area parahippocampalis). The boundaries used to define the area of the hippocampus (Hp) were as reported by Sherry et al. (1989). Volume estimates used the formula for the volume of a truncated cone (Sherry et al., 1989).

We also estimated the volume of the region adjacent to the hippocampus, the hyperpallium apicale (HA) (formerly hyperstriatum accessorium, Reiner et al., 2004), using the same method as for the hippocampus. The boundaries used to define the area of the HA were as reported by Hoshooley and Sherry (2004). We also estimated the volume of the whole telencephalon using alternate NeuN-stained sections (720 μm apart) and a 1.25× objective lens. For both the HA and the telencephalon, bilateral area measurements were made and the volumes determined using the formula for the volume of a truncated cone.

To estimate hippocampal neuron number, we captured grayscale images of every other NeuN labeled hippocampal section (mean of seven sections per bird) using either a 20× or 40× objective lens, depending on the size of the hippocampus in a given section. For sections in which the hippocampus was relatively large, images were captured using the 20× objective and for sections in which the hippocampus was smaller, images were captured with the 40× objective. For each section of hippocampus, an image was captured for one hemisphere, determined randomly. Images captured using the 20× objective lens measured 0.51 × 0.37 mm^2 and images captured using the 40× objective measured 0.24 × 0.17 mm^2. For each objective the measured area was considered the counting frame. For each section of hippocampus, the image and thus the counting frame was adjusted to include approximately equal proportions of the V-shaped, cell-dense region of the hippocampus, and the adjacent parahippocampal area as described in Hoshooley and Sherry (2004). Scion Image (Scion Corporation PC-based version of NIH Image by Wayne Rasband) was used to count the number of neurons present in the counting frame using the density slice function. For this procedure, all of the objects in the image to be counted as neurons were highlighted on the basis of gray scale contrast. For all images, the threshold level for highlighting dark neurons against a light background was adjusted according to the level of background staining for every image assessed. Adjusting the threshold for every image ensures that labeled cells are distinguished from artifacts of histological processing. The number of neurons counted for each section was divided by the volume of the counting frame (including section thickness) and the neuronal densities calculated for each counting frame were averaged to estimate neuron density in the hippocampus. To estimate the total number of hippocampal neurons, we multiplied neuron density by hippocampal volume.

To estimate the number of new neurons recruited into the hippocampus and the HA, we examined all sections labeled for BrdU and counted all new neurons appearing in that brain region according to the morphological criterion of Gould et al. (1999), namely darkly stained, round or oval nuclei ~10 μm in diameter. Using these criteria it is likely that both mature and immature adult generated neurons would be counted. There are two types of errors that may occur using the morphological criteria outlined above. It is possible that some large glial nuclei may be counted as neu-

ronal. It is also possible that some small BrdU-labeled neuronal nuclei, not meeting our size criterion, would be excluded from the counts. In the sections examined (every 12th section, 360 μm apart), new neurons were counted exhaustively in both hemispheres. This method of counting is appropriate for estimating neuronal recruitment and has been used previously for cell counts in the chickadee hippocampus (Hoshooley and Sherry, 2004; Hoshooley et al., 2005). Justification for this counting method comes from Tramontin et al. (1998), who demonstrated that similar estimates of neuron number in avian song control nuclei are obtained using either stereological methods or the systematic nonstereological method used in the current study. To estimate the density of BrdU labeling, we divided the average number of labeled neurons per section of a given brain region by the average sectional volume of the region. To estimate the total number of new neurons in the hippocampus and HA, we multiplied the density of BrdU labeling in each area by the volume estimated for that area. For the hippocampus, the percentage of all neurons that were new was also calculated. To estimate the percentage of new hippocampal cells expressing a mature neuronal phenotype, we analyzed 150 BrdU-Cy3-labeled cells for coimmunoreactivity with NeuN in eight chickadee and six house sparrow brains showing highest levels of neuronal recruitment in DAB stained sections. A BrdU-Cy3-labeled cell was recorded as coimmunoreactive for NeuN if a Cy3-labeled nucleus was surrounded by a green-FITC cytoplasmic halo and both nuclear Cy3-labeling and nuclear and cytoplasmic FITC-labeling came into and out of focus together.

Statistical Analyses

Separate two-way analyses of variance (ANOVAs) were used to examine all dependent variables. Where overall analyses revealed a significant interaction, Tukey's HSD post-hoc tests were conducted to assess simple main effects. All analyses with $p < 0.05$ were considered statistically significant.

RESULTS

The primary objective of this study was to compare seasonal hippocampal neuron recruitment in food-storing and non-food-storing birds. Hippocampal neuron recruitment was assessed by examining three variables: density of new hippocampal neurons (number of new neurons per mm^3), total number of new hippocampal neurons (Hp volume $\times$ density), and the percentage of hippocampal neurons that were new. For all three variables the same pattern of results was obtained. Chickadees showed significantly more hippocampal neuron recruitment than house sparrows at both times of year for all three measures of neuron recruitment (density $F(1,20) = 6.386$, $p = 0.020$, total $F(1,20) = 9.264$, $p = 0.006$, percent new $F(1,20) = 6.778$, $p = 0.017$). There was no significant main effect of season (density $F(1,20) = 0.514$, $p = 0.482$, total $F(1,20) = 0.217$, $p = 0.647$, percent new $F(1,20) = 0.723$, $p = 0.405$). There was no significant interaction between species and season (density $F(1,20) = 0.231$, $p = 0.636$, total $F(1,20) = 0.244$, $p = 0.627$, percent new $F(1,20) = 0.160$, $p = 0.694$ see Fig. 1). Double-labeling for BrdU and NeuN (Fig. 2) in chickadees, revealed that 32% of all new hippocampal cells expressed a mature neuronal protein 6 weeks after cell division. There were far fewer BrdU labeled cells in the house sparrow hippocampus but of these, 32% were coreactive for BrdU and NeuN. To compare counts of neuronal recruitment obtained using BrdU DAB staining and morphological criteria to counts obtained using double-labeling for BrdU and NeuN, we calculated the correlation between the two methods for total counts of Hp neuronal recruitment for five birds (mean of 6.6 sections per bird). Pearson correlation showed a highly significant positive relationship between the two counting methods, $r = 0.908$, $p = 0.001$, $n = 5$.

Neuronal recruitment in the HA was assessed by examining the density of new neurons and the total number of new neurons. There was no significant main effect of species (density $F(1,20) = 2.003$, $p = 0.172$, total $F(1,20) = 1.769$, $p = 0.198$), no significant main effect of season (density $F(1,20) = 1.619$, $p = 0.218$, total $F(1,20) = 0.984$, $p = 0.333$), and no significant interaction between species and season (density $F(1,20) = 0.208$, $p = 0.653$, total $F(1,20) = 0.038$, $p = 0.847$).

The hippocampus was significantly larger in chickadees than in house sparrows $F(1,20) = 20.748$, $p = 0.001$, see Figure 3. The hippocampus was also significantly larger in the spring than in the fall, $F(1,20) = 5.139$, $p = 0.035$, and there was no significant interaction between species and season, $F(1,20) = 0.001$, $p = 0.974$. The HA, however, was significantly larger in house sparrows than in chickadees, $F(1,20) = 12.440$, $p = 0.002$. There was no significant main effect of season, $F(1,20) = 0.507$, $p = 0.485$ and there was no significant interaction between species and season, $F(1,20) = 1.545$, $p = 0.228$ for the HA. The telencephalon was significantly larger in house sparrows than in chickadees, $F(1,20) = 115.786$, $p = 0.001$. There was no significant main effect of season, $F(1,20) = 0.735$, $p = 0.402$ but there was a significant interaction between species and season, $F(1,20) = 7.004$, $p = 0.015$. The telencephalon was larger in house sparrows in spring than in fall ($p < 0.05$). There was no seasonal difference in telencephalon volume in chickadees.

To correct for species differences and seasonal changes in overall telencephalon size, we determined

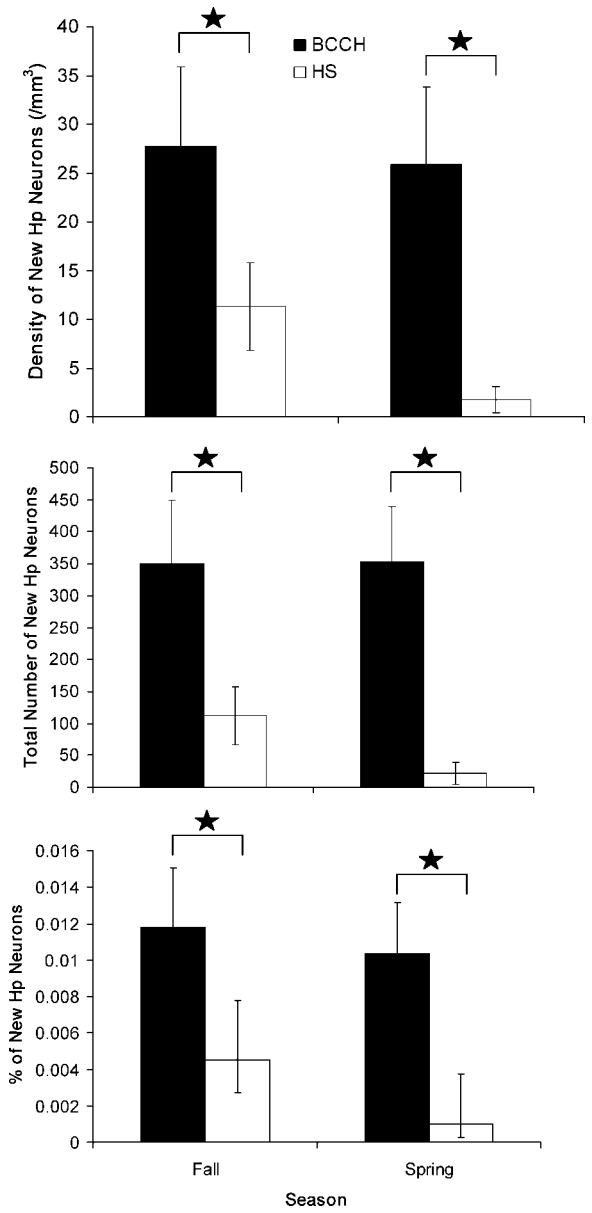

Figure 1 Mean density of new hippocampal neurons (top), total number of new hippocampal neurons (middle), and percentage of hippocampal neurons that were new for fall and spring in chickadees and house sparrows. Fall sample sizes were: chickadee ($n = 9$) and house sparrow ($n = 5$). Spring sample sizes were: chickadee ($n = 6$) and house sparrow ($n = 4$). Error bars are ± 1 SEM. Stars indicate significance at $p < 0.05$.

the relative size of both the hippocampus and HA as proportions of telencephalon volume. The relative hippocampal volume was larger in chickadees than in house sparrows, $F(1,20) = 161.807$, $p = 0.001$. There was also a significant main effect of season, $F(1,20) = 9.574$, $p = 0.006$, with relative hippocampus volume being larger in spring than in fall. There was a significant interaction between species and season, $F(1,20) = 6.805$, $p = 0.017$. Post hoc tests showed that the relative volume of the chickadee hippocampus was greater in spring than in fall ($p < 0.001$), while there was no seasonal difference in house sparrows. For the HA as a proportion of telencephalon volume there was no significant main effect of species, $F(1,20) = 0.087$, $p = 0.337$, or season, $F(1,20) = 0.967$, $p = 0.771$, and no significant interaction between species and season, $F(1,20) = 0.320$, $p = 0.578$.

Finally, we examined hippocampal neuron number by assessing the density of all hippocampal neurons and the total number of hippocampal neurons (density $\times$ structure volume) for both species. Hippocampal neuron density was greater in chickadees than in

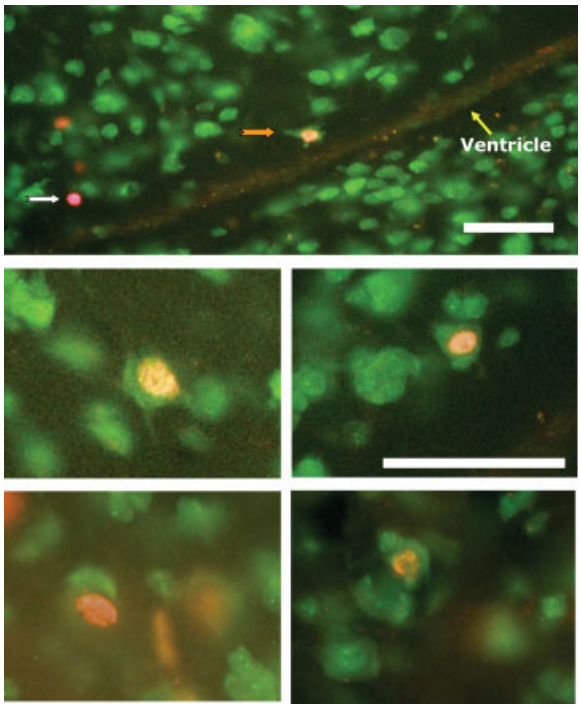

Figure 2 (Top) Chickadee hippocampal neurons labeled for NeuN (green, FITC) and BrdU (red, Cy3) at $\times 20$ objective magnification. New neurons originate at the ventricle and move dorsally into the hippocampus, above the ventricle in this image. Orange arrow indicates a neuron double-labeled for NeuN and BrdU. White arrow indicates a cell nucleus labeled for BrdU in a cell that is not colabeled for NeuN. (Middle) Chickadee hippocampal neurons colabeled for BrdU and NeuN at $\times 40$ objective magnification. (Bottom) House sparrow hippocampal neurons colabeled for BrdU and NeuN at $\times 40$ objective magnification. Both scale bars equal 50 μm.

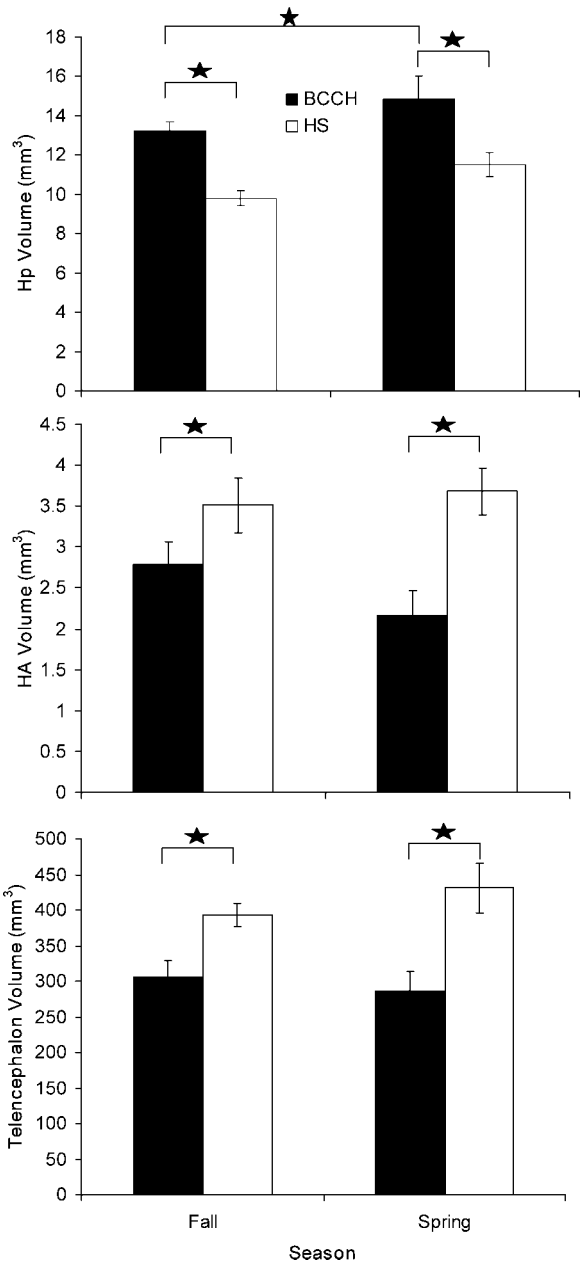

Figure 3 Mean hippocampal volume (top), hyperpallium apicale volume (middle), and telencephalon volume (bottom) for fall and spring in chickadees and house sparrows. Fall sample sizes were: chickadee ($n = 9$) and house sparrow ($n = 5$). Spring sample sizes were: chickadee ($n = 6$) and house sparrow ($n = 4$). Error bars are ± 1 SEM. Stars indicate significance at $p < 0.05$.

house sparrows, $F(1,20) = 7.880$, $p = 0.011$, see Figure 4. There was no significant main effect of season, $F(1,20) = 2.295$, $p = 0.145$. There was, however, a significant interaction between species and season, $F(1,20) = 10.743$, $p = 0.004$. Hippocampal neuron density in house sparrows was greater in fall than in spring ($p < 0.01$), while there was no seasonal difference in hippocampal neuron density in chickadees. Total hippocampal neuron number was significantly greater in chickadees than in house sparrows, $F(1,20) = 39.969$, $p = 0.001$. There was no significant main effect of season, $F(1,20) = 1.426$, $p = 0.246$ but there was a significant interaction between species and season, $F(1,20) = 6.579$, $p = 0.018$. The total number of hippocampal neurons in chickadees was greater in spring than in fall ($p < 0.01$), while there was no seasonal difference in total hippocampal neuron number in house sparrows.

DISCUSSION

We found significantly more hippocampal neuron recruitment in the chickadee than in the house sparrow at both times of year examined. This overall differ-

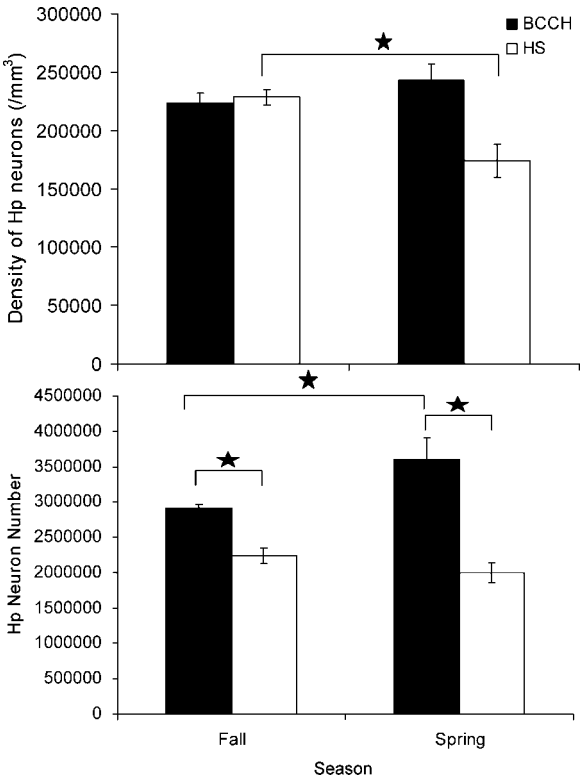

Figure 4 Mean density of hippocampal neurons (top) and total number of hippocampal neurons (bottom) for fall and spring in chickadees and house sparrows. Fall sample sizes were: chickadee ($n = 9$) and house sparrow ($n = 5$). Spring sample sizes were: chickadee ($n = 6$) and house sparrow ($n = 4$). Error bars are ± 1 SEM. Stars indicate significance at $p < 0.05$.

ence in recruitment levels did not extend into the adjacent HA. We did not find significant differences in hippocampal neuron recruitment in chickadees between the times of year examined and thus did not replicate an earlier report of enhanced fall recruitment in chickadees (Barnea and Nottebohm, 1994). Assessment of the developmental progress of new hippocampal cells in chickadees and house sparrows revealed that one-third of 6-week-old hippocampal cells were expressing a protein indicative of a mature neuronal phenotype. Finally, we found a significant increase in the volume of the hippocampus and in the number of Hp neurons in chickadees in the spring group, while Hp neuron number and size were the same at both times of year examined in house sparrows.

The absence of a fall increase in Hp neuron recruitment may have been due to the fact that our birds were held in captivity for 6 weeks between BrdU administration and sacrifice. Barnea and Nottebohm (1994) examined both wild and captive chickadees and birds in both conditions demonstrated the highest levels of recruitment in October. Birds in captivity for 6 weeks, however, showed an overall suppression of recruitment at all time-points compared to birds released into the wild after birth marker administration and caught again 6 weeks later.

It is possible that chickadees in our study were engaged in the same level of caching activity at both times of year. We did not collect caching data and it is therefore not possible to know if this was the case, but observational reports from field studies indicate that caching occurs through fall, winter, and early spring (Odum, 1942; Haftorn, 1956; Ludescher, 1980; Nakamura and Wako, 1988). In the year we conducted our study, environmental conditions presumed to affect caching rates, such as temperature (Brodin and Clark, 1997; Pravosudov and Grubb, 1997; Pravosudov, 2003), may have caused chickadees to store and retrieve food at similar levels in fall and spring, leading to similar levels of hippocampal neuron recruitment at both time points. Other factors which are known to affect the recruitment of new neurons into the hippocampus such as dominance status (Pravosudov and Omanska, 2005), may also have contributed to the difference between our results and Barnea and Nottebohm's (1994).

Although we did not observe seasonally fluctuating neuron recruitment in the hippocampus of either species, we did find significantly greater hippocampal neuron recruitment in food-storing chickadees than in nonstoring house sparrows. Chickadees and house sparrows are similar in a number of behavioral and ecological traits. Both are residents of the north temperate zone and are nonmigratory. Both species form dominance-structured winter flocks and have quite broad diets. Only chickadees however, engage in food-storing. While it is possible that another differentially expressed factor may account for heightened hippocampal neuron recruitment in chickadees, our results support the idea that elevated neuron recruitment in the chickadee hippocampus may be related to food-storing activity and the hippocampus-dependent spatial memory processing associated with food-storing.

By staining for the neuron specific marker, NeuN, we were able to define the structural boundaries of the hippocampus and thus were able to obtain estimates of both volume and neuron number for Hp. We found the hippocampus of the chickadee to be larger than that of the house sparrow at both times of year we examined. Greater absolute size of the chickadee hippocampus occurs even though chickadees are smaller in body size than house sparrows and have a smaller telencephalon and HA. This finding accords with a large body of results documenting greater hippocampal size in food-storing species (Krebs et al., 1989; Sherry et al., 1989; Hampton et al., 1995; Lucas et al., 2004). We also found significant seasonal fluctuation in the size of the hippocampus in chickadees. In this study, the chickadee hippocampus was larger in our spring group than in our fall group. This finding differs from the results of previous studies examining seasonal change in chickadee Hp volume. One study has found the hippocampus to be largest in October (Smulders et al., 1995), while others have found no volumetric change in either a natural population (Hoshooley and Sherry, 2004) or in birds subjected to photoperiodic manipulations (Krebs et al., 1995; MacDougall-Shackleton et al., 2003; Hoshooley et al., 2005). Our results showing increased hippocampal neuron number in the chickadee hippocampus in spring differ from previous results showing increased chickadee Hp neuron number in the fall (Smulders et al., 2000) and also differ from previous results in which we found no seasonal fluctuation in Hp neuron number (Hoshooley and Sherry, 2004). Increased Hp volume and neuron number in spring could arise by various processes. In our population of birds Hp neuron recruitment could have been high during the midwinter period we did not examine, November 25th–February 28th, leading to increased neuron number and volume in the spring. It is also possible that apoptosis, programmed cell death, changes seasonally. If apoptosis was high in summer this could lead to the lower neuron numbers and volume in fall that we observed. A low level of apoptosis in the fall or winter would cause more

neurons to be present in the spring. It is also possible that both heightened neuron recruitment in the winter and increased apoptosis in the summer act together to produce the pattern of results observed in our population of birds. Resolving these different possibilities regarding seasonal change in the chickadee hippocampus will require examination of environmental, energetic, social, and behavioral factors that may affect hippocampal neuron recruitment in food-storing birds.

The results of this study show that hippocampal neuron recruitment occurs at a higher rate in food-storing chickadees than in non-food-storing house sparrows at both times of year we examined. This finding may represent another way in which the hippocampus of food-storing birds is specialized to meet the cognitive demands associated with food-storing behavior and further suggests that hippocampal neuron recruitment may be related to spatial memory processing.

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