Evidence for Evolved Adaptive Specialization in the Food-Storing Black-Capped Chickadee (*Poecile atricapillus*)

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INTRODUCTION

- Food-storing birds can remember numerous cache locations over many hours or even months. Successful retrieval of caches relies on hippocampus (HP) dependent spatial memory (Vander Wall, 1990).
- The HP, in turn, shares reciprocal connections with the septum, a structure also involved with memory.
- The survival value of HP-dependent recall for cache locations may have created an evolved brain specialization in food-storers (Hampton & Shettleworth, 1996).
- HP and septal volume in food-storing birds is larger than that of non-storers (Krebs, Sherry, Healy Perry, & Vaccarino, 1989; Sherry, Vaccarino, Buckenham, & Herz, 1989; Shiflett, Gould, Smulders, and DeVoogd, 2002).
- Although food-storing behavior varies seasonally, and seasonal changes in HP volume have been observed, the data are not always consistent. While Smulders, Sasson, and DeVoogd (1995) reported a fall peak in HP volume coinciding with the peak in storing behavior, others report spring increases in HP volume (Hoshooley and Sherry, 2007).
- In food-storing birds, septal volume has also been found to increase during October, suggesting that an evolved specialization in food-storers may include both the HP and the septum (Shiflett et al., 2002).
- Fall peaks in cell count have also been observed in the food-storing black-capped chickadee (Smulders, Shiflett, Sperling, and DeVoogd, 2000).
- While food-storing black-capped chickadees have more adult HP neurogenesis than non-storing house sparrows, the seasonal data are not always consistent either. While Barnea and Nottebomhm (1994) demonstrated a peak in HP neurogenesis during the fall, Hoshooley and Sherry (2007) reported that neurogenesis remained constant across both seasons.
- The current study sought to resolve these discrepancies by comparing volumes and cell proliferation in the HP, septum, and stem cell-rich subventricular zone (SVZ) of food-storing black-capped chickadees and non-storing dark-eyed juncos captured during the fall and spring in coastal Maine.

METHODS

Subjects

- Seventeen adult food-storing black-capped chickadees (*Poecile atricapillus*) and sixteen adult non-storing dark-eyed juncos (*Junco hyemalis*) were captured in the fall (October, November 2005), and spring (April, May 2006) at two field sites in Maine: The Coastal Studies Center in Orr's Island, and Coleman Farm in Brunswick.

BrdU Administration, Perfusion, and BrdU Immunohistochemistry (IHC)

- To label mitotic cells, all birds received one intramuscular injection of BrdU 48 hrs after trapping.
- On day 10 of captivity, all birds were euthanitized and transcardially perfused with 0.1 M phosphate buffered saline followed by 4% paraformaldehyde. Brains were postfixed in 4% paraformaldehyde for 24 hours, transferred to 0.1M phosphate buffer, embedded in 8% gelatin, and then cut into 5 equivalent sets of 40μm coronal sections using a vibratome.
- All slices containing the HP or telencephalon were used for volume analysis and BrdU IHC.
- BrdU IHC was used to visualize newly born cells. Following standard IHC procedures previously reported by Lee et al. (2007), primary incubation was achieved with 1:500 anti-BrdU (Roche). Secondary incubation with 1:200 biotinylated horse anti-mouse IgG (Vector) was followed by incubation in 1:200 avidin-biotin-peroxidase complex (Vector) and detection was accomplished using diaminobenzidine (Sigma).

Volume Analysis

- Sections were individually digitized using a Polaroid SprintScan scanner mounted with a PathScan Enabler (Meyer Instruments Inc.). HP, septum, and telencephalon (TEL) of each hemisphere were separately outlined and measured using NIH Image software.
- Volumes of HP, septum, and TEL were computed using the formula for a truncated cone (Krebs et al., 1989). Relative volumes for HP and septum were determined by dividing the volume of HP or septum by TEL volume.

Cell Counts

- BrdU-IR cells were visualized using DIC illumination on a Nikon E-800 microscope using NeuroLucida software (MicroBrightField, Inc.).
- BrdU-IR cells were counted in the HP, septum, proximal SVZ (pSVZ; adjacent to the HP), and distal SVZ (dSVZ; not adjacent to the HP).
- Density of BrdU-IR cells (expressed as the number of cells/mm²) were calculated and compared between groups in order to control for species differences in brain region size.

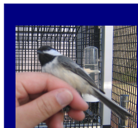

Figure 1. Black-capped chickadee

Figure 2. Dark-eyed junco

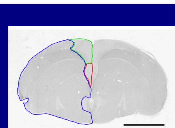

Figure 3. The three brain structures measured for volume analysis are outlined separately: the **HP**, **septum**, and **TEL**. Bar = 5mm.

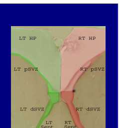

Figure 4. Counting areas

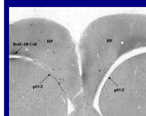

Figure 5. HP and pSVZ in a black-capped chickadee

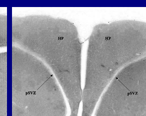

Figure 6. HP and pSVZ in a dark-eyed junco

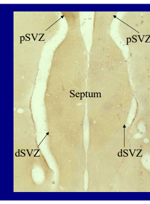

Figure 7. Black-capped chickadee pSVZ, dSVZ, and septum.

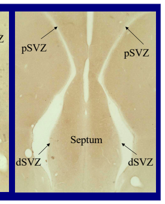

Figure 8. Dark-eyed junco pSVZ, dSVZ, and septum.

RESULTS

Volume Analysis

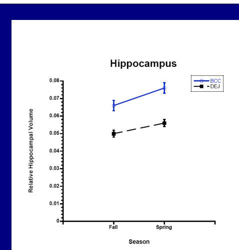

Figure 9. Across both seasons, relative HP volume was significantly larger in the black-capped chickadee compared to the dark-eyed junco ($p<0.05$). However, within each species, relative HP volume was significantly larger in the spring compared to the fall.

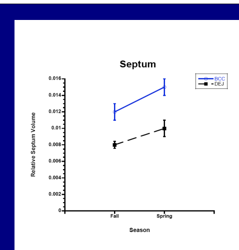

Figure 10. Across both seasons, relative septal volume was significantly larger in the black-capped chickadee compared to the dark-eyed junco ($p<0.05$). However, within each species, relative septal volume was significantly larger in the spring compared to the fall.

Cell Proliferation

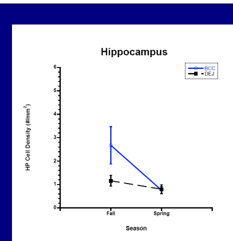

Figure 11. Fall-caught black-capped chickadees had significantly more BrdU-IR cells in the HP compared to spring-caught black-capped chickadees, fall-caught dark-eyed juncos, and spring-caught dark-eyed juncos ($p<0.05$).

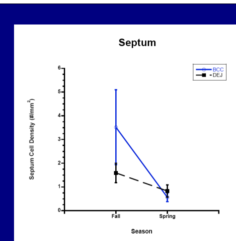

Figure 12. Fall-caught black-capped chickadees had significantly more BrdU-IR cells in the septum than spring-caught black-capped chickadees, but there were no differences between black-capped chickadees and dark-eyed juncos ($p<0.05$).

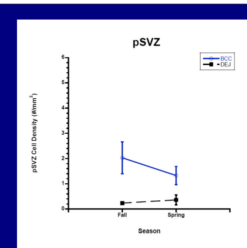

Figure 13. Fall-caught black-capped chickadees had significantly more BrdU-IR cells in the pSVZ compared to both fall- and spring-caught dark-eyed juncos ($p<0.05$).

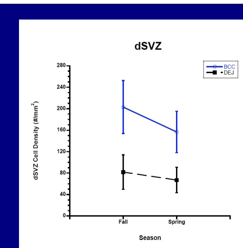

Figure 14. Fall-caught black-capped chickadees had significantly more BrdU-IR cells in the dSVZ compared to both fall- and spring-caught dark-eyed juncos ($p<0.05$).

CONCLUSIONS

Volumes

- Regardless of season, black-capped chickadees had significantly larger relative HP and septal volume compared to dark-eyed juncos.
- Relative HP and septal volume in both species increased during the spring, partially confirming the findings by Hoshooley and Sherry (2007) but refuting the results by Smulders et al. (1995).

Cell Proliferation

- Compared to non-storing dark-eyed juncos, food-storing black-capped chickadees had more newly born (BrdU-IR) cells in the HP, pSVZ, and dSVZ indicating that these structures may have evolved a species-specific specialization.
- Strikingly, non-storing dark-eyed juncos showed no seasonal differences at all.
- In contrast, compared to those caught in the spring, fall-caught black-capped chickadees had significantly more newly born cells in both the HP and septum.
- Seasonal increases in the pSVZ and dSVZ were not observed. Since black-capped chickadees had higher rates of cell proliferation in both regions of the SVZ compared to dark-eyed juncos, this may be a species-specific specialization. However, since it is not seasonally mediated, it is possible that this specialization may not be related to food-storing. Alternatively, the process of cell division could be followed by migration out of the SVZ and into the HP much more rapidly in fall-caught black-capped chickadees.
- Taken together, results indicate that cell proliferation is enhanced in food-storers, especially during the fall, and may reflect a selective adaptation in the brain designed to meet the cognitive demands of food-storing.

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