Hippocampus and Septum Volumes Show Season, Sex, and Species Differences in Black-capped Chickadees and Dark-eyed Juncos

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Introduction

- Resident bird species, unlike migrants, need to create a local energy source for the winter.
- Food-storing birds have larger hippocampus (HP) (Krebs *et al.*, 1989; Sherry *et al.*, 1989) and septum volumes (Shiflett *et al.*, 2002) relative to brain size than non-storing birds.
  - Food-storing birds form spatial memories, an HP- and septum-dependent type of memory, based on a single visit to a site.
- Chickadees have a larger HP and septum in the fall, coinciding with a peak in food-storing behavior, compared to the spring (Odum, 1942; Shiflett *et al.*, 2002; Smulders *et al.*, 1995).
  - The non-storing song sparrow does not show a seasonal change in HP volume (Lee *et al.*, 2001).
  - However, a recent study by Hoshooley and Sherry (2007) showed that HP volume in Chickadees is larger in the spring compared to the fall.
- Chickadees show more neurogenesis in the fall compared to the spring (Barnea and Nottebohm, 1994; Smulders *et al.*, 2000).
- During the fall, HP volume was shown to be correlated with neurogenesis; the larger the HP volume, the more new neurons born (Hoshooley and Sherry, 2004).

Objective

- To examine how HP and septum volumes in food-storing Chickadees compare to those in non-storing Juncos during both the fall and the spring.
- To clarify discrepancies between previous studies regarding how HP volume changes between fall and spring seasons in food-storing Chickadees. To examine species and seasonal differences in cytogenesis in the hippocampus and stem cell rich subventricular zone (SVZ)
- To control for confounding variables (habitat, food availability, photoperiod, temperature, etc.) by using birds from the same area at the same time of year so differences between the species can be attributed to species differences and not differences in environment.

Methods

Trapping

- Wild adult birds were trapped in the fall (October to November, 2005) and in the spring (April - May 2, 2006) at two field sites in southern Maine. In the fall, 11 juncos (*Junco hyemalis*) and 11 chickadees (*Poecile atricapillus*) were trapped and, in the spring, 8 juncos and 10 chickadees were trapped.
- The chickadees weighed 9.0-14.0 g whereas the juncos weighed 18.0-24.0 g. Birds were pair-housed in an outdoor aviary and had *ad libitum* access to water and black oil sunflower seeds. Nesting boxes were placed in the cages to provide the birds with shelter.

BrdU Injections

- Birds were given one intramuscular injection of 5 mL/g body weight of BrdU in 0.1M phosphate buffer (10 mg/mL) 48 hours after trapping.

Histology

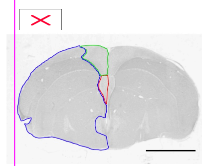
- On day 10 of captivity, birds were perfused transcardially with phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA). The brains were post-fixed for 24 hours in 4% PFA and then stored in 0.1M PBS until they were cut.
- Tissue was sectioned at 40 µm and divided into 5 equivalent sets with each set representing a 1-in-5 series with 200µm between slices.
- All slices containing the HP or telencephalon (TEL) were mounted for volume analysis (approximately 38-51 slices total).

Volume Measurement

- Sections were individually digitized using a Polaroid SprintScan scanner mounted with a PathScan Enabler (Meyer Instruments Inc.). The HP, septum, and telencephalon (TEL) of each hemisphere were separately outlined and measured using NIH Image software (version 1.62).
- 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. Brain volume divisions are shown in Figure 1C.
- All measurements were made blind to species.

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, proximal SVZ (pSVZ), and distal SVZ (dSVZ).
- Density of BrdU-IR cells (expressed as the number of cells/µm2) was calculated and compared between groups.



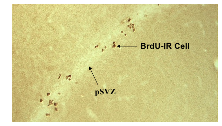


Figure 1 - Image A Black-capped chickadee (*Poecile atricapillus*), member of the *Paridae* family. Chickadees, like most birds in their family, are not sexually dimorphic. Chickadees are resident species in coastal Maine and exhibit food-storing behavior. **Image B** Dark-eyed junco (*Junco hyemalis*), member of the *Fringillidae* family. Juncos are non-storing, short-range migrants that cohabit with chickadees for several weeks in the fall and spring. Unlike chickadees, juncos are sexually dimorphic; however, their color variation is more subtle than other birds in the *Fringiliidae* family. **Image C** The three brain structures measured for volume analysis are outlined separately. The **hippocampus (HP)** boundaries are determined by cell density differences between the HP, septum, and telencephalon (TEL). The **septum** is bounded dorsally by the HP and laterally by the lateral ventricle. Cell density differences are also used to determine the septum boundaries. The **TEL** is used to determine relative volumes. Bar = 5mm. **Image D** Picture of BrdU labeled cells in the right proximal SVZ (pSVZ).

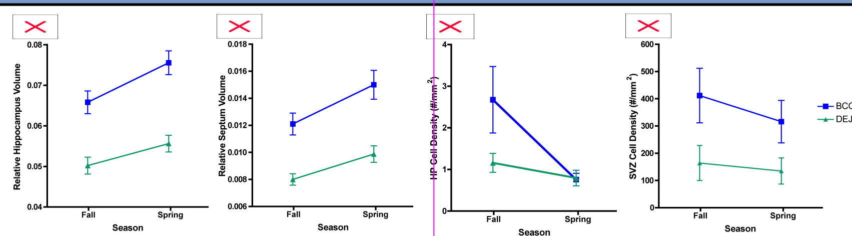


Figure 2 Graph A - The relative hippocampus (total HP volume/total TEL volume) was significantly larger in the Chickadees compared to Juncos ($p<0.05$). Additionally, relative hippocampus volume was significantly larger in the spring compared to fall in the Chickadee ($p<0.05$); while in the Juncos, there was a trend for an increase in relative hippocampus volume ($p=0.087$). **Graph B -** The relative septum (total septum volume/total TEL volume) volume was significantly larger in Chickadees compared to Juncos across the two seasons ($p<0.05$). In both species, relative septum volumes were significantly larger in the spring compared to the fall ($p<0.05$). **Graph C** - In the HP, chickadees have significantly higher densities of BrdU-labeled cells compared to Juncos during the fall ($p<0.05$); however, there was no species difference during the spring. Additionally, the fall chickadees had higher HP cell densities compared to chickadees trapped in the spring ($p<0.05$). **Graph D -** In the subventricular zone (SVZ), chickadees have significantly higher densities of BrdU-labeled cells compared to juncos across both seasons ($p<0.05$).

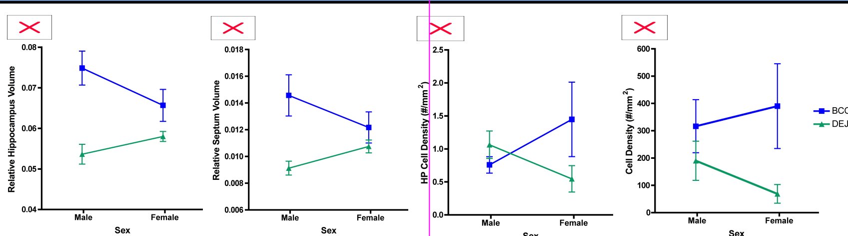


Figure 3 Graph A - Male chickadees had larger relative HP volumes than female chickadees and female juncos had larger relative HP volumes than male junco, but the sex differences were not significant within either species. However, there was a trend for an interaction effect between species and sex ($p=0.0568$). **Graph B -** While male chickadees on average had larger relative septum volumes compared to female chickadees, this result was not significant. However, female juncos showed a trend for having larger relative septum volumes compared to male juncos ($p=0.0735$). Additionally, there was a significant interaction effect between species and sex ($p<0.05$). **Graph C -** Female chickadees had higher HP cell densities compared to male chickadees and male juncos had higher HP cell densities compared to female juncos; however, there were no significant sex differences within either species. Additionally, here was a trend for an interaction effect between species and sex for cell density ($p=0.0834$). **Graph D -** Female chickadees seemed to have slightly higher SVZ BrdU-IR cell densities compared to male chickadees and male juncos seemed to have higher SVZ BrdU-IR cell densities compared to female juncos; however, neither species showed significant sex differences. There appeared to be a trend for an interaction effect between species and sex, but this was also found to be non-significant.

Conclusions

Species Differences

- Food-storing black-capped chickadees have significantly larger relative HP and relative septum volumes compared to non-storing dark-eyed juncos.
- Black-capped chickadees have significantly more BrdU-IR cells in the HP and SVZ compared to dark-eyed juncos.

Seasonal Differences

- Relative hippocampus and relative septum volumes are larger during the spring compared to the fall in both species.
  - This finding is contrary to many previous studies, but similar to those in the Hoshooley & Sherry (2007) study.
- In the HP, black-capped chickadees have significantly more cytogenesis during the fall compared to dark-eyed juncos; however, in the spring, cytogenesis levels in the chickadees drop to be approximately equal to the juncos.
  - This seasonal change in HP cytogenesis is opposite to the seasonal change seen in HP volume.
- In the SVZ, black-capped chickadees have significantly more cytogenesis than dark-eyed juncos across both seasons.
- No interaction effect between species and season for relative HP volume, relative septum volume, HP cell density, or SVZ cell density.

Sex Differences

- Males fluctuate in relative volumes more than females.
  - Males show a trend of having larger hippocampus volumes in the spring compared to fall, while the females remain constant. (Data not shown.)
  - Males had significantly larger septum volumes in the spring compared to fall; again, the female volumes remained fairly constant. (Data not shown.)
- HP cell densities show a trend for an interaction effect between species and sex.
- SVZ cell densities, like HP cell densities, seem to show a trend for an interaction effect between species and sex, but these results were not significant.
- Sex difference data is confounded because there were no female juncos during the fall.

Implications

A mechanism other than cytogenesis causes the increase in hippocampus and septum volumes from the fall to the spring.

- **Cell death** levels may be high during late summer to pare down cells created for non-food-storing types of memory. As a result, the peak in cytogenesis in food-storing birds during the fall may not increase HP and septum volumes. HP and septum volumes may decrease again later in the spring/summer when food-storing birds no longer rely on food caches.
- **Cell size** may be larger during the spring compared to the fall. This would result in higher volumes in the HP and septum without increasing the density of BrdU-IR cells.
- **Dendritic arborization** may be higher during the spring compared to the fall. During the spring, birds need to acquire new spatial memories, such as nest location or areas with high numbers of insects. As a result, the cells in the HP and septum may make new dendritic connections, resulting in larger volumes but not altering the density of BrdU-IR cells.

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