

Hippocampal volume does not change seasonally in a non food-storing songbird

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Seasonal differences in hippocampal morphology have been reported in food-storing birds. Non food-storing species have not been investigated however. It is therefore unclear whether seasonal changes in the hippocampus are specifically related to food-storing or reflect a more general seasonal mechanism that occurs in both food-storing and non food-storing birds alike. We determined the volumes of the hippocampal formation and remaining telencephalon in the non-storing male song sparrow (*Melospiza melodia morphna*) in two experiments comparing birds collected in the spring and fall of 1992–94 (Experiment 1)

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and 1997 (Experiment 2). Although pronounced seasonal changes in song control nuclei such as the HVC and RA were previously reported for the same brains used in Experiment 1, we found that hippocampal volume did not change with season in either Experiment 1 or 2 for these song sparrow brains. These results suggest that seasonal changes in the hippocampus do not occur in this non food-storing species and may be specific to food-storing birds. *NeuroReport* 12:1925–1928 © 2001 Lippincott Williams & Wilkins.

INTRODUCTION

Studies of the natural behaviour of animals with highly specialized capabilities, such as food-storing songbirds, may add to our general understanding of the neurobiological bases of memory formation, especially with regard to the role played by the hippocampus [1]. Food-storing birds have relatively larger hippocampal volumes than their non food-storing counterparts in a wide variety of species [2,3]. Volumetric differences are not accompanied by differences in cell density but rather by a greater number of cells as well as qualitatively different cells such as larger, calbindin-immunopositive neurons [4,5].

Observations in the wild suggest that many food storing birds, including chickadees and titmice, show seasonal peaks in food caching, mostly during the fall and early winter months [6,7]. In captivity, willow tits engage in seasonal caching in their outdoor aviary [8]. European jays also observed in outdoor aviaries spend more time caching, cache more items, and leave their caches for longer periods of time during the fall than in the spring [9].

There is some evidence that these seasonal changes in food storing may correlate with seasonal changes in brain morphology. In the black-capped chickadee (*Parus atricapillus*), hippocampal volume is reported to be larger during

the fall when food storing is at its peak [10]. A seasonal increase in hippocampal neurogenesis is also observed in wild-caught black-capped chickadees in late summer just before the seasonal peak in storing [11]. Although overall levels of neurogenesis are higher in younger birds, older birds continue to show these seasonal changes [12]. Furthermore, both juveniles and adults show an increase in the number of small and large cells (primarily neurons) in the fall [13]. These post-maturational alterations in hippocampal morphology may occur in response to general changes in the environment itself (e.g. food availability, temperature, daylength) and/or specific seasonal behavioural changes that depend upon the hippocampus, such as memory-based retrieval of food stores. Seasonal changes in the hippocampus of non-storing brood parasites have been reported [14,15]. However, studies investigating seasonal changes in hippocampal volume and neurogenesis of food-storing birds have not typically included a non-storing species as a control. It remains to be seen whether changes in hippocampal morphology are specific to birds that cache or the result of a more general seasonal mechanism affecting both food-storing and non food-storing birds alike.

In the present study, we investigated the possibility that

hippocampal volume changes are the result of a general seasonal mechanism by using adult male western song sparrows (*Melospiza melodia morphna*). A review of the literature reveals no evidence of food caching behavior in this species [16–18]. In song sparrows, changes in season result in concomitant changes in gonadal steroids, nuclear and cellular attributes of song nuclei, and song behavior [19]. In the fall, testosterone concentrations are lower, and the higher vocal center (HVC) and robust nucleus of the archistriatum (RA) song nuclei are significantly smaller than during the spring breeding season. Also in the fall, male song sparrows have the same repertoire size but sing songs that are structurally more variable than those sung during the spring when testosterone concentrations are higher and song nuclei larger. Although the song sparrow brain is capable of substantial seasonal plasticity, it is unknown what effect, if any, season has on the hippocampus in this non food-storing species. Therefore, for Experiment 1, we performed additional analyses on brain tissue from a subset of the birds reported previously [19] and determined hippocampal and telencephalic volumes for birds captured during late spring and late fall. We conducted Experiment 2 to further test the possibility that season influences hippocampal volume in this species by determining hippocampal and telencephalic volumes for song sparrows collected 3 years later as part of a subsequent study on the effects of estrogen on aggression [20,21].

MATERIALS AND METHODS

Experiment 1: Thionin-stained tissue was obtained from the laboratory of E. Brenowitz. Tissue for Experiment 1 was first used as part of a separate study investigating seasonal mediation of gonadal hormones, song nuclei, and song behavior; results from those studies have been published previously [19]. Subject information and tissue preparation procedures are summarized below; for details see the previous report [19].

Adult male song sparrows were collected in 1992–94 from three field sites in western Washington State: Skagit State Wildlife Recreation Area, Lee Forest, and Montlake Fill in Seattle. Males were captured using a mist net and playback of male song; a blood sample was immediately collected. On the day of capture, birds were deeply anesthetized with methoxyflurane (Metofane; Pitman-Moore, Mundelein, IL) and perfused with heparinized avian saline followed by 10% neutral buffered formalin (NBF); brains were removed and stored in 10% NBF. Only adult birds having completely pneumatized skulls were included in the study. Brain sections from birds collected at two times of the year were used for the present analysis: (1) spring (April–early May) during the peak of the breeding season, when testes were fully recrudescing ($n=6$); and (2) fall (December) when territorial behavior and spontaneous song were relatively infrequent ($n=6$).

Brains were embedded in gelatin and cryoprotected in 10% NBF containing 20% sucrose (4°C) for 2–3 days. The brains were frozen on dry ice and cut in 50 μm coronal sections on a sliding microtome. Sections were mounted on gelatin-subbed slides, dried overnight, stained in thionin, dehydrated in a graded ethanol series, cleared in xylene,

and coverslipped in DPX mountant (BDH Laboratory Supplies, Poole, UK).

In coronal section, the dorsal, ventral, and medial boundaries of the avian hippocampal formation (HF) correspond to the surface of the brain, the septum and the lateral horns of the ventricle, and the mid-line, respectively. The Nissl-defined lateral boundary is characterized by a change in cell density [2] and coincides with the boundary defined by calbindin [4,22] and acetylcholinesterase labeling [22,23]. Medial to the boundary, cell area distributions are bimodal with peaks at 20 μm^2 and 130–150 μm^2 . Lateral to the boundary, cell areas range between 20 and 30 μm^2 only. Using these criteria, Nissl-defined boundaries were traced for HF and remaining telencephalon minus HF (TEL) from every fourth 50 μm section using a 13 \times magnified image projected by a Bausch and Lomb microprojector. The traced outlines were digitized using a scanner, and the areas were calculated using NIH Image software (version 1.58). Volumes of HF and TEL were computed using the formula for a truncated cone [2,3]. All measurements were made blind to season.

Experiment 2: Adult male song sparrows were collected in 1997 from three field sites in western Washington State: Skagit State Wildlife Recreation Area, Big Beef Creek Reserve, and Montlake Fill. Males were captured using a mist net and playback of male song. On the day of capture, the birds used in the present study received subcutaneously an empty silastic implant and an osmotic minipump that was filled with avian saline; these birds served as a control group for a separate study investigating estrogen regulation of aggression [20,21]. After implantation, each bird was released back onto its territory. Birds were recaptured 9–17 days later, deeply anesthetized and perfused within 1 hour of capture using the procedures described above. Brain sections from birds collected at two times of the year were used: (1) spring (late May–early June), $n=5$, and (2) fall (November–mid December), $n=5$.

Tissue was processed using the same procedures described in Experiment 1. Nissl-defined boundaries were traced for only one hemisphere of the HF and remaining telencephalon minus HF (TEL); neither the HF nor TEL differ in size between the two sides of the brain [19]. The traced outlines were digitized using a scanner, and the areas were calculated using NIH Image software (version 1.58). Volumes of the single hemisphere of HF and TEL were computed using the formula for a truncated cone [2,3] then multiplied by 2 to approximate total volume of the regions. All measurements were made blind to season.

RESULTS

Experiment 1: Mean volumes of HF, TEL, and relative HF (HF/TEL) determined for spring and fall birds were compared using a *t*-test (Table 1). There was no significant effect of season on any measure: TEL ($t=1.404$, $df=10$, $p=0.191$), HF ($t=0.289$, $df=10$, $p=0.779$), or relative HF volume ($t=1.270$, $df=10$, $p=0.233$). One additional comparison was made between seasonal groups using a more conservative measure of relative HF volume. When obtaining tissue slices, occasionally the rostral- and caudal-most extent of TEL were not included; although this happened infrequently, it is still a potential source of bias and would

Table 1. Volume of brain regions.

Region	Spring	Fall	t	df	p
Experiment 1	n = 6	n = 6			
Telencephalon	563.22 ± 18.060	617.55 ± 34.241	-1.404	10	0.191
Hippocampus	19.20 ± 0.441	19.55 ± 1.129	-0.289	10	0.779
HF/TEL	0.034 ± 0.002	0.032 ± 0.001	1.270	10	0.223
*HF/TEL	0.041 ± 0.002	0.038 ± 0.001	1.340	10	0.210
Experiment 2	n = 5	n = 5			
Telencephalon	710.41 ± 33.253	722.88 ± 32.000	-0.270	8	0.794
Hippocampus	22.10 ± 0.802	21.94 ± 0.644	-0.148	8	0.887
HF/TEL	0.031 ± 0.001	0.030 ± 0.001	-0.548	8	0.599
**Experiments 1 and 2, ANOVA main effect of season	n = 11	n = 11			
Telencephalon	630.12 ± 28.760	665.43 ± 27.947	1.251	1	0.278
Hippocampus	20.52 ± 0.615	20.64 ± 0.752	0.015	1	0.905
HF/TEL	0.033 ± 0.001	0.031 ± 0.001	1.687	1	0.210

Values are means ± s.e.m. All volumes are in mm³. *HF/TEL was determined using only those slices in which both TEL and HF were present; sections cut from the rostral-most extent of the brain before HF appeared and the caudal-most extent of the brain after HF disappeared were excluded for all birds. **ANOVA main effect of experiment is not summarized in table; results indicated that HF/TEL did not differ but TEL and HF were significantly larger in Experiment 2 possibly due to methodological differences between years. Interactions were nonsignificant.

result in an underestimation of TEL and therefore an overestimation of HF/TEL. *HF/TEL was therefore determined by eliminating any slice that did not include both HF and TEL. That is, sections cut from the rostral-most extent of the brain before HF appeared and the caudal-most extent of the brain after HF disappeared were excluded for all birds. As with all other measures, no volume difference was found between seasons using the new estimate of *T ($t=1.691$, $df=10$, $p=0.122$), *HF ($t=0.323$, $df=10$, $p=0.753$), or *HF/TEL ($t=1.340$, $df=10$, $p=0.210$).

Experiment 2: Mean volumes of HF, TEL, and relative HF (HF/TEL) determined for spring and fall birds were compared using a *t*-test (Table 1). In replication of the results of Experiment 1, there was no significant effect of season on any measure: TEL ($t=0.270$, $df=8$, $p=0.793$), HF ($t=0.148$, $df=8$, $p=0.886$), or relative HF volume ($t=0.548$, $df=8$, $p=0.598$).

An ANOVA was performed on all data from Experiments 1 and 2 (Table 1) to investigate the main effects of season (spring *vs* fall) and experiment (1 *vs* 2). Interactions between the main effects were not significant. Again, there was no significant effect of season on any measure: TEL ($F=1.251$, $df=1$, $p=0.278$), HF ($F=0.015$, $df=1$, $p=0.905$), or relative HF volume ($F=1.687$, $df=1$, $p=0.211$). There was however a significant effect of experiment on both TEL ($F=17.877$, $df=1$, $p<0.001$) and HF volume ($F=10.525$, $df=1$, $p=0.005$) but not on relative HF volume ($F=2.338$, $df=1$, $p=0.144$). Both TEL and HF were significantly larger in Experiment 2; this may have been due to methodological differences between the studies including perfusion technique and tissue preparation which may affect the extent of brain shrinkage, or due to sampling differences which could include the possibility that the birds caught in 1997 were larger than those caught in 1994. The critical measure, relative HF volume, was not found to vary significantly between experiments nor between seasons.

DISCUSSION

Relative hippocampal volume was reported to be larger during the fall when food storing is at its peak in black-capped chickadees [10]. In late summer, just prior to the chickadee's peak in food storing, a seasonal increase in hippocampal neurogenesis is also observed [11]. Non food-storing species were not investigated in either study, leaving it unclear as to whether the results were due to food-storing or a more general seasonal mechanism operating on both food-storing and non food-storing birds. Although substantial changes in androgen levels and song-related nuclei such as the HVC and RA are associated with season [19], hippocampal volume in the non food-storing song sparrow did not change with season in either Experiment 1 or 2. This observation suggests that a general seasonal effect does not explain seasonal hippocampal growth in food-storing birds. If not a general seasonal mechanism, then changes in the hippocampus of food-storing birds may result from species differences in hippocampal plasticity in response to seasonal pressures to engage in food storage and retrieval. In fact, some evidence supports the suggestion that there are indeed species differences in seasonal plasticity of the hippocampus of stors and non-stors. Following training on a spatial learning task, the hippocampus of juvenile food-storing marsh tits increases to the same size as that of conspecifics that received food-storing experience, whereas juvenile non-storing blue tits show no such change [24].

Although song nuclei are larger in the spring, coincident with greater stereotypy of song behavior, the hippocampus of food-storing songbirds is larger in the fall, coincident with peak storing behavior. Because the peaks in song and food-storing behaviors, as well as the size of the brain regions involved in these behaviors, occur in different seasons, fluctuations in plasma levels of gonadal hormones *per se*, such as testosterone, cannot adequately explain the seasonal fluctuations of these different brain regions. High levels of testosterone correlate positively with song nuclei growth but negatively with hippocampal growth.

Seasonal and/or species differences in androgen sensi-

tivity may underlie the different patterns of seasonal plasticity in the song system and hippocampus. Song behavior is regulated by both androgens and estrogens [25,26]. Androgen receptors (AR) are present in most song nuclei, and estrogen receptors (ER) are found in HVC [27–29]. Seasonal changes in the morphology of the song nuclei are primarily regulated by changes in plasma testosterone levels [19]. AR levels, and therefore presumably androgen sensitivity, also change seasonally in the song control system. In the HVC of white-crowned sparrows (*Zonotrichia leucophrys gambelli*), AR+ cell density and number, the percentage of AR+ cells, and the staining intensity of these cells all increased during the spring breeding season, when plasma testosterone levels were high [29].

The hippocampus contains both AR and ER, like the song nuclei [27,29]. It is unknown whether there are seasonal changes in steroid receptor containing cells in the hippocampus comparable to those seen in the song nuclei. There are suggestions, however, that steroid metabolism may differ between the song system and the hippocampus. The activity of the steroid metabolizing enzymes aromatase, 5 α -reductase, and 5 β -reductase in the hippocampus differ between storing and non-storing songbirds, but do not differ in the song nuclei of these same songbirds [30]. This observation is consistent with the hypothesis that cells in the song nuclei and hippocampus respond differently to seasonal changes in circulating gonadal steroid levels, despite the fact that both brain regions contain receptors for these hormones. An interesting extension of the present study would be to compare seasonal patterns of morphology and steroid metabolism of cells in the song nuclei and hippocampus in food-storing and non-storing songbirds.

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