Published in final edited form as:

Dev Neurobiol. 2009 December ; 69(14): 972–981. doi:10.1002/dneu.20736.

Captivity Reduces Hippocampal Volume but not Survival of New Cells in a Food-Storing Bird

Bernard A. Tarr,

Cornell University, Psychology

Jeremy S. Rabinowitz,

Cornell University, Psychology

Mubdiul Ali Imtiaz, and

Cornell University, Psychology

Timothy J. DeVoogd

Cornell University, Psychology

Abstract

In many naturalistic studies of the hippocampus wild animals are held in captivity. To see if captivity itself affects hippocampal structure, adult black-capped chickadees (*Parus atricapilla*) were caught in the fall, injected with bromodeoxyuridine to mark neurogenesis and alternately released back to the wild or held in captivity for 4–6 weeks. Wild birds were recaptured and perfused simultaneously with their captive counterparts. The hippocampus of the captive birds was 23% smaller than the wild birds, with no hemispheric differences in volume within groups. There was no statistically significant difference in the size of the telencephalon between groups, or in the number and density of surviving new cells. Proximate causes of the hippocampal volume change could include stress, lack of exercise, diminished social interaction or limited caching opportunity; a hippocampal-dependent activity. The results suggest the avian hippocampus - a structure essential for rapid, complex relational and spatial learning - is both plastic and sensitive, much as is the case in mammals, including humans.

Keywords

Captivity; Hippocampal Volume; Neurogenesis; Stress; Food-Storing Bird

INTRODUCTION

The avian hippocampus is homologous to the mammalian hippocampus, as evidenced by embryological, cytoarchitectural, histochemical, electrophysiological and neuronal connectivity studies (Craigie, 1935; Kuhlenbeck, 1938; Krayniak and Siegel, 1978a, b; Casini et al., 1986; Erichsen et al., 1991; Krebs et al., 1991; Montagnese et al., 1996; Margrie et al., 1998; Siegel et al., 2000; Redies et al., 2001). Anatomical subdivisions in the

structure appear to differ substantially between birds and mammals however, and consequently the existence of structural homologies at the level of these subdivisions remains unclear (Atoji and Wild, 2006). Notwithstanding this, as in mammals, one of the primary functions of the avian hippocampus is spatial learning and memory (Krebs, 1990; Vander Wall, 1990; Shettleworth, 1995; Hampton and Shettleworth, 1996; Shiflett et al., 2003). Food-storing birds such as the black-capped chickadee (*Poecile atricapilla*), cache and later retrieve up to thousands of food items during the fall and winter - a behavior that may have evolved by sustaining birds through protracted periods of intemperate weather (Odum, 1942; Haftorn, 1954; Smith, 1967; Pravosudov, 1986; Sherry, 1989; Clayton and Krebs, 1994a). And if the hippocampus in food-storing birds is lesioned, cache recovery is reduced to chance (Krushinskaya, 1966; Sherry and Vaccarino, 1989).

While the mammalian hippocampus is critical for rapid learning of complex spatial and temporal relations, it is sensitive to a broad array of variables, including stress (Gould et al., 1997; McEwen, 1999; Bremner, 2006; Szeszko et al., 2006), hypoxia (Cervosnavarro and Diemer, 1991; Ogawa et al., 2007; Maiti et al., 2008), isolation housing (Bianchi et al., 2006; Scaccianoce et al., 2006; Stranahan et al., 2006; Ibi et al., 2008), dementia (Caselli et al., 2006; Hua et al., 2008), post-traumatic stress disorder (Karl et al., 2006), various psychiatric disorders (Sheline et al., 2003; Campbell et al., 2004; Videbech and Ravnkilde, 2004; Duman and Monteggia, 2006; Dhikav and Anand, 2007), and exercise (Kiraly et al., 2005). Stress and elevated glucocorticoids reduce hippocampal cell proliferation and survival (Tanapat et al., 2001; Mirescu and Gould, 2006; Mineur et al., 2007; but see Thomas et al., 2006, 2007), apical dendrite branching (Uno et al., 1994; Magarinos and McEwen, 1995a, b; Magarinos et al., 1996; McKittrick et al., 2000; Sousa et al., 2000; but see Woolley et al., 1990; Galea et al., 1997), synapse density (Sousa et al., 2000; Sandi et al., 2003; Stewart et al., 2005), relative concentration of neuronal processes (Tata et al., 2006), levels of neurotrophins (Duman and Monteggia, 2006) and hippocampal volume (Czeh et al., 2001; Sheline et al., 2003; Campbell et al., 2004; Videbech and Ravnkilde, 2004; Heine et al., 2004; Stewart et al., 2005). Social isolation elicits similar effects in mammals (Bianchi et al., 2006; Scaccianoce et al., 2006; Ibi et al., 2008), and birds (Barnea et al., 2006). In young food-storing birds, even the forced lack of use of the hippocampus can result in attrition or its failure to grow (Clayton and Krebs, 1994b), and neonatal nutritional deficiencies can cause enduring spatial memory deficits (Pravosudov et al., 2005). Exercise is typically neuroprotective in mammals, increasing hippocampal neurogenesis (van Praag et al., 1999, 2005; Brown et al., 2003; Rhodes et al., 2004; Eadie et al., 2005; Yasuhara et al., 2007), neurotrophin levels (Neeper et al., 1996; Cotman and Berchtold, 2002; Vaynman et al., 2004; Rex et al., 2007), and spine and dendritic density (Eadie et al., 2005; Redila and Christie, 2006; Stranahan et al., 2007). Hippocampal-dependent learning increases cell proliferation and survival (Gould et al., 1999; Leuner et al., 2004; Dalla et al., 2007; Sisti et al., 2007; but see van Praag et al., 1999), spine density, dendrite complexity and size of perforated postsynaptic densities (Leuner et al., 2003; Kozorovitskiy et al., 2005; Marrone, 2007). In humans, there is a positive correlation between proficiency on hippocampal-dependent tasks and hippocampal volume (Maguire et al., 2006; Schumann et al., 2007; Yonelinas et al., 2007; but see Van Petten, 2004; Amat et al., 2008), though it remains unclear whether this reflects genetic variation or learning-

induced changes in the hippocampus. Evidence for a link between hippocampal-dependent learning and structural changes in the hippocampus remains tenuous in wild adult food-storing birds, at best, save perhaps for increased neurogenesis (cell survival in particular) (Barnea and Nottebohm, 1994; Smulders et al., 1995; Smulders et al., 2000b; Hoshoooley and Sherry, 2004; Hoshoooley et al., 2007).

Despite structural and functional similarities however, to date hippocampal vulnerability has been studied almost exclusively in rodents and humans, with obvious limitations to human experimental study. The study of the hippocampus in species other than rodents offers several advantages - it is easier to study relations between natural behaviors and neurobiology in species that are common and easily observed; it may be easier to relate human hippocampal function to diurnal species that use vision extensively; and it may facilitate the identification of patterns in neurobiology to behavior relations across a range of species (Smulders, 2007). Also, if findings in species distantly related to humans converge on findings in humans it would suggest the principles of hippocampal function evolved early, and have been conserved in subsequent vertebrate lineages.

Therefore, given the structural and functional similarities (homology) of the avian and mammalian hippocampus, we wanted to see if the avian hippocampus is similarly vulnerable. This question is of particular importance in studies of wild food-hoarding birds, since in most cases some degree of captivity is involved. It is likely that laboratory-housed wild birds are more stressed, less physically active, more socially isolated and faced with fewer hippocampal task-specific demands than in the wild.

In this study we find that simply being in captivity results in hippocampal atrophy, with no difference in new cell survival after 4 to 6 weeks.

METHODS

Subjects

Black-capped chickadees were caught under state and federal permits, in Ithaca, NY. Mist nets were set and birds captured in the last week of November and first week of December. 17 birds were captured in 2003 (group 1) and 8 birds were captured in 2004 (group 2). At capture, all birds were banded for identification and injected with 5 μ l BrdU (calculation based on a typical chickadee body weight of 13 g and 75 μ g/g), and alternately released to the wild or held in captivity, such that each alternation constituted a pairing of one bird from each experimental group. Of the first group 9 were released post-injection and 8 housed in the animal facility, and 4 were released back to the wild and 4 housed at the animal facility of the second group. At the animal facility the birds were housed in the same room (2.85 m wide $\times$ 3.90 m long $\times$ 2.50 m high), one bird per wire cage (60.96 cm wide $\times$ 40.64 cm long $\times$ 40.64 cm high), in rows of three.

To acclimatize to the relative warmth and confines of the animal facility, caged birds were kept outside in a large enclosed area (5.45 m wide $\times$ 6.20 m long $\times$ 2.30 m high) for the first night. They were then moved into animal housing where they remained until the end of the experiment. Water was provided ad libitum and the birds were fed a daily diet of

mealworms, and a mixed food containing ground beef, carrot baby food, hardboiled eggs, wheat germ, and turkey starter pellets. A 10 h – 14 h light-dark cycle was maintained. Temperatures in the housing and testing rooms were set as low as possible, to 10 – 16 degrees Celsius. Six birds were re-captured from the first wild group and all 4 from the second wild group making 20 subjects in total (10 pairs).

Histology

After 4 to 6 weeks each bird from the wild was taken to the lab with its caged counterpart, weighed, anaesthetized by intramuscular injection of 0.07 ml Chloropent (Fort Dodge Labs), and transcardially perfused with 0.8% NaCl (+ 0.1% NaNO₂) in 0.1 M NaPB and then 4% paraformaldehyde (+ 0.1% NaNO₂) in 0.1 M NaPB. After perfusion, the gonads were removed and weighed. The brain was removed from the skull, weighed, and placed for an hour in 4% paraformaldehyde in Hartmann's PBS, then transferred to 30% sucrose in 0.1 M NaPBS for cryoprotection for 1 – 2 days. The brains were then embedded in 10% gelatin/30% sucrose, frozen and sectioned at 40 µm. One series of every fourth slice of tissue was mounted on microscope slides for Nissl staining, providing a cross-section of the entire brain from front to back at 160 µm intervals. Another tissue series was mounted on electrostatic slides for anti-BrdU staining. Once stained, all tissue was cover-slipped with Permount (Fisher, Pittsburgh, PA).

Volumetric Analysis and Cell Counting

A camera lucida attached to a light microscope (40x objective) was used to trace the perimeter of the hippocampus from each hemisphere in all the sections in which they appeared [typically 31 sections, see Fig. 2].

NIH Image 1.63 was used to determine the areas of the hippocampus tracings and of the telencephalon (including the HF). The volume of the hippocampal formation was subtracted from telencephalon volume (Tel-HF) for all the statistical analyses. Large to medium-sized round or oval-shaped BrdU-labeled nuclei within the hippocampus were counted by eye in every slice in a mounted series for group 1 (n = 12). Labeled cells exhibiting characteristics of glia – small triangular or irregular shaped cells, were not counted. In mammals, several studies have demonstrated that only a very small percentage of BrdU-immunoreactive cells in the hippocampus (typically < 15%) stain for non-neuronal markers such as von Willebrand factor (endothelial cells) or glial fibrillary acidic protein (astroglia) (Gould et al., 1999; Stranahan et al., 2006). The great majority (typically > 75%) stain for neuronal markers such as neuron specific enolase (NSE), neuronal nuclei (NeuN), and class III beta-tubulin (TuJ1) (Gould et al., 1997; Leuner et al., 2004, 2006; but see Rakic, 2002).

Control cell counts of immuno-labeled nuclei were counted in an extra-hippocampal area comprising two contiguous 1mm × 1mm sized counting grids in the hyperpallium apicale adjacent to the lateral ventricles, immediately superior to the junction of the hippocampus and septum, in every slice in which hippocampal cell counts were performed.

Group 2 was not processed for neurogenesis. The brain sections from each bird were coded to ensure the experimenters were blind to the treatment groups and the codes not revealed until the analyses completed.

Statistical Analyses

General linear regression was used to test for effects of several categorical (year, sex, housing) and continuous (body weight, brain weight and Tel-HF) variables on HF volume. An indicator variable was used for year, since the volume regressions included samples from two years. The sex of one bird and the brain weights of two others were not determined at sacrifice, in both cases from the second year (sample), therefore only affected the volume regressions. This was remedied by the interpolation of brain weight values by simple linear regression and all the regressions were run including and excluding these two subjects. Furthermore, in every case the regressions were run with the “sexless” bird coded as male (0), then as female (1). Each regression was effectively run 4 times, therefore. All the same procedures were followed and all the same variables, excluding Tel-HF were regressed against Tel-HF to test for general brain effects.

For the BrdU staining we used general linear regression to test for effects of several categorical (sex, housing) and continuous (body weight, brain weight and Tel-HF) variables on HF BrdU+ cell counts, HF BrdU+ cell density (BrdU+ cell counts divided by hippocampal volume), extra-hippocampal control cell counts (CNTRL), and the ratio of hippocampal BrdU+ cell counts to control cells counts (HF BrdU+/CNTRL). The indicator variable for year was not necessary, since staining for neurogenesis was only performed in the birds from the first year (sample). To check for any region-specific differences in HF BrdU+ cell counts, HF BrdU+ cell density, extra-hippocampal control cell counts, and HF BrdU+ cell counts/CNTRL, we divided the brain into 3 regions: rostral ($n = 11$ slices), middle ($n = 10$ slices) and caudal ($n = 10$ slices). We then established a dummy variable to distinguish the regions and an interaction variable to capture any differential effect of captivity between them. Finally, to test for within-group hemispheric differences in HF volume, HF BrdU+ cell counts, HF BrdU+ cell density, extra-hippocampal control cell counts and HF BrdU+ cell counts/CNTRL, we performed pair-wise mean comparisons (student-t tests) for each housing condition.

RESULTS

Hippocampal Volume

The hippocampal formation was 23% smaller in lab-housed birds than wild birds. The average hippocampal volume for captive birds was $10.74 \pm 0.40 \text{ mm}^3$ vs. $13.90 \pm 0.61 \text{ mm}^3$ for wild birds (Mean $\pm$ SEM). In all the regressions, all the coefficients were non-significant ($p > 0.1$) except housing ($p < 0.05$), which accounted for a minimum of 62% of the variance in HF volume [$F(6,12) = 3.20$, $R^2 = 0.62$, $p < 0.05$]. The estimated co-efficient of the housing variable was -2.92 mm^3 (95% CI: -0.87 mm^3 , -4.97 mm^3). To generate an unbiased measure of percentage volume change, a regression on the logarithm of the HF volume data was performed using the same dependent variables and produced the same results.

There was no statistically significant difference in HF volume between the two hemispheres for birds in either housing condition ($p > 0.05$).

Telencephalon Volume

Tel-HF volume did not differ between housing groups [$p > 0.1$; Fig. 3]. The average Tel-HF volume for captive birds was $291.00 \pm 8.06 \text{ mm}^3$ vs. $297.91 \pm 10.84 \text{ mm}^3$ for wild birds (Mean $\pm$ SEM). While the co-efficient for year was initially significant, once the two subjects with interpolated brain weights were dropped, year was no longer a significant predictor of Tel-HF.

Neurogenesis

Survival of new cells in the HF was unaffected by 6 weeks of captivity [Fig. 4]. There was no housing-group difference in any measure of BrdU+ cell counts ($p > 0.05$), including any region-specific differences in the hippocampus, extra-hippocampal control cell counts, or ratio of the two. There were no significant main effects or interactions between BrdU measures and housing ($p > 0.1$), and BrdU measures did not differ between hemispheres in either housing group ($p > 0.1$). The average number of BrdU+ cells for captive birds was 231 ± 56 vs. 250 ± 34 for wild birds (Rostral: 64 ± 13 vs. 89 ± 18 , Middle: 96 ± 26 vs. 92 ± 12 , Caudal: 71 ± 19 vs. 68 ± 8 , Captive vs. Wild, Mean $\pm$ SEM). In captive birds BrdU+ cell density was $21 \pm 5 \text{ cells/mm}^3$ vs. $18 \pm 3 \text{ cells/mm}^3$ for wild birds (Rostral: $21 \pm 4 \text{ cells/mm}^3$ vs. $22 \pm 5 \text{ cells/mm}^3$, Middle: $22 \pm 6 \text{ cells/mm}^3$ vs. $17 \pm 3 \text{ cells/mm}^3$, Caudal: $18 \pm 4 \text{ cells/mm}^3$ vs. $16 \pm 2 \text{ cells/mm}^3$, Captive vs. Wild, Mean $\pm$ SEM). The average number of BrdU+ cells was 57 ± 21 for captive birds vs. 58 ± 10 for wild birds in the extra-hippocampal control region (Rostral: 21 ± 9 vs. 23 ± 6 , Middle: 17 ± 6 vs. 17 ± 2 , Caudal: 20 ± 6 vs. 17 ± 3 , Captive vs. Wild, Mean $\pm$ SEM). In captive birds, the ratio of HF BrdU+ cells to extra-hippocampal control cells was 4.6 ± 0.1 vs. 4.7 ± 0.5 for wild birds (Rostral: 5.0 ± 1.7 vs. 7.4 ± 3.9 , Middle: 10.4 ± 4.2 vs. 5.5 ± 0.6 , Caudal: 4.2 ± 1.1 vs. 4.3 ± 0.7 , Captive vs. Wild, Mean $\pm$ SEM).

DISCUSSION

In this study we find that 6 weeks captivity in the fall causes a 23% reduction in hippocampal volume but no difference in new cell survival in adult black-capped chickadees, a wild food-storing bird. Data in mammals suggest the results could be due to stress, social isolation, lack of exercise or reduced opportunity to cache and a lack of hippocampal stimulation at a time when birds are normally optimally active in this regard. Data in wild food-storing birds are scarce, and in most studies interventions are confounded with laboratory housing. And since lab-housed wild birds are likely more stressed, less active, more socially isolated *and* faced with fewer hippocampal task-specific demands than in the wild, it is difficult to discern the effects of each of these factors individually, on the hippocampus. Furthermore, in the absence of data, the influence of lab-housing-related perturbations to other “normal” ambient conditions cannot be discounted, either. Aside from a more regular supply of food (ad libitum food), likely most lab-housing facilities are warmer, more humid and generally just more “spring-like” than the wild in winter. In lab-housed food-storing birds at least, existing data suggest that while a more constant supply of food reduces baseline plasma corticosterone (Pravosudov et al., 2001), proficiency on spatial memory tasks, and accuracy of cache retrieval (Pravosudov and Clayton, 2001), there is no change in hippocampal volume or total neuron number (Pravosudov et al., 2002a). And

changes in photoperiod do not affect baseline plasma corticosterone (Pravosudov et al., 2002b), hippocampal neurogenesis (Hoshooley et al., 2005) or volume of the HF (Krebs et al., 1995; MacDougall-Shackleton et al., 2003).

In lab-housed mountain chickadees (*Poecile gambeli*), psychosocial stress causes reduced cell proliferation (Pravosudov and Omanska, 2005a) and poorer spatial memory-test performance (Pravosudov et al., 2003), but is not associated with a change in hippocampal volume (Pravosudov and Omanska, 2005a) or baseline corticosterone levels (Pravosudov et al., 2003). A two-fold increase in baseline corticosterone does not alter telencephalon volume, hippocampal volume, cell proliferation, or cell survival in lab-housed mountain chickadees implanted with continuous time-release corticosterone pellets (Pravosudov and Omanska, 2005b). In male dark-eyed juncos (*Junco hyemalis*), a non-caching species, captivity during the breeding season (a time when the birds are normally actively navigating and defending a territory) is associated with reduced hippocampal volume (Smulders et al., 2000a). Adult black-capped chickadees (*Poecile atricapillus*) housed on short days cache more, but do not have a larger hippocampus or more hippocampal neurogenesis than birds on long days (Hoshooley et al., 2005). And there is no increase in hippocampal volume, neuron density or total neuron number in adult willow tits (*Parus montanus*) permitted to cache for 15 minutes a day (Cristol, 1996). In both cases, however, such lab exercises might be insufficiently demanding (relative to actions of animals in the wild) to generate meaningful (detectable) differences in hippocampal volume.

Field data are more scarce, even. In a 1996 study, Healy et al. found that migration experience in garden warblers (*Sylvia borin*) increases relative hippocampal volume, neuron number and neuron density. But perhaps only birds with a sufficiently large and developed hippocampus survive migration? And though captive experienced garden warblers did not have a smaller hippocampus than their free-living counterparts, brains were collected several months subsequent to the previous migration. Evidence for caching-related differences in neurogenesis and hippocampal volume are mixed in black-capped chickadees, perhaps related to variable periods of lab-housing immediately prior to sacrifice (Barnea and Nottebohm, 1994; Smulders et al., 1995, 2000b; Hoshooley and Sherry, 2004; Hoshooley et al., 2007).

While the exact reasons remain unclear, therefore, we find that 4 – 6 weeks captivity in the fall/winter period causes dramatic hippocampal atrophy with no difference in new cell survival in the adult black-capped chickadee; a food-hoarding bird. These results provide further evidence of the vulnerability of the avian hippocampus, and suggest it is similar to the mammalian hippocampus in this respect. As in mammals, it appears the cost of hippocampal plasticity in food-storing birds is increased sensitivity, or perhaps susceptibility to insult. These results may help explain why previous researchers have not found a link between caching and hippocampal plasticity in adult birds in the lab. If activity-dependent structural changes do occur in adult wild food-storing birds, and caching exercises sufficient to engage them are employed in the lab, captivity alone may eliminate any detectable differences between lab-housed experimental and control groups. Future study will determine if as brief a period as one to two weeks is sufficient to lead to structural changes in the hippocampus. Furthermore, while the neurogenesis data is perhaps best regarded as

tentative – neuronal counter-stain absent – that there was no difference in any measure of new cell survival yet substantial structural atrophy suggests either the attributes of existing cells such as synapses, dendrites and axons are easily compromised, or that new neurons in captive birds survive but do not elaborate to the extent they do in the wild; or both.

Acknowledgments

Grant Information: This research was supported by National Institutes of Health Grants MH56093 to Timothy J. DeVoogd.

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Figure 1.
Average HF volume by slice from immediately rostral to the hippocampus to immediately caudal of the hippocampus (left to right).

Figure 2. Micrographs (same magnification) demonstrating the difference in the size of the HF in a bird from each housing condition. Neither brain pictured was at the volumetric extreme of its group.

Figure 3. Mean telencephalon volume less hippocampal volume by slice from the rostral-most extent of the telencephalon to the caudal-most extent (left to right).

Figure 4.
Average HF BrdU+ cell density by slice (moving caudally left to right).