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Short communication

Female hatchling American kestrels have a larger hippocampus than males: A link with sexual size dimorphism?



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ABSTRACT

The brain and underlying cognition may vary adaptively according to an organism's ecology. As with all raptor species, adult American kestrels (*Falco sparverius*) are sexually dimorphic with females being larger than males. Related to this sexual dimorphism, kestrels display sex differences in hunting and migration, with females ranging more widely than males, suggesting possible sex differences in spatial cognition. However, hippocampus volume, the brain region responsible for spatial cognition, has not been investigated in raptors. Here, we measured hippocampus and telencephalon volumes in American kestrel hatchlings. Female hatchlings had a significantly larger hippocampus relative to the telencephalon and brain weight than males (~12% larger), although telencephalon volume relative to brain weight and body size was similar between the sexes. The magnitude of this hippocampal sex difference is similar to that reported between male and female polygynous *Microtus* voles and migratory and non-migratory subspecies of *Zonotrichia* sparrows. Future research should determine if this sex difference in relative hippocampus volume of hatchling kestrels persists into adulthood and if similar patterns exist in other raptor species, thus potentially linking sex differences in the brain to sex differences of space use of adults in the wild.

Neuroecology posits that the brain and underlying cognition are adaptively specialized to meet specific ecological functions [1]. A classic example of this principle is hippocampus volume, which is larger in species, sexes or populations that require greater spatial memory [2,3,4]. For instance, species [5] or populations [6] that store or rely more on stored food, typically have a larger hippocampus than those that rely less on stored food. Furthermore, migratory white-crowned sparrows (*Zonotrichia leucophrys gambelii*) were reported to have a larger hippocampus than a non-migratory subspecies (*Z. l. nuttalli*; [4]). In addition, several studies have reported sex differences in hippocampus volume. Males that have larger home ranges than females were reported to have a larger hippocampus than females in multiple species of rodents [3,7,8]. In rats, a male-biased sex difference in the volume of the dentate gyrus granule cell layer, a part of the hippocampus that influences spatial performance, was present in pre-pubescent individuals and at the same magnitude (8–9%) as in adults [9]. A female-biased sex difference in hippocampal volume was recorded in brood-parasitic cowbirds (*Molothrus* spp.) in which only females search for

host nests, but not in a species in which both sexes search for host nests (e.g., *M. rufocaxillaris*) nor in a related species that is not a brood parasite (e.g., *Agelaioides badius*) [10,11, but see 12]. As such, sex differences in hippocampus volume can depend on an individual's use of space, regardless of its sex. Raptors (birds of prey) offer a unique opportunity to further test these hypotheses because, unlike most birds and mammals, reversed sexual size dimorphism occurs in these species (females are larger than males), which could be associated with sex differences in spatial cognition. Yet, to our knowledge, sex-specific variation in hippocampus volume has not been examined in raptors.

The origin and maintenance of sexual size dimorphism in raptors remain elusive. A review examining several hypotheses for sexual size dimorphism in falcons, hawks, and owls found the strongest support for the related intersexual-competition and small-male hypotheses [13]. The intersexual-competition hypothesis proposes that the different sizes of the sexes have evolved to reduce intersexual competition for food [13]. The small-male hypothesis proposes that smaller males evolved to be more efficient foragers, because they expend less energy during

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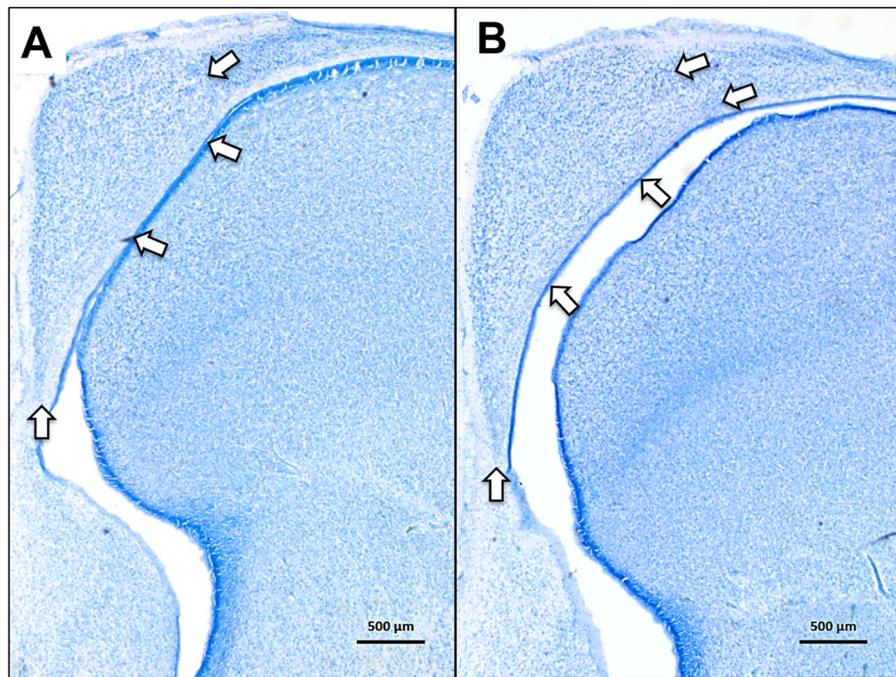


Fig. 1. Coronal section of hippocampus near the anterior commissure in male (left; A) and female (right; B) hatchling American kestrels. The boundaries of hippocampus are indicated by arrows.

hunting than larger males. This facilitates hunting more agile prey [13]. Larger female raptors can more easily handle larger prey that have larger home ranges, which may require females to have a larger hippocampus than males to allow for better spatial cognition. Another aspect relevant to spatial cognition in raptors is that females tend to migrate further than males [14,15].

We examined sex differences in hippocampus volume relative to telencephalon volume (measure of forebrain size) and brain weight of hatchling American kestrels (*Falco sparverius*; hereafter “kestrel”). As adults, kestrels show moderate sexual size dimorphism, with females being 15–20% larger than males at the level of the subspecies [16,17]. Kestrels are North America’s smallest falcon, with males weighing 100 g and (non-breeding) females 120 g, feeding primarily on insects and other invertebrates, as well as on small rodents and birds (e.g., voles, sparrows, warblers) [17]. However, kestrels can hunt larger prey, including squirrels and woodpeckers [16,17]. Compared to females, male kestrels hunt more birds [18], a more agile prey type than mammals such as rodents. This prey-type related difference linked to female and male kestrel sex supports the small-male and intersexual competition hypotheses described above [13]. We might expect that the behaviour of each sex related to spatial cognition would be specialized, with female kestrels specializing in larger-bodied prey and so potentially having to hunt over a larger area than males. Under this paradigm, females would be expected to have a larger hippocampus volume than males. In addition, female kestrels migrate longer distances than males, which could require greater spatial memory and navigational skills [4,19]. Therefore, because there are sex differences in prey specialization and migration distances, we predicted that female kestrels would have a larger relative hippocampus than males.

All animal handling procedures and protocols used in this present study were approved by the United States Geological Survey Patuxent Wildlife Research Center (USGS PWRP) Animal Care and Use Committee. The present study was part of a larger study examining the effects of the lipophilic flame retardant 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) (CAS# 183658-27-7) on hatchling kestrels. Eggs were collected fresh daily from a captive colony at the USGS PWRP. Eggs were set on their sides to incubate and rotated 180° hourly;

incubation occurred at 37.5 °C at 10L:14D (photoperiod), with humidity set between 35–40% and readjusted accordingly to maintain a mean weight loss of 16% over incubation [20]. On embryonic day 5 (ED5), eggs of known fertility were injected into the aircell with either organic safflower oil (Irresistible Brand®, BioOrganic) (controls) or one of three fixed doses (11, 55, or 137 ng/g egg) of EH-TBB (> 99% purity; both from Wellington Laboratories Inc., Guelph, ON, Canada). Immediately following injection, the holes were sealed with a 6.35 mm diameter piece of adhesive material from a waterproof clear bandage (CVS Pharmacy, Laurel, MD, USA). On ED24, incubation temperature was set at 37 °C and relative humidity at 70% until hatching (ED28). On ED28, hatchlings were sacrificed by decapitation and their brains removed. Brains were placed in 10% formalin for two weeks, immersed in 30% sucrose until they sank to the bottom of the vial (approximately 30 h), and frozen at -80 °C. Brains were shipped frozen on dry ice to McGill University and stored at -80 °C until processing.

Brains were sectioned on a cryostat (thickness = 40 µm), every fifth section collected into 0.1 M phosphate buffered saline, and free-floating sections mounted onto gelatin-coated slides. Dried, mounted slides were Nissl stained with thionin, serially dehydrated in ethanol, cleared in solvent, and coverslipped with Permount (Fisher Scientific, Ottawa, ON). Entire brain sections were scanned with a high-resolution (4800 dpi) flatbed scanner and images of the hippocampus were captured with a Zeiss Imager M2 light microscope set to a 2.5X objective. The microscope was equipped with an AxioCam HRC digital camera (Carl Zeiss AG) connected to a computer with Zen 2 (Blue Edition [2012]; Carl Zeiss AG) image analysis software. The lateral boundary selected for the hippocampus of kestrels matched well the change in cell size and cell density between area parahippocampalis (APH; lateral part of the hippocampus) and hyperpallium apicale (region lateral to hippocampus) in passerines (Fig. 1) [12]. The same observer (M.F.G.), blind to the treatment groups and sex of the subjects, traced the hippocampus from every collected section, and telencephalon from every second collected section, using ImageJ (NIH). The cross-sectional surface areas were measured and the frustum volume between each surface area measurements was calculated to estimate volume of hippocampus and telencephalon. Section intervals for area measurements were 200 µm

and 400 μm for hippocampus and telencephalon, respectively. We measured 12–20 sections for hippocampus and 11–17 sections for telencephalon.

We conducted statistical analyses using SAS[®], Version 9.4 (SAS Institute; Cary, North Carolina). Our main statistical analysis consisted of a general linear model (Proc GLM) that examined the effects of sex, treatment (vehicle control, low EH-TBB, mid EH-TBB, or high EH-TBB), and their interaction on mean hippocampus volume (mean of left and right hemispheres). We previously determined with a linear mixed model (Proc Mixed), which included hemisphere as a repeated measure, that there was no significant difference between the left and right hemispheres for hippocampus volume ($F_{1,27} = 2.63, p = 0.12$). To the general linear model for mean hippocampus volume, we added two covariates to control for brain size; mean telencephalon volume (with mean hippocampus subtracted) and brain weight. We also conducted a second neuroanatomical analysis examining the effects of the same factors on mean telencephalon volume, corrected for brain size via brain weight and body size (crown-to-rump length). Crown-to-rump length is an accepted measure of body size in birds, including those exposed to stressors [21,22] because it avoids the potential problem of fluctuating asymmetry as a confounding factor, such as may occur with tarsus length. To measure crown-to-rump length, each hatchling was placed on a centimeter ruler, aligning the body from the head to the feet along the same plane of the ruler. The same two observers handled and recorded the readings directly from the ruler. The ruler was screwed into a wooden block, which creates a fixed point against which the head of the animal was placed. Finally, we ran a general linear model examining the effects of sex, treatment, and their interaction on crown-to-rump length to determine whether sexual-size dimorphism was present at hatching. We confirmed that residuals from all analyses were normally distributed using a Shapiro-Wilk test. All values are presented as means \pm standard error of the mean (SEM), except for hippocampus volume, which is presented as marginal means (least square means in SAS[®]) to control for covariates. Statistical significance for all tests was set at $p < 0.05$.

There was no effect of treatment or interaction between treatment and sex in our analyses for hippocampus ($0.46 \leq p \leq 0.61$), telencephalon ($0.13 \leq p \leq 0.65$), and crown-to-rump length ($0.18 \leq p \leq 0.46$). Because EH-TBB had no effect on the relevant brain and body size parameters, we included birds exposed to EH-TBB with the vehicle control birds in our analyses to increase statistical power. Sample sizes were as follows: 10 females and 11 males from the treated group and 3 females and 4 males from the vehicle control group.

For hippocampus volume, there was a significant effect of sex ($F_{1,18} = 13.02, p = 0.002$), with females having a larger hippocampus than males relative to the telencephalon (Fig. 2). Telencephalon volume minus hippocampus volume was a significant covariate in the model ($F_{1,18} = 9.96, p = 0.006$), whereas brain weight, which averaged 578.58 ± 9.76 g for females and 593.54 ± 13.81 g for males, was not statistically significant ($F_{1,18} = 2.00, p = 0.17$). The female hippocampus corrected for telencephalon and brain size ($3.01 \text{ mm}^3 \pm 0.07$), was approximately 12% larger than that of males ($2.64 \text{ mm}^3 \pm 0.07$; Fig. 2).

Unlike with hippocampus volume, there was no significant effect of sex on telencephalon volume ($F_{1,18} = 0.03, p = 0.86$) (Fig. 2). Crown-to-rump length was not a significant covariate in the model for telencephalon volume ($F_{1,18} = 2.34, p = 0.14$), although brain weight tended toward significance ($F_{1,18} = 3.93, p = 0.06$). Finally, female kestrels had a significantly longer straight crown-to-rump length (6.05 ± 0.07 cm) than males (5.82 ± 0.06 cm) ($F_{1,21} = 5.83, p = 0.02$).

This female-biased sex difference in hippocampus volume in hatchling kestrels (Fig. 2) suggests that females may rely more on spatial memory than males, as reported in other wildlife species. In voles (*Microtus* sp.), sex-specific patterns of space use related to mating system predicted the size of the hippocampus [3] and spatial ability, as

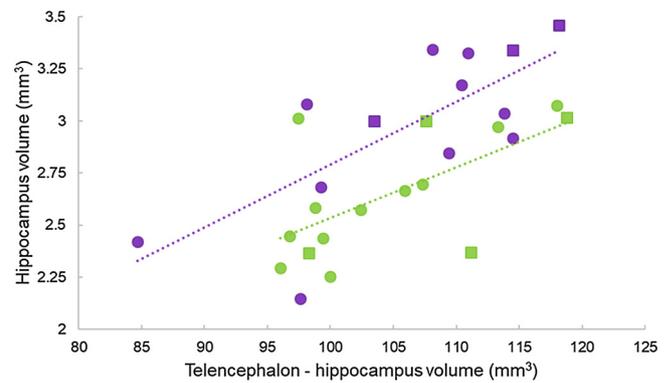


Fig. 2. Scatterplot of hippocampus volumes and associated telencephalon volumes (telencephalon volume minus hippocampus volume; mm^3) for female (dark purple; $n = 13$) and male (light green; $n = 15$) hatchling American kestrels. Individuals only exposed to safflower oil are indicated by squares, whereas individuals exposed to 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EH-TBB) are indicated by circles. Females had a significantly larger relative hippocampus (dark purple trend-line), than males (light green trend-line), with no effect of EH-TBB. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

measured by their performance in a maze test [23]. The home range size of polygynous male meadow voles (*M. pennsylvanicus*) was found to encompass the home ranges of many females, and males had a significantly larger hippocampus and better spatial ability than females [3,23]. In contrast, male and female monogamous pine voles (*M. pinetorum*) were found to share similarly sized home ranges, and had a similar hippocampus size and spatial ability [3,23]. Indeed, male meadow voles had a hippocampus that was $\sim 10\%$ larger than that of females [3], a sex difference consistent with what we report here in hatchling kestrels ($\sim 12\%$; Figs. 1 and 2).

We report a sex difference in crown-to-rump length, suggesting that reverse sexual size dimorphism in kestrels is present at hatching. However, the effect is weak as we may expect in hatchlings for this species with moderate reverse size dimorphism, with only $< 4\%$ difference between female and male mean crown-to-rump lengths. Previous research with American kestrels showed that sexual size dimorphism becomes more evident later during nestling development, with a sex difference in growth appearing at about 7 d of age, producing differences in body weight between the two sexes at about 16–21 d [24]. Yet, we observed a strong sex difference in hippocampus volume at hatching ($\sim 12\%$), suggesting that the hippocampus volume may be under strong selection to be larger in females throughout development.

The source of this sex difference in hippocampus volume, at the cellular level, is unknown. Changes could be due to multiple factors. To better understand the connection between hippocampus and memory, Roth and Pravosudov [6] advocated the quantification of specific features of the hippocampus, such as the size and number of neurons and spacing between neurons, measures that can be assessed using NeuN immunohistochemistry [25]. As such, future research should measure fine-scale cellular characteristics of the hippocampus concurrently with neuroanatomy [6].

In conclusion, we found a significant female-biased sex difference in hippocampus volume relative to the telencephalon in hatchling kestrels. This difference ($\sim 12\%$) was at a magnitude similar to that previously reported between female and male *Microtus* sp. of voles ($\sim 10\%$) [3], migratory and non-migratory subspecies of white-crowned sparrows ($\sim 13\%$) [4], and between female and male brown-headed cowbirds ($\sim 15\%$) [10]. Our findings in the present study concern hatchlings whereas the findings for cowbirds, sparrows, and voles concern adults [3,4,10,11]. Although the sex difference in hippocampus volume we report in hatchling kestrels may persist into adulthood [9], we recommend that future research examine hippocampus volume in adult

kestrels. A second step would be to link sex differences in the hippocampus with sex differences in spatial behaviour in the wild and spatial cognition. There is a paucity of information on spatial memory in raptors, including kestrels. With the rapid advent of new technology, it should soon be feasible to use GPS loggers on smaller individuals like kestrels, to track their fine-scale movements. Although our study included large sample sizes for a neuroanatomy study, it only included one species. Future work should include multiple species of raptors with varying sexual size dimorphism across the species, to determine if sex differences in hippocampus volume occur in other raptors and whether they correspond to the pattern of sexual size dimorphism evident across raptor species. The study of neuroecology in raptors is an exciting avenue for future research.

Disclaimer

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Conflict of interest

The authors declare no conflict of interest.

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