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Hummingbirds have a greatly enlarged hippocampal formation

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Both field and laboratory studies demonstrate that hummingbirds (Apodiformes, Trochilidae) have exceptional spatial memory. The complexity of spatial–temporal information that hummingbirds must retain and use daily is probably subserved by the hippocampal formation (HF), and therefore, hummingbirds should have a greatly expanded HF. Here, we compare the relative size of the HF in several hummingbird species with that of other birds. Our analyses reveal that the HF in hummingbirds is significantly larger, relative to telencephalic volume, than any bird examined to date. When expressed as a percentage of telencephalic volume, the hummingbird HF is two to five times larger than that of caching and non-caching songbirds, seabirds and woodpeckers. This HF expansion in hummingbirds probably underlies their ability to remember the location, distribution and nectar content of flowers, but more detailed analyses are required to determine the extent to which this arises from an expansion of HF or a decrease in size of other brain regions.

Keywords: hummingbirds; spatial memory; hippocampus; comparative method

1. INTRODUCTION

Hummingbirds visit hundreds of flowers per day [1]. In order to feed efficiently, they must remember what flowers they have visited, the locations of high nectar-rewarding flowers and a host of additional spatial–temporal information. A combination of field and laboratory studies demonstrate that hummingbirds can remember the nectar quality and content of individual flowers [2], nectar-refilling rates [3], spatial location and distribution of flowers [1], avoid revisiting recently sampled flowers [4,5] and rely on ‘episodic-like’ memory for daily foraging [3].

Although several studies have investigated the behaviour of hummingbirds with respect to spatial–temporal memories [1–6], nothing is known about

the morphology of the hippocampal formation (HF), which plays a critical role in spatial memory [7,8]. Variation in the size of the HF, relative to the telencephalon, in birds often reflects differences in spatial memory; individuals and species with better spatial memory tend to have larger HFs. For example, food-caching songbirds have relatively larger HFs than songbirds that do not cache food [7,8]. Given this relationship between the relative size of the HF and spatial memory, we predicted that hummingbirds would have HFs that are enlarged in a way similar to that of food-caching parids and corvids. Here, we provide the first evidence that hummingbirds do indeed have greatly enlarged HFs compared with other birds.

2. MATERIAL AND METHODS

(a) Specimens

Hummingbird specimens were wild caught in Panama or sent to us from other researchers. Rufous-tailed (*Amazilia tzacatl*) and long-tailed hermits (*Phaethornis superciliosus*) were trapped with mistnets near the Smithsonian Tropical Research Institute in Gamboa, Panama. Once captured, the hummingbirds were given an overdose of isoflurane, decapitated and immersion-fixed in 10 per cent neutral-buffered formalin (pH 7.4). In addition, we received immersion-fixed heads of Anna’s (*Calypte anna*) and rufous hummingbirds (*Selasphorus rufus*) from Drs Ken Welch Jr., and Raul Suarez (University of California, Santa Barbara). We also examined the brains of two window-killed common swifts (*Apus apus*) that were immersion-fixed in 4 per cent paraformaldehyde and sent to us by Dr Gerard Gory (Museum d’Histoire Naturelle de Nimes, France). Swifts (Apodidae) are the sister group to hummingbirds [9,10] and examining even one species will provide some insight into determining whether any changes in HF arose solely in hummingbirds or are common to all members of the clade (Apodiformes).

(b) Hippocampal formation measurements

All specimens were embedded in gelatin, serially sectioned at a thickness of 40 µm in the coronal plane on a freezing microtome and free-floating sections collected in 0.1 M phosphate-buffered saline. Every section was mounted onto gelatinized slides in two alternating series, stained with thionin and coverslipped with Permount.

To measure volumes of both the HF (electronic supplementary material, figure S1) and the telencephalon, we used the Cavalieri method [11], as implemented in Stereo Investigator (MicroBrightfield, Inc., Colchester, VT, USA), because this method is free of measurement bias, is replicable across species and individuals and is frequently used for HF measurements in birds [7,12,13]. We measured the HF and telencephalon on every fourth section of the hummingbird and swift specimens with a 200 µm grid using the same HF borders as earlier studies [14,15]. The error coefficients [11] of the HF and telencephalon measurements were 0.021 (range = 0.014–0.040) and 0.009 (range = 0.008–0.024), respectively.

Data for 77 additional species were gleaned from the literature (see electronic supplementary material). Although there are criticisms regarding the combination of data from disparate sources in comparative studies [7,16], it is only by including data for a broader range of species that we could determine with confidence whether hummingbirds have larger HFs than other birds. Furthermore, all of the literature data that we compiled used the same criteria to determine the borders of the HF [15,17].

(c) Statistical analyses

Relative HF volume was examined in two different ways. First, we calculated the relative size of the HF as a percentage of total telencephalic volume and performed an analysis of variance (ANOVA) with the following groups: food-caching songbirds, non-caching songbirds, seabirds, woodpeckers and hummingbirds. The swift was excluded from the ANOVA because we had data only for a single species. Prior to running the ANOVA, we log-transformed the percentages such that the data were normally distributed (Shapiro–Wilk; $p > 0.05$). To account for phylogenetic effects on relative HF size, we constructed a phylogeny of all 82 species based on several recent studies (see electronic supplementary material) and set all branch lengths to 1. Using this phylogeny, we ran a phylogenetic-generalized least-squares (PGLS) analysis on relative HF volume (expressed as a percentage) and the groups listed above for the ANOVA with REGRESSION v. 2.M [18]. Following Lavin *et al.* [18], we tested several models of evolutionary change

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and selected the model with the lowest Akaike information criterion (AIC) value. Second, we \log_{10} -transformed the volume of the HF and plotted it against the \log_{10} -transformed volume of the telencephalon, minus that of the HF. We then constructed phylogeny-corrected 95% prediction intervals in the PDAP:PDTree module of MESQUITE [19,20] in a fashion similar to recent allometric studies [19,21]. The swift was included in the calculation of the prediction intervals to determine whether it was a significant outlier compared with the other taxa included in our analysis.

3. RESULTS

On average, hummingbirds have a HF that comprises 12.81 per cent of telencephalic volume (range = 10.42–14.41%). This is two to five times larger than that for any of the other species sampled and much larger than that of the common swift (5.63%). The conventional ANOVA ($F = 13.96$, d.f. = 4, 76, $p < 0.01$, AIC = 22.98) and the PGLS analysis (Ornstein–Uhlenbeck model, $F = 4.51$, d.f. = 4, 76, $p < 0.01$, AIC = -38.81) yielded a significant difference across the five groups. Post hoc Tukey–Kramer tests revealed that hummingbirds have significantly larger relative HF volumes than woodpeckers, seabirds and songbirds and confirmed the significantly larger relative HF of caching compared with non-caching songbirds. No other differences were detected.

Similar results were obtained in our comparison of HF volume plotted against telencephalic volume (figure 1*b*). The hummingbirds lie above the 95% prediction interval calculated using both conventional statistics and phylogeny-based methods. Indeed, the long-tailed hermit has a telencephalic volume similar to the non-caching songbird, the American redstart (*Setophaga ruticilla*), but the hermit has a hippocampal volume that is almost 10 times larger than that of the redstart (8.89 and 0.99 mm³, respectively). Thus, regardless of how we express relative HF volume, hummingbirds have much larger HF volumes than all other birds examined to date.

4. DISCUSSION

All of our analyses indicate that hummingbirds have a greatly enlarged HF (electronic supplementary material, figure S1). The magnitude of difference in relative HF volume between hummingbirds and other birds is greater than any differences that could have arisen from histological processing or measurement error.

The expansion of the HF in birds is generally associated with better spatial memory [7,8,14,15] and given the ability of hummingbirds to remember a range of spatial–temporal features of their flowers, the gross enlargement of the HF of hummingbirds probably reflects their spatial memory. That said, there are other factors that could have contributed to HF enlargement in hummingbirds. For example, hummingbirds hover and have a unique brain morphology. Hovering flight is associated with the enlargement of at least one visual region in the avian brain [21], but this region is not directly connected with the HF [22]. Hummingbirds have relatively small telencephala and enlarged cerebella [23]. Other species included in our analyses also have small telencephala, but lack an enlarged HF (e.g. common swift), so this HF expansion is unlikely to have arisen from a scaling effect. It does, however,

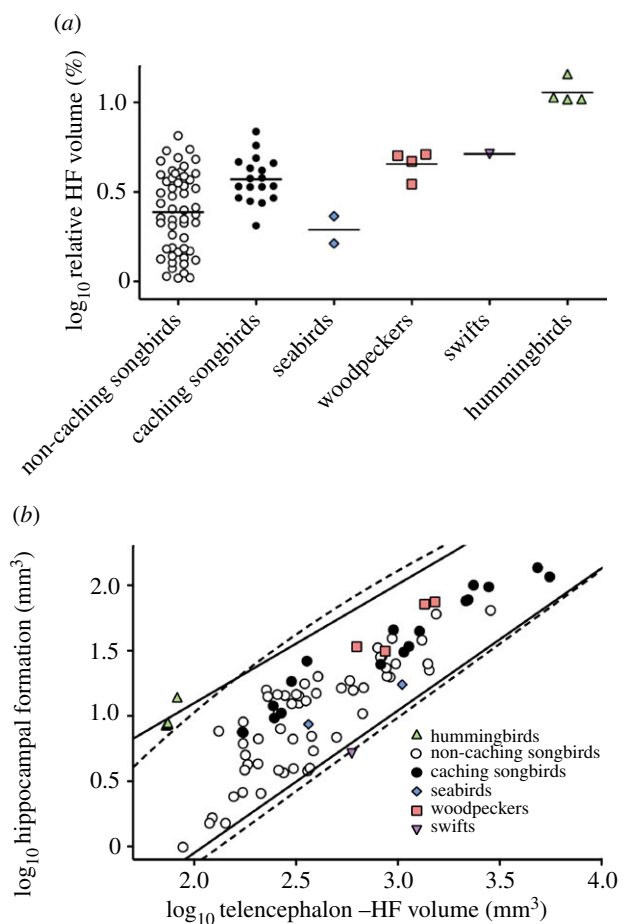


Figure 1. (a) Scatterplots of relative hippocampal formation (HF) size, as expressed as the log-transformed percentage of telencephalon volume, of food-caching and non-food-caching songbirds, woodpeckers, seabirds, swifts and hummingbirds. The lines depict the means of each group. (b) Scatterplots of \log_{10} -transformed HF volume plotted against \log_{10} -transformed telencephalon (minus HF) volume of hummingbirds and all other birds. The lines indicate the 95% CI: solid lines denote conventional statistics; dashed lines denote incorporating phylogeny.

suggest that hummingbirds may have undergone a reduction in the size of other telencephalic regions. That is, hummingbirds might not have expanded the HF so much as reduced the size of the rest of the telencephalon. Future studies should therefore examine all telencephalic brain regions across a range of species, including swifts, to determine the extent to which HF expansion is offset by the reduction of other regions.

Animal protocols in Panama were approved by the University of Mississippi Institutional Animal Care and Use Committee (IACUC) and the Smithsonian Tropical Research Institute IACUC. We thank the STRI and the visitor and scientific support staff for managerial and logistical support and Autoridad Nacional del Ambiente and Autoridad del Canal de Panama for granting us permission to conduct research in Panama. All protocols also adhered to the Canadian Council of Animal Care Guidelines. We wish to thank Drs Ken Welch Jr., Raul Suarez and Gerard Gory for donating specimens, several anonymous reviewers for their comments and NSERC for funding to D.R.W., D.M.S. and A.N.I. and the University of Mississippi Office of Research and Sponsored Programmes, and Department of Biology for funding to L.B.D. and S.R.W.

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