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Sexually Dimorphic Neural Phenotypes in Golden-Collared Manakins *(Manacus vitellinus)*

Lainy B. Day[a] Leonida Fusani[c] Carol Kim[b] Barney A. Schlinger[b]

[a]Department of Biology, University of Mississippi, Oxford, Miss., and [b]Departments of Integrative Biology and Physiology and Ecology and Evolutionary Biology, UCLA, Los Angeles, Calif., USA; [c]Department of Biology and Evolution, University of Ferrara, Ferrara, Italy

Key Words
Manakin · *Manacus vitellinus* · Sex differences · Hippocampus · Arcopallium · Cerebellum · Ventrolateral mesopallium · Entopallium

Abstract

Male golden-collared manakins *(Manacus vitellinus)* perform a high-speed acrobatic courtship display punctuated by loud 'snaps' produced by the wings. Females join males on display courts to select individuals for copulation; females follow displaying males but do not perform acrobatics or make wing snaps. Sexually dimorphic courtship displays such as those performed by manakins are the result of intense sexual selection and suggest that differences between sexes exist at neural levels as well. We examined sex differences in the volume of brain areas that might be involved in the male manakin courtship display and in the female assessment of this display. We found that males had a larger hippocampus (HP, spatial learning) and arcopallium (AP, motor and limbic areas) than females when adjusted for the size of the telencephalon (TELE) minus the target area. Females had a larger ventrolateral mesopallium (MVL) both when adjusting for the size of the remaining TELE and by direct comparison. The entopallium (E) was not sexually dimorphic. The E is part of the avian tectofugal pathway and the MVL is linked to this pathway by reciprocal connections. The MVL likely modulates visually guided behavior via descending brainstem pathways. We found no sex differences in the volume of the cerebellum or cerebellar nuclei. We speculate that the HP is important to males for cross-season site fidelity and for local spatial memory, the AP for sexually driven motor patterns that are complex in males, and that the MVL facilitates female visual processing in selecting male display traits. These results are consistent with the idea that sexual selection has acted to select sex-specific behaviors in manakins that have neural correlates in the brain.

In many species, males and females perform vastly different behaviors. Such behavioral sex differences are frequently associated with sexually dimorphic brain regions. As expected, sex differences are frequently detected in brain regions regulating primary sex-specific behaviors, such as in those controlling copulation [e.g. Arnold and Gorski, 1984]. Robust differences between the sexes can also be seen in circuits that are indirectly related to reproduction. For example, in songbird species where only males sing, volumes of several song control nuclei, HVC, area X, and the robust nucleus of the arcopallium (RA) are larger in males than in females, where-

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Lainy B. Day
Department of Biology, University of Mississippi
Post Office Box 1848
Oxford, MS 38677-1848 (USA)
Tel. +1 662 915 5444, E-Mail lainyday@olemiss.edu

as there is no dimorphism in species in which both sexes sing [Nottebohm and Arnold, 1976; Kirn et al., 1989; Brenowitz, 1997; Tramontin et al., 1998; Airey et al., 2000]. The size of the hippocampus (HP), a region that plays a prominent role in spatial ability in birds and mammals, is related to sex-specific demands for locating dispersed resources [Jacobs et al., 1990; Gaulin, 1992; Galea et al., 1996; Reboreda et al., 1996; Day et al., 2008]. For instance, hippocampal size is larger in female than in male cowbirds in species where males do not accompany females during nest searching for brood parasitism [Reboreda et al., 1996]. Sex-specific behaviors can drive sex-specific neuroanatomy. In the same cowbird species where sex differences in nest searching favor a larger HP in female cowbirds, differences in singing behavior favor a larger HVC in males [Hauber et al., 1999]. We sought to examine these sex-specific neural phenotypes in golden-collared manakins. While many species perform sexually dimorphic courtship displays, we know little about sex differences in the neural circuitry underlying complex physical courtship displays like those of the golden-collared manakins.

At the beginning of each dry season in Panama (January), colorful male golden-collared manakins (Suboscines, *Manacus vitellinus,* manakin from here out) locate the site of their lek from the previous year(s), reclear their arena on the forest floor and begin performing their elaborate, acrobatic courtship display. This remarkable display includes powerful jumps between saplings and/or the ground, half-flips in mid-air and the production of loud snaps with their wings [described in Chapman, 1935; Prum, 1998; Fusani et al., 2007b; Schlinger et al., 2008a, b]. These displays are performed at great speed with exquisite neuromuscular control and serve to attract multiple females for mating. The cryptically colored green female manakins are thought to roam the forests until attracted to the leks by the noise of displaying males. Females do not perform the complex display of males [Fusani et al., 2007b] but appear able to discriminate the features of the display that are performed at unusually high speed. We have evidence that females favor males that perform postural display elements tens of milliseconds faster than other males [Fusani et al., 2007a, b; Barske et al., 2011].

These patterns of behavior suggest that there may be unique demands placed on the neural circuits subserving complex motor control, spatial learning and memory, and rapid visual processing in manakins. Furthermore, in light of their unique behavioral repertoires, males and females may express different properties in these circuits. We have already described the sex steroid dependence of the male display [Day et al., 2006, 2007a, b; Fusani et al., 2007a; Schlinger et al., 2008a, b] as well as some sex-specific spinal and muscular adaptations [Schultz and Schlinger, 1999; Schultz et al., 2001; Feng et al., 2010]. To test the idea that there are sex-specific adaptations in brain circuitry, we measured the volume of several brain regions involved in motor control (cerebellum, CB, cerebellar nuclei, CN, and arcopallium, AP), spatial memory (HP) and visual processing (entopallium, E, and ventrolateral mesopallium, MVL). The CB and AP are of special interest because of their high expression of androgen receptors (ARs) on which androgens likely act to help control male behavior [Day and Olster, 2003; Fusani et al., 2003]. In addition, the AP of oscine songbirds contains nucleus RA that functions in the premotor control and sensory feedback of song [Roberts et al., 2008]. As wing snaps, an acoustic signal like song, are a conspicuous element of male manakin courtship, we were curious whether there were specializations in the AP of the suboscine manakin similar to the existence of the RA in the AP of oscine songbirds. Therefore, in addition to measuring the volume of the AP, we also examined neuron size and cell density in five regions of the AP to quantify anatomical distinctions in these areas. The E is part of the avian tectofugal pathway and MVL is linked to this pathway by reciprocal connections with E [Krützfeldt and Wild, 2004, 2005]. We were influenced in our investigation of these telencephalic visual nuclei by preliminary findings suggesting that the telencephalon (TELE) adjusted for whole brain (WB) volume was larger in female than in male manakins. Pondering this result in light of female manakins' discrimination of tenths of milliseconds differences in males' displays, we predicted that the E, a large telencephalic nucleus believed to be involved in pattern discrimination, motion perception, and visual recognition including intraspecies recognition [Nguyen et al., 2004; Yamamoto and Watanabe, 2007; Watanabe et al., 2008] would be larger in females than in males. The function of the second visual nucleus, MVL, is less well understood but is likely related to modulation of visual guided behavior via descending striatal-brainstem pathways [Krützfeldt and Wild, 2004]. We did not expect sexual dimorphism in MVL.

Materials and Methods

Animals

All birds were captured in the forest near Gamboa, Panama, under permits from local authorities and approved protocols from the Animal Care and Use Committee of University of California, Los Angeles, and Smithsonian Tropical Research Institute. We collected 12 golden-collared manakins (n = 6 males, n = 6 females) during the peak of the breeding season in mid-April. Birds were held briefly in captivity for behavioral observation prior to sacrifice (males mean = 7 days, SE ±1.06, females mean = 4 days, SE ±1.95). Housing conditions are described elsewhere [Day et al., 2006, 2007b]. Briefly, birds were housed individually in cages approximately 360 × 290 mm in a room with open screened windows and natural light that was supplemented by fluorescent lighting, manually turned on and off to approximate sunrise and sunset times – 12 h daylight. Food and water were available ad libitum.

Histology and Morphometrics

Birds were deeply sedated with isoflurane as confirmed by toe pinch and perfused transcardially with 0.9% saline followed by neutral buffered formalin (NBF). Brains were removed and postfixed in NBF overnight then infiltrated in succession with 20 and 30% sucrose in 0.1 M phosphate buffer. Brains were frozen and maintained on dry ice until transported to UCLA for transfer to an ultrafreezer at –80°C. Brains were cut in the coronal plane on a cryostat at 30 μm; every 5th section was slide-mounted and Nissl-stained with thionin. Slides were coded so that the investigators making measurements were unaware of the sex of the individuals. All birds were used for volume measurements while 4 randomly chosen individuals of each sex were used for AP cell size and density measurements.

We measured the area of the HP, MVL, E including the perientopallial belt, CB, CN, and AP on every section collected (150 μm apart, fig. 1). The TELE and WB were measured on every other collected section (300 μm apart). We calculated volume from areas as distance between sections times the sum of the areas. For TELE and WB, we randomly chose from the first or second slice collected and then measured every other collected slice from this starting slice. Regional areas were determined by capturing images of slices with either MRGrab (UCLA) or Axiovision (University of Mississippi) software (Carl Zeiss, Inc., Thornwood, N.Y., USA) via light microscopes (Zeiss) equipped with AxioCam digital cameras (Zeiss) and using Axiovision software to measure surface area. For TELE and WB, images were captured with a stereoscope (Zeiss). For area measurements, we used a magnification that would allow the whole region of interest to fit within the software capture frame (HP, AP, MVL, and E ×2.5, TELE and WB ×0.8, and CB ×1.25, each with a ×10 phototube); higher magnification (up to ×20) was used to assess boundaries when necessary. Both hemispheres were measured for CB, CN, and WB. For other regions, we measured the left and right hemispheres of 3 subjects. We found no trend for hemispheric specialization for any region. We thus measured only one hemisphere for the remainder of the birds, randomly selecting the left or right side for measurement, unless the quality of the histological preparation biased measurement to one side. When adjusting TELE size for WB size, we multiplied the single TELE hemisphere measured by 2 so that measurements reflected the proportion of the WB occupied by the whole TELE. The area of the ventricles was subtracted from each slice of the TELE and WB to correct for any differences between subjects in ventricular expansion during perfusion. Boundaries of each brain region were determined using previously established neuroanatomical boundaries and the revised avian brain nomenclature [Reiner et al., 2004] was applied. Each region was measured throughout its rostral-caudal extent. We used canary, zebra finch, chicken, and pigeon atlases [Karten and Hodos, 1967; Stokes et al., 1974; Kuenzel and Masson, 1988; Nixdorf-Bergweiler and Bischof, 2007] along with papers specific to our regions of interest [Sherry et al., 1989; Timmermans et al., 2000; Laverghetta and Shimizu, 2003; Krützfeldt and Wild, 2004, 2005; Day et al., 2005; Guirado et al., 2005; Yamamoto et al., 2005; Sadananda et al., 2007; Day et al., 2008; Feenders et al., 2008; Watanabe et al., 2008; Atoji and Wild, 2009; Campanella et al., 2009; da Silva et al., 2009; Saint-Dizier et al., 2009] to identify region boundaries. Specific boundaries for each region are given below.

Hippocampus

In birds, the HP is bounded by the midline, the lateral ventricle, and the brain surface. It can be distinguished laterally from the apical part of the hyperpallium (HA) by an obvious increase in cell density where HA begins (fig. 1a) [Sherry et al., 1989; Day et al., 2005].

Cerebellum

The CB is physically separated from other brain regions in rostral and caudal coronal sections (fig. 1b) and in middle rostrocaudal sections; it can be separated from the remainder of the metencephalon at the breaks between the CN and the nucleus angularis and vestibular nuclei [Karten and Hodos, 1967; Arends and Zeigler, 1991]. White matter of the ventral portion of the CB, such as the cerebellar commissure and lateral cerebellar-vestibular process, was included in cerebellar measurements with dorsal boundaries drawn starting from the flocculus, following a line between the cerebellar tracts and hindbrain nucleus somewhat dorsally, and then dipping ventrally to include the lingula. We measured the entire CB including the CN and surrounding deep white matter. Coronal sectioning made measurement of CB folia impractical. To measure the CN themselves, we measured only the cell-dense areas that define the CN. We outlined the CN as a unit when they were spatially contiguous and separately where cell-dense regions were separated by white matter, then summed all these cell-dense areas to get a total CN area (fig. 1c).

Arcopallium

In comparing gross anatomy, the rostral appearance of the AP in the golden-collared manakin is most similar to that of the canary, appearing to be bordered ventrolaterally by the subpallial amygdaloid area (SpA) [Reiner et al., 2004] (fig. 1d) and dorsomedially by the nucleus taeniae of the amygdala (TnA) [Stokes et al., 1974]. More caudally, the lateral boundary of the AP is the frontoarcopallial tract with the dorsal arcopallial lamina curving from ventrolateral to dorsal and then moving back ventromedially to establish the more dorsal boundary of the AP (fig. 1d). Where the AP neared the ventricle, we used the ventricle as the ventral border. This means that on some slices we included portions of the occipitomesencephalic tract in our measurements. We also included a small cytoarchitectonically distinct region of ventral cells in our measure of whole AP. This may correspond to Zeier and Karten's [1971] archistriatum posterior, now the posterior nucleus of the pallial amygdala (PoA) [Reiner et al., 2004], and thus be amygdalar in nature [Campanella et al., 2009; da Silva et al., 2009; Saint-Dizier et al., 2009]. We did not include the other AP amygdaloid regions, SpA or TnA, in our measurements of AP volume.

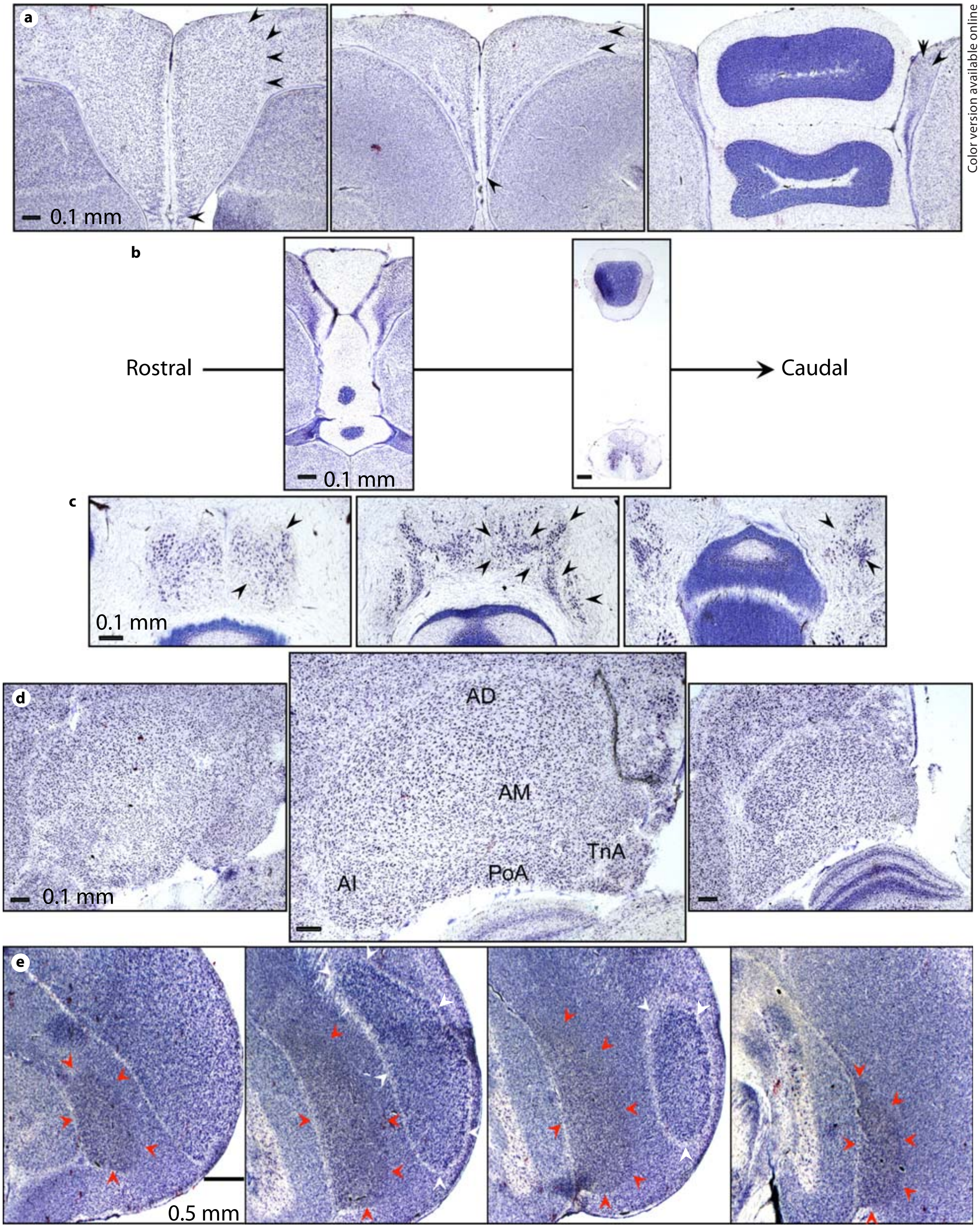

Fig. 1. Photomicrographs of brain regions measured. Each panel contains representative sections of the brain region from rostral to caudal with arrows indicating borders where needed. **a** Hippocampus. **b** Most rostral and most caudal section of CB. **c** Cerebellar nuclei. **d** Arcopallium. **e** Entopallium (dark arrows) and caudal MVL (light arrows).

We compared cell size and density in the AP for PoA, TnA, and three other regions of the AP; dorsal (AD), lateral/intermediate (AI), and intermediate/medial (AM) portions that correspond to the dorsal arcopallium, intermediate arcopallium, and medial arcopallium of Zeier and Karten [1971], respectively (fig. 1d). Other than the TnA, these subregions of the AP do not maintain sharp boundaries throughout their extent in Nissl-stained sections. However, the medial core of each of these 5 regions can be readily distinguished and gross examinations suggest differences in cell size and density that distinguish the regions. To determine if these regions were significantly cytoarchitectonically distinct, we sampled cell size and density across the rostral-caudal extent of each region.

We used a NIH Image J to measure cell size and perform neuron counts. Neurons were measured at ×800 (×100 objective, ×8 phototube, frame size 1,795.58 μm^2). Neurons, compared to glia, were characterized as having dark staining, nongranular cytoplasm, one nucleolus and/or an obvious axon hillock [Smith et al., 1997; Tramontin et al., 1998; Wissman and Brenowitz, 2009]. We measured the area of 50 neurons throughout the rostral-caudal extent of each region of the AP. Of the 15–20 slices of the brain that contained whole AP, about 5 slices clearly contained the putative AD, AI, and AM, about 2 or 3 slices contained PoA, and 3 or 4 slices contained TnA. We distributed the 50 cells to be measured across the slices containing the appropriate AP region using 10 counting frames (each fame was 7,499.54 μm^2 viewed at ×320, ×40 objective, ×8 phototube). We avoided the edges of regions but otherwise used a random sampling scheme within brain areas. To minimize overcounting errors, we counted nucleoli. This technique produces counts that are as accurate as the optical dissector [Tramontin et al., 1998]. We estimated the density of neurons by dividing the number of cells by the volume sampled (area·volume·frames sampled = 2,249,862 μm^3).

Entopallium and Mesopallium

In manakins, the E has clear boundaries (fig. 1e). It appears slightly less chromatic than the surrounding Nissl-stained nidopallium. In some sections, the lateral border is more chromatic than the rest of the E but still less chromatic than the surrounding nidopallium. This lateral entopallial region also appears to have less cell packing density then the E. We included this region which may correspond to the perientopallial belt described in zebra finches [Krützfeldt and Wild, 2004] and seen in the chicken atlas [Kuenzel and Masson, 1988]. In the mesopallium, we measured a region of the MVL that is teardrop-shaped rostrally and oval caudally [Laverghetta and Shimizu, 2003; Krützfeldt and Wild, 2004, 2005]. We initiated measurement at the point when the mesopallial lamina (LaM) widens considerably and pinches off this teardrop-shaped portion of the mesopallium. From this point, the mesopallium has very clear boundaries, being surrounded by the ventral lateral portion of LaM and by the superior frontal lamina. The region we measured is similar in shape and position to the region called MVL in zebra finches that receives heavy inputs from the E [Krützfeldt and Wild, 2004, 2005]. The region we measured in manakins also appears similar to the mesopallium ventral near the E (MVe) as defined in Feenders et al. [2008] for several species. MVe shows vision-related ZENK expression [Feenders et al., 2008]. Unlike Feenders et al. [2008], we did not include portions of the mesopallium that are more rostral and dorsal to where the LaM pinches off the MVL. In manakins, we did not see a distinct darker tear-shaped mesopallial region prior to where LaM pinched off the area we labeled MVL. Included in our measurements are likely visual regions and mesopallial regions that are sensory integration areas associated with behavioral flexibility [Timmermans et al., 2000; Feenders et al., 2008; Mehlhorn et al., 2010].

Telencephalon

For telencephalic boundaries on sections in which the telencephalic hemispheres were joined with the diencephalon, we used prominent markers such as the occipitomesencephalic tract and the anterior commissure to identify boundaries [Brenowitz et al., 1995].

Statistical Analyses

We first compared the size of brain regions between sexes using t tests. Because individual variation in brain and body size can affect the volume of other brain regions, we used regression analyses to examine relationships between regions of interest and the relevant larger brain region in which they were contained. Although tarsus size was significantly larger in males than females, no brain volumes correlated with tarsus size, so this variable was not included further. Given significant relationships between TELE or WB size and the target region of interest, we then used general linear modeling (GLM) to adjust telencephalic brain regions for telencephalic size, CB for WB size, and CN for CB size. To assure independence of variables in the GLM, the volume of the region of interest was always subtracted from the volume of the overall region for which it was adjusted. To confirm that we met the assumptions for the GLM, we checked for lack of significant p values for Levene's test of equality of error variances between sexes, and ran GLM models first with the sex by covariate interaction term included to look for homogeneity of slopes. We then ran the GLM without the interaction term. Only the GLM for AP had a significant interaction term showing that the effect of sex differences in AP volume adjusted for TELE size interacts with sex differences in TELE size. Given this slope heterogeneity, we also examined AP volume differences by the more traditional method of dividing the AP volume by the TELE volume and comparing the proportions between the sexes using the Mann-Whitney U test. There was a significant linear relationship between TELE and the remainder of WB volume for males but not for females as described further in the Results section below. To compare cell size and density in regions of AP, we used sex by region repeated-measures ANOVA followed by paired comparisons between regions. For all comparisons, alpha or the multiple comparison corrected alpha was set at $p < 0.05$. Error bars and error values reported in the text are means ± SEM. Analyses were performed in SPSS 14 (SPSS Inc., Chicago, Ill., USA) and StatView 5 (SAS).

Results

Relationships between Brain Regions

There was a significant relationship between all telencephalic brain regions and the size of the TELE minus that brain region (HP vs. TELE: $R^2 = 0.53$, $n = 12$, $p <$

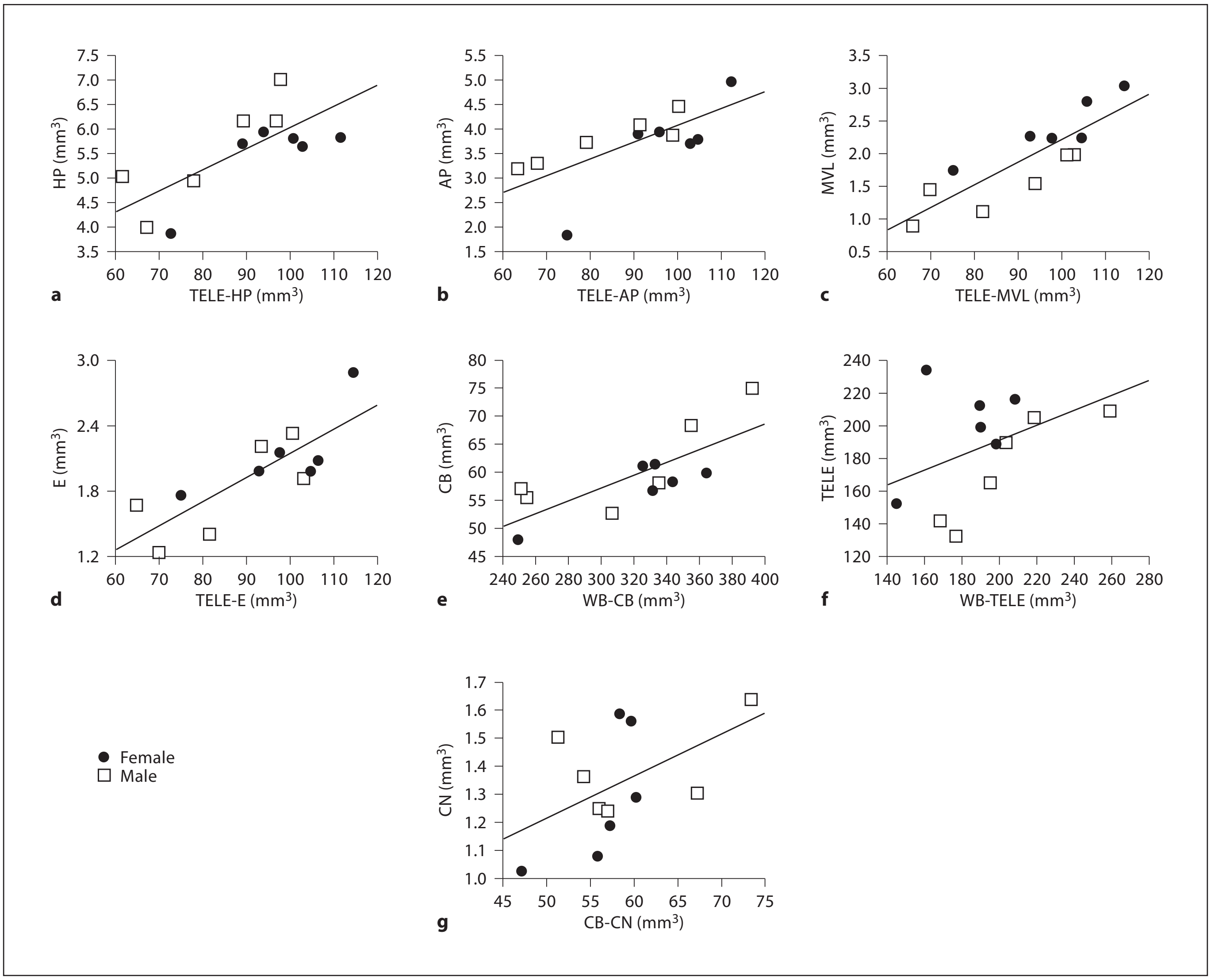

Fig. 2. Relationships between brain region volumes.

0.007, AP vs. TELE: $R^2 = 0.50$, n = 12, $p < 0.01$, MVL vs. TELE: $R^2 = 0.72$, n = 12, $p < 0.005$, E vs. TELE: $R^2 = 0.64$, n = 12, $p < 0.002$) as well as between CB and WB size minus the CB (CB vs. WB: $R^2 = 0.59$, n = 12, $p < 0.004$). The size of the CN did not correlate with cerebellar volume minus CN volume (CN vs. CB: $R^2 = 0.27$, n = 12, $p < 0.08$). This was true for both males ($R^2 = 0.19$, n = 6, $p < 0.38$) and females ($R^2 = 0.50$, n = 6, $p < 0.11$) examined independently. TELE size was not significantly related to WB minus TELE (fig. 2f, $R^2 = 0.18$, n = 12, $p < 0.17$). However, this seemed to be due to 1 female that had the largest TELE (234 mm^3) but did not have the largest WB minus TELE measure (WB 394 mm^3). This female was not an extreme outlier (less than 2 SDs from the mean) such as would promote exclusion from the analyses; however, we did determine that with this female removed, the relationship between TELE and the remainder of the WB was significant ($R^2 = 0.85$, n = 12, $p < 0.0001$). This result suggests that, in general, TELE volume and WB volume are correlated in manakins. While inclusion of this female in the GLM does not cause significant heterogeneity of slopes, we ran the GLM for TELE adjusted for the remainder of the WB with and without this somewhat unusual female.

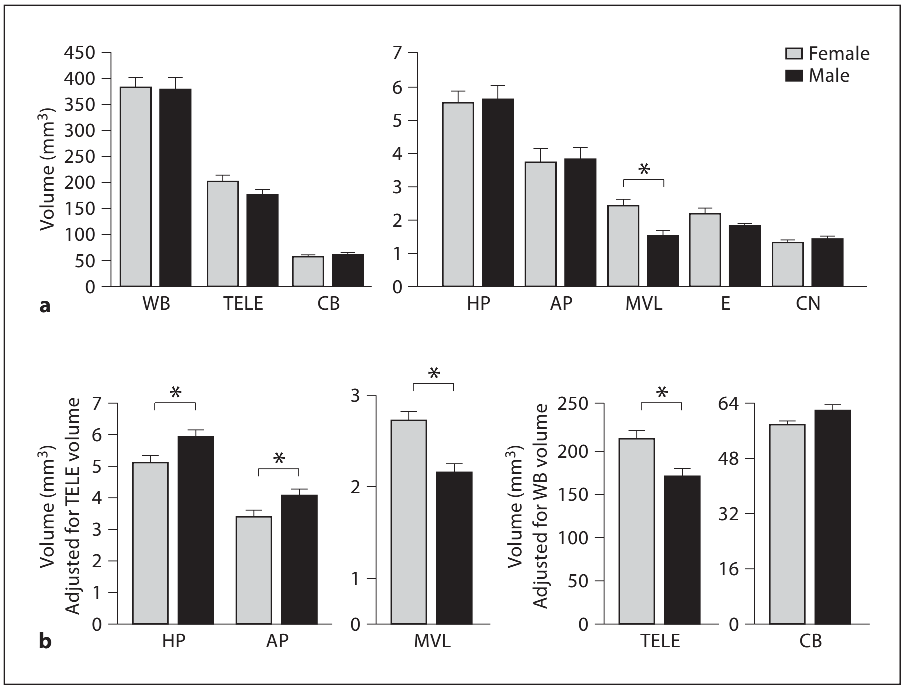

Fig. 3. Average volume of brain regions for male and female golden-collared manakins. **a** Unadjusted volumes. **b** Regions are adjusted for the size of the TELE or WB. Significant comparisons are indicated by asterisks.

Sex Differences in Brain Region Volumes

We found that the TELE was larger in females than males when adjusted for WB volume whether we included the female with the largest TELE (see above, ($F_{(1,9)} = 9.99$, $p = 0.01$) or excluded this female ($F_{(1,8)} = 15.91$, $p = 0.004$). We found that the HP (fig. 3b, $F_{(1,9)} = 5.6$, $p < 0.04$) and AP (fig. 3b, $F_{(1,9)} = 4.8$, $p < 0.05$) was larger in male than female manakins while the MVL (fig. 3b, $F_{(1,9)} = 15.09$, $p < 0.004$) was significantly larger in females than males, with each of these brain regions adjusted for TELE volume minus the region of interest. MVL was the only region that showed significant sexual dimorphism without adjusting for differences in overall brain size; females had a larger MVL than males (fig. 3a, $t_{(10)} = 3.45$, $p = 0.006$). Adjusting for WB size, males showed a nonsignificant trend for a larger CB (fig. 3b, $F_{(1,9)} = 3.51$, $p = 0.09$). Unexpectedly, neither corrected nor uncorrected volume of E differed between males and females (fig 3a, adjusted volumes not shown). Because there was heterogeneity of slopes in the GLM for AP, we also confirmed that the proportion of TELE taken up by the AP ($Z_{(10)} = 2.16$, $p < 0.03$) was larger in males than females. Figure 4b shows that 1 female with a somewhat small AP and TELE contributed to the heterogeneity of slopes between groups. When we removed this female from the analyses, males maintained a larger AP divided by TELE ($Z_{(9)} = 2.19$, $p < 0.03$). Neither WB nor TELE unadjusted for WB size were sexually dimorphic.

Cell Size and Density in the AP

There were no sex differences in estimates of AP neuron soma size or neuron density. However, there were regional differences in both cell size (fig. 4a, $F_{(4,24)} = 7.95$, $p < 0.0005$) and cell density (fig. 4b, $F_{(4,24)} = 46.95$, $p < 0.004$), confirming that these regions of the AP are cytoarchitechtonically distinct. Cells were larger in TnA than in PoA, AD, or AI and larger in AM than in AD (fig. 4a, $p < 0.05$ for all Bonferroni corrected). We found that the AM had significantly less cell density than the PoA (fig. 4b, $p < 0.05$ for all Bonferroni corrected). In addition, there was a significant interaction between sex and cell density across brain regions (fig. 4b, $F_{(4,24)} = 13.34$, $p < 0.03$) showing that the pattern of cell density in the 5 regions of the AP is not the same in males and females. As can be seen in figure 4b, males have greater cell density compared to females in all regions except TnA. In males, TnA is the least dense region while TnA is the densest region in females. The interaction remains significant if we examine only the nonamygdalar portions of AP (as we did in our volume measurements) by excluding the amygdalar TnA from the analysis.

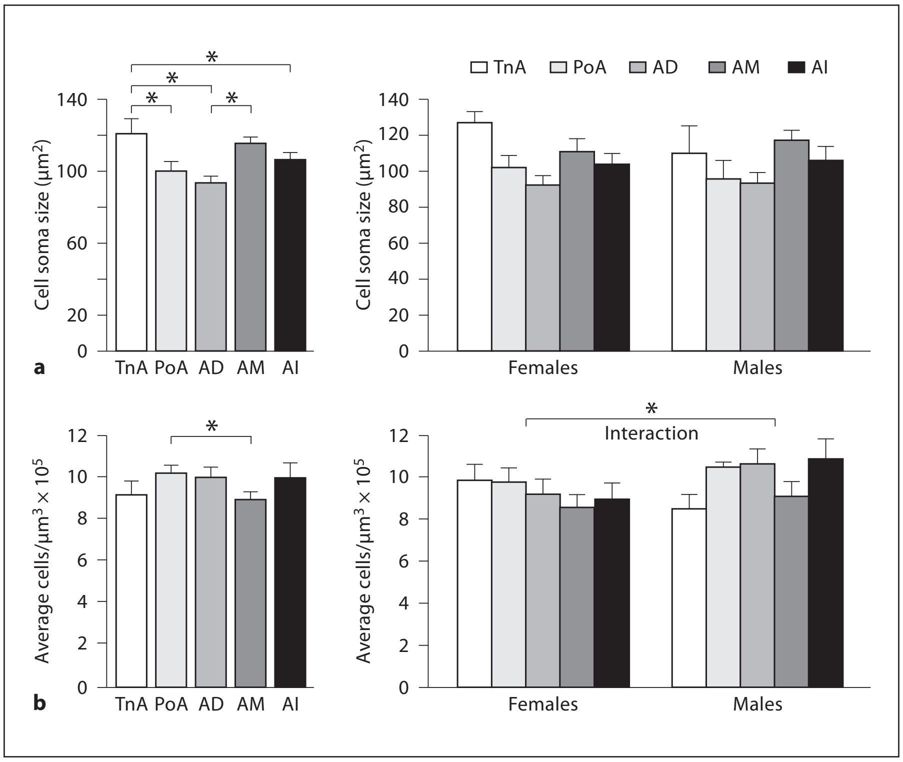

Fig. 4. Average cell soma size (**a**) and cell density (**b**) in 5 regions of the AP for both sexes in the first bar graph and for males and females separately in the second graph in each panel. Asterisks indicate significant differences.

Discussion

We found that adult, breeding golden-collared manakins possess sexually dimorphic neural phenotypes in brain regions subserving motor, visual and cognitive functions. Females had a larger TELE than males when TELE is adjusted for WB size. Adjusting for TELE size, males compared to females had a larger HP, a brain region that is known to support spatial learning and memory. In addition, males had a larger AP, a complex heterogeneous region with both amygdalar and sensorimotor functions. By contrast, females had a larger MVL, a nucleus in the tectofugal visual pathway that may modulate visually guided behavior. We did not find the expected female advantage in the size of E, the tectofugal nucleus known to contribute to intraspecies, individual recognition [Yamamoto and Watanabe, 2007]. We found no evidence of a distinct region in the AP akin to the oscine RA, but we did find sex differences in patterns of cell density across AP regions suggesting sex-specific specializations. No differences were seen in CB or CN volumes. We discuss each of these results in the context of the unique demands of courtship behavior on males and females of this species.

Hippocampus

Male manakins had a larger HP than females. The HP, or the nonmammalian homologue in other vertebrates, plays a role in complex forms of learning and memory, particularly in spatial learning [Rodriguez et al., 2002; Day, 2003; Goodrich-Hunsaker and Hopkins, 2010; Sherry and Hoshooley, 2010]. Sex differences and species differences in hippocampal volume occur in both mammals and birds and favor the sex or species that has more demand on spatial memory [Sherry et al., 1993; Galea et al., 1996; Hampton and Shettleworth, 1996; Reboreda et al., 1996; Lavenex et al., 2000; Day et al., 2008]. In both mammals and birds, the HP is known to be particularly plastic [Clayton, 1995, 1996; Maguire et al., 2000; van Praag et al., 2002; Galea et al., 2006]. Experience with spatial-memory intensive behaviors appears to be involved with hippocampal expansion [Abbott et al., 1999; Galea et al., 2006; Pravosudov et al., 2006; Mehlhorn and Rehkamper, 2009; Sherry and Hoshooley, 2010]. For example, in cowbird species where females must locate nests for brood parasitism and females have a larger HP than males [Reboreda et al., 1996], females prevented from nest searching and nonbreeding females have a reduced HP compared to breeding females allowed to search for a nest [Day et al., 2008].

We suspect that male manakins' experience with space use influences sexual dimorphism in hippocampal volume. Male manakins create arenas by clearing patches of forest floor surrounded by small saplings where they display. Breeding males are site faithful, apparently for life, but arenas are abandoned by adult males during the nonbreeding season. Males return to the prior years' location and will even reuse the same saplings as in a prior year despite large changes in surrounding foliage, such as tree falls, large growth of the underbrush, or significant human disturbance. Thus, males are required to remember the location of an approximately 75-cm diameter patch of forest floor despite alteration of local and sometimes distal landmarks. Presumably, females also return to the leks visited in previous years, but they would have the benefit of the sound of the males' calls and mechanical sound displays to guide them to the arenas.

Arcopallium

We were particularly interested in AP morphology because the songbird AP contains RA, a premotor output nucleus of the vocal song production pathway. In songbirds, RA is typically larger in males that sing than in females that do not [Ball and MacDougall-Shackleton, 2001]. In addition, much of the AP contains ARs, and RA is dense in AR expression, particularly in male songbirds [Bernard et al., 1999; Metzdorf et al., 1999; Ball and MacDougall-Shackleton, 2001; Fusani et al., 2003]. In many non-songbirds, including suboscine passerines, the non-amygdaloid AP is AR sparse and no discrete AR-rich region exists [Bernard et al., 1999; Metzdorf et al., 1999; Ball and MacDougall-Shackleton, 2001; Fusani et al., 2003]. Distinctively, in golden-collared manakins, most of the AP is dense in ARs [Fusani et al., 2003]. Thus, we predicted that sexually dimorphic features may be conspicuous in the AP of adult manakins. Furthermore, we expected to find a sex-specific portion of the AP homologous to the premotor nucleus RA in oscine songbirds with control not of the syrinx but of muscles responsible for the production of manakin wing snaps. While we found that the AP was larger in males than in females, there were no sex differences in soma size or cell density for any of the regions of the AP, suggesting the absence of a discrete premotor wing-snapping nucleus in the AP [see also Saldanah et al., 2000]. It is possible that the AP as a whole, as reflected in the overall sexually dimorphic volume difference, may participate in the androgen-dependent motor functions of male courtship. Future studies are needed to assess the connectivity of the AP with spinal circuits innervating wing and leg muscles used in the male courtship display.

While we think it likely that the relatively enlarged male AP contributes to the motor control of courtship behavior, given that AP has sensorimotor functions (anterior AP), the enlarged AP may have multiple limbic functions as well [posterior AP; Zeier and Karten, 1971; Sadananda et al., 2007; Saint-Dizier et al., 2009]. In quail and zebra finches, the AM and AI are activated by copulation [Charlier et al., 2005; Sadananda et al., 2007]. The AM receives projections from visual and auditory areas and, in turn, projects to the medial preoptic area [Charlier et al., 2005]. These patterns of innervation and activation suggest a role in the motivation to engage in courtship. Male manakins invest tremendous time and energy in courtship. Thus, it is plausible that the neural source of this motivation to court, parts of the AP, would be enlarged and especially active in male manakins.

We found an interaction of sex with AP cell density. Males had greater density in AD, AI, AM, and PoA than in TnA, whereas this pattern was the opposite in females (fig. 4b, cell soma size is similar in males and females). The functional meaning of these patterns of cell density is unclear. In songbirds, high cell density does not contribute to increases in volume in RA; instead, increased volume occurs by increases in soma size and a decrease in cell density [Meitzen and Thompson, 2008]. Given that Nissl-stained borders of these AP regions are not clear in manakins, we did not measure the AP regions independently and thus cannot estimate total cell numbers for each region. Lesion studies, tract-tracing studies, and immediate early gene studies are called for to expand our understanding of the connectivity and functionality of these regions in manakins and other species. Certainly, we can conclude from our findings that sex differences in the AP in manakins are more regionally diffuse than sex differences found in RA in many songbird species where males sing and females do not [Nottebohm and Arnold, 1976; Kirn et al., 1989; Brenowitz, 1997; Tramontin et al., 1998; Airey et al., 2000].

Ventral Lateral Mesopallium and Entopallium

We compared the volume of two telencephalic nuclei in the tectofugal visual pathway, MVL and E [Krützfeldt and Wild, 2004, 2005]. Whereas E volume was similar between males and females, both the uncorrected volume and the TELE-adjusted volume of MVL were larger in females than males. Behavioral studies suggest that E is associated with pattern discrimination [Watanabe et al., 2008], individual recognition [Yamamoto and Watanabe, 2007], reaction to looming stimuli [Xiao and Frost, 2009], and sexual imprinting [Sadananda and Bischof, 2006;

Sadananda et al., 2007]. Based on these functions, we anticipated that the E might be enlarged in females to enhance their visual processing speed to detect small differences in the speed of male displays [Fusani et al., 2007; Barske et al., 2011]. E could still contribute more to female than male visual processing; it is numerically, but not significantly, larger in females. However, both males and females likely need to process the abundant visual properties of male displays: males to perform them and females to follow and observe the males.

We did not anticipate the robust sexual dimorphism found in MVL volume. The function of MVL is less well understood than that of E. Whereas some portions of MVL may have sensory integration functions [Feenders et al., 2008; Atoji and Wild, 2009; Mehlhorn et al., 2010], the caudal region to which we restricted our measurements probably functions in visual processing as this region is known to receive input from E in zebra finches [Krützfeldt and Wild, 2004]. The larger volume of caudal MVL in manakins suggests that females have greater demands on the MVL than do males. There are no obvious behavioral or ecological differences that would differentially impact visual processing of males and females except that females observe displaying males and seem able to ascertain the speed of display elements to assess male quality [Barske et al., 2011; Fusani et al., 2007b]. Thus, we speculate that an enlarged MVL may assist females in this rapid visual discrimination task.

Cerebellum and Cerebellar Nuclei

Previous studies have shown that CB volume can be related to emergent features of male courtship, such as the cross-species correlation of CB size in male bowerbirds with the complexity of the bowers they build to attract females [Day et al., 2005]. In that the CB participates in motor learning, planning, postural adjustment, as well as the timing and sequencing of movements, we predicted that male manakins would have a larger CB than females in association with the complex courtship display they perform. Although the mean CB size (relative to WB) was somewhat larger in male than female manakins, the difference was not significant ($p = 0.09$). Given the low power for these analyses ($1 - \beta = 0.4$), not too much can be made of this negative result. We are currently examining species differences in the size of the whole CB, cerebellar folia, and CN within the manakin family in relation to the complexity of display across species; findings may help us better interpret CB involvement in manakin display.

Similarly, there was no correlation between the size of the CN and the CB ($p = 0.08$). Given that the CN receive collaterals from inputs to the CB and are the only output area of the CB (other than the vestibular nuclei) one expects a strong relationship between these regions as has been shown at the intraspecies level in primates [Matano et al., 1985]. The lack of relationship found in the present study has been confirmed in another sample of golden-collared manakins and in other manakin species [Wilkening and Day, 2010]. Perhaps inclusion of the white matter input and output pathways which were not included in our measurement of the cell-dense portion of the CN or cell counts of CN and CB would reveal a stronger relationship between CB and CN. We are currently performing Purkinje cell counts and CN cell counts in a number of manakin species to further investigate the relationship between CB and CN in manakins.

General Patterns, Whole Brain and Telencephalon

Females have a larger TELE relative to WB and males have a larger body size than females but do not have a larger WB size. Why this might be is unclear. Perhaps males incur greater energetic costs than females due to their sustained displaying and reduce the sizes of their TELE, an energetically costly region. The increased size of MVL is not sufficient to explain the size of the TELE in females (maximum HP + AP = 11.4 mm^3, maximum MVL + E = 5.9 mm^3). Perhaps females have some other sex-specific specializations of the TELE of which we are unaware that increase the size of the TELE relative to WB. Whatever the reason for differences in the size of the TELE, the patterns of similarities and differences in the size of particular brain regions relative to other brain regions between males and females demonstrate sex-specific allometric scaling patterns among regions. Because of these differences, it is difficult to know which adjusted volumes have actually been selected and which are only byproducts of some scaling factor. Based on the known functions of the regions that we found to be sexually dimorphic, we have suggested ecological correlates for regional sex specializations, but these inferences should be verified with further study to rule out nonbehavioral adaptations.

Conclusions

Our results demonstrate that there are neural phenotypes specific to the sexes of golden-collared manakins. The functional domains of the specialized brain regions suggest that females could have greater capacity for sensory integration of visually guided behaviors while males have greater capacity for spatial (HP) and some limbic or sensorimotor aspects of AP function. Other than the association of the HP with some aspects of spatial learning, which has been well studied, more studies on functions of these sexually dimorphic brain regions and on the specific connectivity of these regions in manakins and other birds are needed before we can truly understand the sex differences we found here. We acknowledge that sex differences in the volume of brain regions do not necessitate sex differences in functional capacity. However, they do suggest that there are sex-specific adaptations to the way that behaviors are neurally controlled [Gahr et al., 2008]. We have predicted or, in the case of MVL, posited the behavioral forces selecting for these neural sexual dimorphisms. These can now be further investigated. Manakins offer a unique opportunity to explore adaptive neural specializations for highly developed, steroid-driven, neuromotor behavior.

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