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Neural Specialization for Hovering in Hummingbirds: Hypertrophy of the Pretectal Nucleus Lentiformis Mesencephali

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ABSTRACT

Hummingbirds possess an array of morphological and physiological specializations that allow them hover such that they maintain a stable position in space for extended periods. Among birds, this sustained hovering is unique to hummingbirds, but possible neural specializations underlying this behavior have not been investigated. The optokinetic response (OKR) is one of several behaviors that facilitates stabilization. In birds, the OKR is generated by the nucleus of the basal optic root (nBOR) and pretectal nucleus lentiformis mesencephali (LM). Because stabilization during hovering is dependent on the OKR, we predicted that nBOR and LM would be significantly enlarged in hummingbirds. We examined the relative size of nBOR, LM, and other visual nuclei of 37 species of birds from 13 orders, including nine hummingbird species. Also included were three species that hover for short periods of time (transient hoverers; a kingfisher, a kestrel, and a nectarivorous songbird). Our results demonstrate that, relative to brain volume, LM is significantly hypertrophied in hummingbirds compared with other birds. In the transient hoverers, there is a moderate enlargement of the LM, but not to the extent found in the hummingbirds. The same degree of hypertrophy is not, however, present in nBOR or the other visual nuclei measured: nucleus geniculatus lateralis, pars ventralis, and optic tectum. This selective hypertrophy of LM and not other visual nuclei suggests that the direction-selective optokinetic neurons in LM are critical for sustained hovering flight because of their prominent role in the OKR and gaze stabilization. J. Comp. Neurol. 500:211–221, 2007. © 2006 Wiley-Liss, Inc.

Indexing terms: accessory optic system; comparative method; optomotor; optokinetic; Trochiliformes

Hummingbirds (order Trochiliformes) are readily distinguished from other birds by their diminutive size and their ability to hover. Although some other birds hover for short periods (i.e., transient hovering in raptors, kingfishers, and sunbirds; Hamas, 1994; Ferguson-Lees and Christie, 2001; Cheke, 2002), the sustained hovering flight of hummingbirds is unique among birds (Altshuler and Dudley, 2002). In fact, the hovering flight of hummingbirds differs from the flight of other birds in a number of respects. Compared with other birds, hummingbirds can beat their wings up to 50 times faster (Schuchmann, 1999), produce force during both up and down strokes rather than just up strokes (Warrick et al., 2005), and generate more lift with their wings during take-offs (Tobalske et al., 2004). Kinematically, the hovering flight of hummingbirds is entirely unlike that of other birds but is remarkably similar to that of some insects (Warrick et al., 2005). To allow the high wing-beat frequency and hovering capability, hummingbirds possess a unique suite of

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morphological and metabolic specializations, including an enlarged heart, modified wing bones and pectoral girdle, specialized musculature, and extremely high metabolic rate (Schuchmann, 1999; Suarez and Gass, 2002). However, we are unaware of any investigations of neural specializations in hummingbirds that might be important for hovering.

A critical aspect of hovering flight is stabilization. To guide the beak into and out of flowers, hummingbirds must be able to maintain a stable position in time and space, despite the disruptive effects that must result from wind gusts and other environmental perturbations. Stabilization is mediated by several vestibular, visual, and proprioceptive reflexes, including the optomotor or optokinetic response (OKR; for reviews see Wilson and Melvill Jones, 1979; Ito, 1984; Melvill Jones, 2000). The OKR, which occurs in both vertebrates and invertebrates, is a visual following reflex in response to a large moving visual stimulus whereby eye, head, and/or body movements are made in the direction of motion to minimize the amount of visual motion across the retina (Miles and Wallman, 1993; Steinman, 2004). A specific, highly conserved, subcortical visual pathway known as the accessory optic system (AOS) is critical for mediating the OKR in vertebrates (Simpson, 1984; Simpson et al., 1988; Grasse and Cynader, 1990; Frost et al., 1994). In birds, the AOS comprises two primary retinorecipient nuclei: the pretectal nucleus lentiformis mesencephali (LM) and the nucleus of the basal optic root (nBOR; Karten et al., 1977; Fite et al., 1981; McKenna and Wallman, 1985a; Gamlin and Cohen, 1988a). Lesions to either nucleus significantly impair or abolish outright the OKR (Fite et al., 1981; Gioanni et al., 1983a,b), and neurons in these nuclei have extremely large receptive fields and exhibit direction selectivity to moving large-field ("optic flow") stimuli (Burns and Wallman, 1981; Morgan and Frost, 1981; Gioanni et al., 1984; Winterson and Brauth, 1985; Wylie and Frost, 1990). Most LM and nBOR neurons prefer extremely slow stimulus speeds (i.e., $\leq$1°/second; Burns and Wallman, 1981; Wylie and Crowder, 2000; Crowder et al., 2003; see also Simpson, 1984), so they are thought to provide the error signal that drives the OKR (Simpson, 1984; Simpson et al., 1988; Miles and Wallman, 1993). That is, during stabilization, even minimal retinal slip would be detected by AOS neurons, which would maintain the OKR and thus promote stabilization. Given this, we hypothesized that both nBOR and LM would be hypertrophied in hummingbirds, compared with other birds, to meet the increased motion processing and OKR demands of hovering flight.

Abbreviations

AOS	accessory optic system
GLv	nucleus geniculatus lateralis, pars ventralis
GT	tectal gray
GTc	caudal tectal gray
GTd	dorsal tectal gray
GTv	ventral tectal gray
ICo	nucleus intercollicularis
LM	nucleus lentiforms mesencephali
LPC	nucleus laminaris precommissuralis
MLd	nucleus mesencephalicus lateralis, pars dorsalis
nBOR	nucleus of the basal optic root
nRt	nucleus rotundus
OKR	optokinetic or optomotor response
PPC	nucleus principalis precommissuralis
SOp	stratum opticum
TeO	optic tectum
VLT	ventrolateral thalamic nucleus

MATERIALS AND METHODS

Specimens

Brains of nine hummingbird species (n = 12) and 28 other bird species (n = 31) were obtained from other researchers, veterinary clinics, and wildlife sanctuaries as well as specimens loaned to us from the National Museum of Natural History, the Field Museum of Natural History, and the Louisiana State University Museum of Natural Science (Table 1). Some of the other groups surveyed were the swifts (Apodiformes, two spp.), which are the sister group to hummingbirds (Sibley and Ahlquist, 1990; Cracraft et al., 2004), and songbirds (Passeriformes, 11 spp.), some of which are similar in size to the larger hummingbirds. Within this sampling were the brains of three transiently hovering species: an Australian songbird, the eastern spinebill (*Acanthorhynchus tenuirostris*), belted kingfisher (*Ceryle alycon*), and American kestrel (*Falco sparverius*). These three species also hover, but for relatively short periods (Hamas, 1994; Higgins et al., 2002; Smallwood and Bird, 2002), and lack the extensive morphological specializations for hovering present in hummingbirds. The eastern spinebill, in fact, feeds on nectar and pollen in a fashion similar to hummingbirds, but it does not always hover while feeding (Higgins et al., 2002).

All of the museum specimens were formalin fixed and subsequently stored in 70% ethanol. Specimens obtained from other sources were either perfused or immersion fixed in formaldehyde. For all specimens, the brains were extracted, embedded in gelatin, and serially sectioned in the coronal plane on a freezing-stage microtome. Forty-micrometer sections were collected in 0.1 M phosphate-buffered saline and mounted onto gelatinized slides. After air drying, the slides were stained with thionin, dehydrated through a graded ethanol series, cleared in Hemo-D, and coverslipped with Permount.

Measurements

We measured the volumes of both of the AOS nuclei, LM and nBOR. The nBOR, which is located just at the base of the brain at the mesodiencephalic border, is easy to delineate and readily distinguishable from adjacent structures (Brecha et al., 1980). To delineate the borders of LM, we adhered to the descriptions provide by Gamlin and Cohen (1988a,b; see Fig. 1). The LM consists of medial and lateral subnuclei, but we simply grouped these together, because the distinction between the two was difficult in some specimens. There are a variety of sizes of neurons in LM, including extremely large multipolar cells that project to the cerebellum (Gamlin and Cohen, 1988b; Pakan et al., 2006). Toward the rostral pole, LM is bordered laterally by the optic tectum (TeO) and medially by the nucleus laminaris precommissuralis (LPC), a nonretinorecipient nucleus consisting of a thin group of small basophilic cells. The nucleus principalis precommissuralis (PPC) resides medial to LPC, and the nucleus rotundus (nRt) is medial to PPC. Dorsally, LM surrounds the isthmooptic tract. The ventrolateral portion of LM is bordered by nucleus geniculatus lateralis, pars ventralis (GLv), but this border is quite distinct. Caudally, the LM is bordered

TABLE 1. List of the Species Surveyed, Sample Sizes, and Volumes (mm^3) of the Brain, Nucleus Lentiformis Mesencephali (LM), Nucleus of the Basal Optic Root (nBOR), Nucleus Geniculatus Lateralis Pars Ventralis (GLv), Optic Tectum (TeO), and Nucleus Mesencephalicus Lateralis, Pars Dorsalis (MLd)

Order	Species	Common name	n	Brain	LM	nBOR	GLv	TeO	MLd
Apodiformes	*Collocalia esculenta* (FMNH SEA132)[1]	Glossy swiftlet	1	121[2]	0.0891	0.1029	0.0707	9.51	0.504
	Collocalia troglodytes (FMNH SEA133)[1]	Pygmy swiftlet	1	130[2]	0.0975	0.0827	0.0822	11.04	0.579
Anseriformes	*Anas platyrhynchos*	Mallard	1	6,392	2.5045	1.7724	1.7537	185.44	8.588
Caprimulgiformes	*Eurostopodus argus*	Spotted nightjar	1	1,013	0.8445	0.7686	0.5799	66.54	3.836
	Podargus strigoides	Tawny frogmouth	1	5,943	1.9140	2.5614	1.3195	328.05	10.75
Charadriiformes	*Limnodromus griseus*	Short-billed dowitcher	1	1,338	0.5944	0.5772	0.5636	51.80	2.002
Ciconiiformes	*Bubulcus ibis*	Cattle egret	1	4,025	1.6398	1.5976	1.7746	239.33	2.315
Columbiformes	*Columba livia*	Pigeon	2	2,282	1.5430	1.5766	1.7776	144.21	4.296
Coraciiformes	*Ceryle alcyon* (USNM430744)[1]	Belted kingfisher[3]	1	1,606[2]	1.6742	1.2015	1.1487	141.32	2.084
Falconiformes	*Accipiter fasciatus*	Brown goshawk	1	4,875	4.4914	3.1292	4.8059	257.02	4.114
	Falco sparverius	American kestrel[3]	2	1,017	1.5788	0.6985	1.7474	79.77	3.510
Galliformes	*Bonasa umbellus*	Ruffed grouse	2	3,146	3.1833	2.2874	4.4699	197.80	9.301
Gruiformes	*Fulica americana*	American coot	1	2,719	1.6739	1.4580	1.1844	138.48	5.917
Passeriformes	*Acanthiza pusilla*	Brown thornbill	1	434	0.2742	0.1581	0.4611	40.76	0.603
	Acanthorhynchus tenuirostris	Eastern spinebill[3]	1	396	0.4746	0.2397	0.5635	29.46	0.822
	Dendroica coronata	Myrtle warbler	1	510	0.2965	0.2744	0.4335	—	—
	Dendroica magnolia	Magnolia warbler	1	530	0.2678	0.2792	0.3419	—	—
	Eopsaltria australis	Eastern yellow robin	1	839	0.7517	—	—	43.49	1.940
	Erythrura gouldiae	Gouldian finch	1	428	0.2492	0.2301	0.3462	20.94	0.625
	Gymnorhina tibicen	Australian magpie	1	4017	1.8225	1.8532	1.6726	219.43	3.559
	Manorina melanocephala	Noisy miner	1	2279	0.8242	0.8262	1.3924	96.01	1.727
	Menura novaehollandiae	Superb lyrebird	1	10163	5.4412	3.1819	5.0576	417.29	4.455
	Pardalotus punctatus	Spotted pardalote	1	401	0.3270	0.2786	0.4930	19.69	0.965
	Regulus satrapa	Golden-crowned kinglet	1	310	0.2278	0.2314	0.4989	—	—
	Taeniopygia bichenovii	Double-barred finch	1	409	0.3209	0.2114	0.3870	28.19	0.715
Psittaciformes	*Nymphicus hollandicus*	Cockatiel	1	2111	0.5983	1.0339	0.9617	68.03	2.232
Strigiformes	*Aegolius acadicus*	Northern saw-whet owl	1	2857	2.5549	—	—	66.99	14.831
	Ninox boobook	Southern boobook owl	1	6339	3.8353	2.2142	2.1530	160.23	10.047
Trochiliformes	*Adelomyia melanogenys* (LSUMZ 129494, 129491)[1]	Speckled hummingbird	2	86[2]	0.2165	0.0794	0.1711	7.22	0.145
	Calypte anna	Anna's hummingbird	1	184	0.4167	0.1695	0.2453	14.28	0.381
	Doryfera ludoviciae (FMNH 320498)[1]	Green-fronted lancebill	1	139[2]	0.2975	0.0944	0.2816	10.95	0.259
	Eugenes fulgens (LSUMZ 64774)[1]	Magnificent hummingbird	1	192[2]	0.4140	0.1148	0.3382	16.46	0.2863
	Eutoxeres condamini (FMNH 315304, FMNH 315300)[1]	Buff-tailed sicklebill	2	254[2]	0.4886	0.1968	0.4049	21.81	0.505
	Glaucis hirsuta (USNM 616825)[1]	Rufous-breasted hermit	1	123[2]	0.3117	0.1114	0.3270	12.96	0.296
	Patagona gigas (LSUMZ 123075)[1]	Giant hummingbird	1	393[2]	0.7522	0.1979	0.4159	31.38	0.800
	Sephanoides sephanoides (FMNH 316786, FMNH 316784)[1]	Green-backed firecrown	2	127[2]	0.2881	0.1050	0.2423	10.19	0.238
	Thalurania furcata (LSUMZ 123339)[1]	Fork-tailed woodnymph	1	116[2]	0.3782	0.0720	0.1779	9.32	0.189

[1]Specimen numbers refer to the following institutions: USNM, National Museum of Natural History (Washington, DC); FMNH, Field Museum of Natural History (Chicago, IL); and LSUMZ, Louisiana State University Museum of Natural Science (Baton Rouge, LA).
[2]Brain and brain region volumes of the museum specimens are affected by shrinkage resulting from long-term storage in ethanol and do not reflect measurements of fresh brains.
[3]Transiently hovering species.

by the retinorecipient rostral tectal gray (GT), which appears continuous with layer 5 of TeO. The GT consists of loosely packed small cells (Gamlin and Cohen, 1988a), but the large multipolar cells seen in LM are few or absent from GT (Pakan et al., 2006). More caudally, the rostral GT becomes divided into dorsal and ventral components (GTd, GTv) separated by the caudal GT (GTc), which appears continuous with layer 8 of TeO. At this point, the LM is usually bisected into a dorsal and a ventral component (see Fig. 2). Still more caudally, the ventral LM continues medially just dorsal to the stratum opticum (SOp).

In addition to LM and nBOR, we measured the volumes of two other retinorecipient nuclei (Cowan et al., 1961; Crossland and Uchwat, 1979), TeO and GLv. The volume of nucleus mesencephalicus lateralis, pars dorsalis (MLd), an auditory midbrain nucleus (Karten, 1967) that does not receive direct projections from the retina, was measured as well. The boundaries of these three structures were based on previous descriptions in the literature, including volumetric studies (Brecha et al., 1980; Knudsen, 1983; Guiloff et al., 1987; Gamlin and Cohen, 1988a; Boire, 1989; Boire and Baron, 1994). Our TeO measurement included all layers of the tectum. GLv was readily distinguished from neighboring structures by the presence of large, darkly stained cells on the dorsal aspect of GLv, known as the parvocellular layer, compared with the overlying ventrolateral thalamic nucleus (VLT). Ventrally, the optic tract bound GLv. MLd was differentiated from the adjacent nucleus intercollicularis (ICo) by the presence of a thin lamina running under the ventral surface and along part of the medial and lateral borders (Knudsen, 1983). The third ventricle provided the dorsal border of MLd.

Digital photos were taken of every second section throughout the rostrocaudal extent of each structure. Measurements were taken directly from these photos with the public domain NIH Image program (http://rsb.info.nih.gov/nih-image/). Volumes were calculated my multiplying the thickness of the section (40 μm) by the sampling interval. All images were obtained with a Canon PowerShot S50 digital camera (Tokyo, Japan) or an Openlab Imaging system (Improvision, Lexington, MA), and Adobe Photoshop (Adobe, San Jose, CA) was used to compensate for brightness and contrast.

To account for allometric effects, we measured the brain volumes of each individual specimen. The brains were first weighed to the nearest milligram. Ideally, the brains would have been weighed following a standard amount of time in fixative. Because of our reliance on specimens provided by museums and other researchers, this was not possible, so the weights were taken immediately prior to histological processing. Brain volume for each specimen was calculated by dividing the mass of the brain by the density of brain tissue (1.036 g/mm^3; Stephan, 1960) as in previous studies (Rehkämper et al., 1991; Ebinger, 1995; Iwaniuk and Nelson, 2002; Iwaniuk et al., 2005, 2006). Although we applied this uniformly across all specimens, it should be noted that the brain volumes provided for the museum specimens are not representative of fresh brain volumes. The museum specimens are all fixed in formaldehyde by immersion, but, after fixation for up to several months, the specimens are then stored in 70% ethanol for long-term storage (Winker, 2000; Livezey, 2003). The museum specimens that were loaned to us had, in fact, been stored in 70% ethanol for between 2 and 50 years. The result of this long-term storage is significant tissue shrinkage, for which we could not adequately account. The brain, and brain region, volumes of the museum specimens therefore do not represent fresh volumes but rather the volumes of the specimens following long-term storage in 70% ethanol.

Because tissue processing can also cause tissue shrinkage, we calculated shrinkage factors for each specimen by comparing brain volumes prior to processing with brain volumes calculated by measuring serial sections of the processed tissue. To calculate the latter, we measured the areas of entire coronal sections apart throughout the brains and multiplied these areas by section thickness (40 μm) and the sampling interval (every fourth section). The difference between the two brain volume measurements yielded a shrinkage factor, which was then applied to the volumes of each measured structure to provide volumetric measurements that were corrected for shrinkage (as in Boire, 1989; Rehkämper et al., 1991; Boire and Baron, 1994; Ebinger, 1995; Iwaniuk et al., 2005). Thus, the volumes of all of the brain regions measured are corrected for tissue shrinkage during processing.

Statistical analysis

To test for significant differences in the relative size of all five structures, we performed analyses of covariance (ANCOVAs) on log-transformed brain region volumes minus the volume of the brain region being examined (Deacon, 1990; Iwaniuk et al., 2005, 2006). The species were first grouped as hummingbirds and nonhummingbirds. We then repeated the analyses with the addition of the transiently hovering species as a third category. Specifically, we tested for differences in the slope (i.e., interaction term) and intercept (i.e., hummingbird/nonhummingbird) of regression lines describing the allometric relationship between each structure and brain volume for hummingbirds and all other birds.

Because comparative analyses using species as independent data points are subject to inflated type II error (Harvey and Pagel, 1991), we compared the results of these tests with conventional critical Fs and phylogeny-corrected critical Fs (Garland et al., 1993). The phylogeny-corrected critical Fs have been used in previous comparative studies of brain–behavior relationships (Pellis and Iwaniuk, 2002; Iwaniuk and Arnold, 2004; Iwaniuk et al., 2005, 2006). For all comparisons, we therefore provide the calculated F, the conventional critical F, and the phylogeny-corrected critical F. The phylogeny-corrected critical Fs were calculated by performing Monte Carlo simulations of the data on top of a phylogenetic tree to derive an F distribution in the PDAP software package (available from T. Garland upon request). We constructed a phylogenetic tree based on the interordinal relationships depicted by Sibley and Ahlquist (1990), with additional resolution provided by more recent studies (Altshuler et al., 2004; Barker et al., 2004). The simulated values were constrained to biologically realistic values set just above and below the largest and smallest values within our data set. Branch lengths were all set equal to one, because the phylogeny was constructed from multiple sources, and this provided adequately standardized branch lengths for other phylogenetically based analyses, such as independent contrasts (Garland et al., 1992). For all simulations, we assumed a gradual model of evolutionary change (i.e., changes occurring along the lengths of the branches), but our results remained the same under other models of evolutionary change.

RESULTS

Figure 1 shows coronal sections through the pretectum for three hummingbird species [*Patagona gigas* (A), *Doryfera ludoviciae* (B), *Thalurania furcata* (C)] and two songbirds [*Acanthorhynchus tenuirostris* (D), *Eopsaltria australis* (E)]. For the hummingbirds, the sections were taken at different rostrocaudal levels (A most rostral, C most caudal). In Figure 1, LM appears much larger relative to the optic tectum (TeO) in hummingbirds than it does in songbirds. In particular, the mediolateral extent of the LM was broader in hummingbirds than in other species. Also, the rostral pole of LM appeared in sections rostral to the TeO in hummingbirds, but not in any other species examined. The LM of the spinebill (D) also appeared slightly larger than that of the other songbirds, but not as large as that of the hummingbirds.

Our statistical analysis corroborated these observations; the hummingbirds had a significantly larger LM, relative to brain volume, than all other birds examined. The regression line describing the relationship between LM and brain volume is significantly higher in hummingbirds than in other birds, despite sharing a similar slope (Fig. 2A, Table 2). Even when compared against species that shared similar brain volumes (two swifts and six songbirds), the hummingbirds had significantly larger relative LM volumes ($F_{1,16} = 76.86$, $P < 0.01$; phylogeny-corrected F = 24.12). Furthermore, when expressed as a percentage of total brain volume, LM was much larger in the hummingbirds (0.19–0.33%) compared with the swifts (0.07%), the songbirds (0.05–0.09%), and the average of all other birds (Fig. 2B).

The magnitude of this difference is also evident in serial sections taken throughout the rostrocaudal extent of LM. As shown in Figure 3, in comparison with a closely related species of similar body and brain size, a swiftlet (Fig. 3B), the LM of the hummingbird (Fig. 3A) appears much broader mediolaterally and extends farther rostrocaudally. In contrast, when the hummingbirds (Fig. 3A,C) are compared with a much larger species (e.g., songbird shown in Fig. 3D), the LM appears to be similar in size. That is,

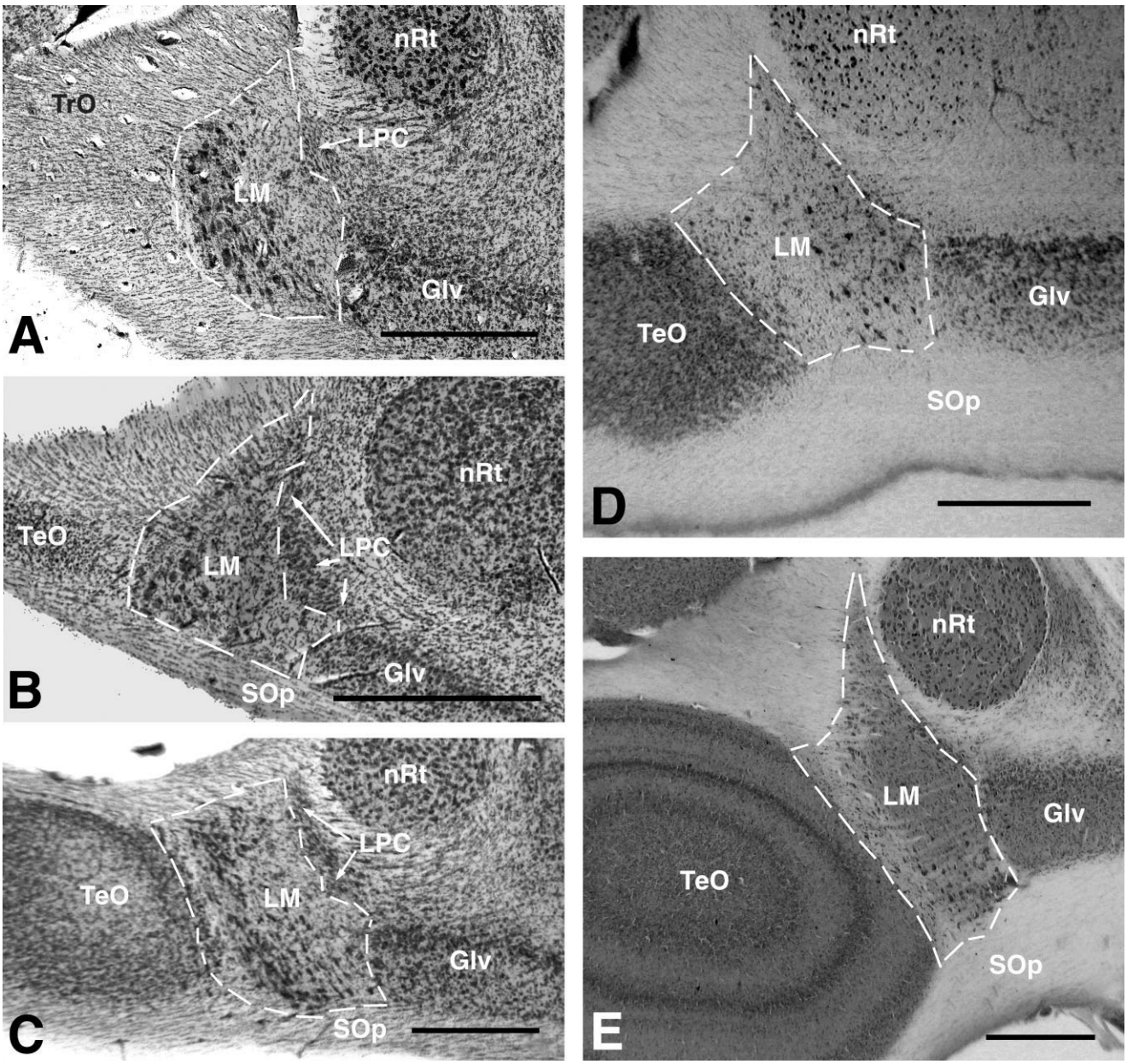

Fig. 1. Photomicrographs showing the location and borders of nucleus lentiformis mesencephali (LM) and neighboring structures. Coronal sections are shown for three hummingbirds. **A:** Giant hummingbird (*Patagona gigas*, LSUMZ123075). **B:** Green-fronted lancebill (*Doryfera ludoviciae*, FMNH 320498). **C:** Fork-tailed woodnymph (*Thalurania furcata*, LSUMZ 123339). In addition, coronal sections for two songbirds are shown. **D:** Transiently hovering and nectarivorous eastern spinebill (*Acanthorhynchus tenuirostris*). **E:** Eastern yellow robin (*Eopsaltria australis*). Although the brains of the songbirds are much larger than those of the two hummingbirds, they share a similar LM volume. For additional abbreviations see list. Scale bars = 0.5 mm.

the volume of LM in hummingbirds is similar to that of a nonhovering species that has a brain almost three times as large. This is corroborated by the data shown in Table 1; an average-sized hummingbird in our sample, such as the rufous-breasted hermit (*Glaucis hirsuta*), has an LM approximately of the same volume as that of songbirds with brains three to four times larger.

Although not as large as that of the hummingbirds, the LM was also larger than average in the transiently hovering species (Fig. 2A,B). The eastern spinebill, belted kingfisher, and American kestrel all had relatively larger LMs than nonhovering species, although the kestrel was the only species that had a relative LM volume approaching that of the hummingbirds (Fig. 2A,B). Including the transient hoverers as an additional category in the ANCOVA yielded a significant difference among the hummingbirds, transient hoverers, and other birds (intercept $F_{2,33} = 31.55$, $P < 0.01$; phylogeny-corrected $F = 15.22$). This reflected significant differences among all three groups (as determined by pairwise post hoc Tukey-

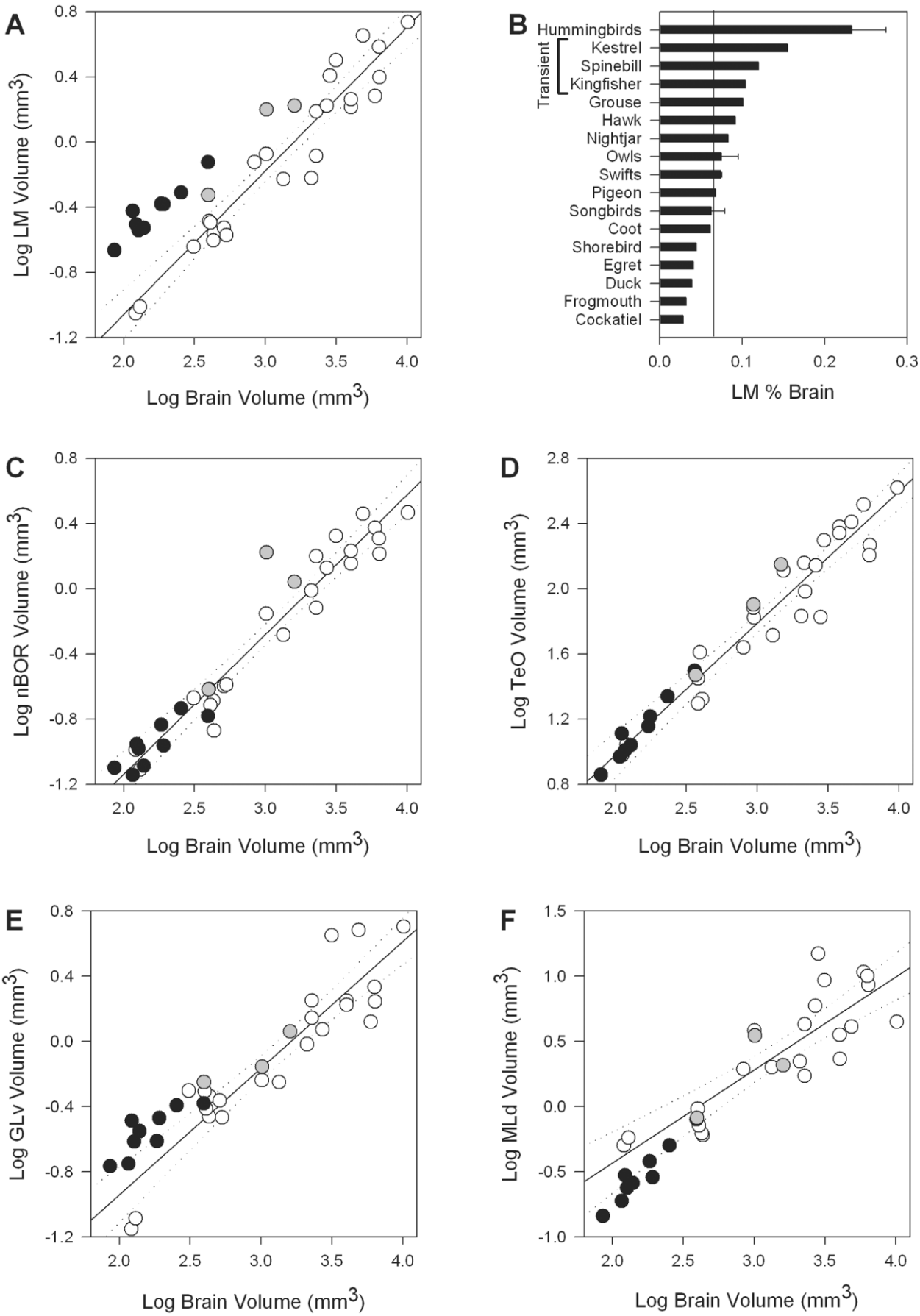
A
Log LM Volume (mm3)
Log Brain Volume (mm3)
B
Hummingbirds
Kestrel
Spinebill
Kingfisher
Transient
Grouse
Hawk
Nightjar
Owls
Swifts
Pigeon
Songbirds
Coot
Shorebird
Egret
Duck
Frogmouth
Cockatiel
LM % Brain
C
Log nBOR Volume (mm3)
Log Brain Volume (mm3)
D
Log TeO Volume (mm3)
Log Brain Volume (mm3)
E
Log GLv Volume (mm3)
Log Brain Volume (mm3)
F
Log MLd Volume (mm3)
Log Brain Volume (mm3)

Figure 2

TABLE 2. Results of Analyses of Covariance (ANCOVAs) Performed on Each of the four Brain Regions

Structure	Effect	df	Calculated F	Critical F	Phylogeny-corrected critical F
LM	Slope	1, 33	0.26	4.14	7.02
	Intercept	1, 34	34.30[1]	4.13	28.92
nBOR	Slope	1, 31	1.61	4.16	7.45
	Intercept	1, 32	0.08	4.15	24.66
GLv	Slope	1, 31	0.34	4.16	6.91
	Intercept	1, 32	4.42	4.15	31.75
TeO	Slope	1, 29	0.38	4.18	6.72
	Intercept	1, 30	0.63	4.17	23.69
MLd	Slope	1, 30	0.35	4.17	5.95
	Intercept	1, 31	4.01	4.16	23.95

[1] $P < 0.05$.

Kramer tests) such that, in terms of relative size, LM gets larger in the following sequence: other species < transient hoverers < hummingbirds. Thus, LM is significantly hypertrophied in all hovering species, but the degree of hypertrophy is significantly greater in the hummingbirds than in the transient hoverers.

Hypertrophy was not observed for nBOR (Fig. 2C), TeO (Fig. 2D), or MLd (Fig. 2E). None of the ANCOVAs yielded a significant difference in the relative size of these three structures between hummingbirds and other birds (Table 2). Significant differences were also not detected when the transient hoverers were included as an additional category or when we constrained the analysis to hummingbirds and similarly sized species (all $P > 0.50$). Thus, regardless of how we analyzed the data, nBOR, TeO, and MLd were not significantly different in hummingbirds from other birds.

By conventional statistics, GLv was significantly larger relative to brain volume in the hummingbirds (Fig. 2F), but this difference was not supported by phylogeny-corrected F values (Table 2). The results were identical when we include transient hoverers as an additional category and when we constrained the analysis to species of similar brain size. That is, GLv was significantly larger using conventional statistics (transient hoverers: $F_{2,31} = 5.17$, $P = 0.01$; similar brain size: $F_{1,16} = 10.80$, $P < 0.01$) but not when compared with the phylogeny-corrected critical Fs (16.21 and 28.06, respectively). Thus, GLv is slightly enlarged in the hovering species, but not to the same extent as LM, and this difference is not supported by phylogeny-corrected statistical analysis.

Fig. 2. Graphs depicting the relative size of nucleus lentiformis mesencephali (LM) and other nuclei in hummingbirds and other birds. In the scatterplot (**A**), the log-transformed volume of LM is plotted against log-transformed brain minus LM volume for all species examined (see Table 1). The hummingbirds are indicated by the black circles, transiently hovering species by the gray circles, and other birds by the white circles. The solid line indicates the least-squares linear regression line for all species, and the dotted lines are the 95% confidence interval around the regression line. **B** is a bar graph of the relative size of LM expressed as a percentage of total brain volume. The solid line indicates the mean for all nonhummingbirds (0.069), the error bars indicate the standard deviations, and the bracket indicates the transient hovering species. The remaining graphs are scatterplots depicting the relative size of the nucleus of the basal optic root (nBOR; **C**), optic tectum (TeO; **D**), nucleus mesencephalicus lateralis, pars dorsalis (MLd; **E**), and nucleus geniculatus lateralis, pars ventralis (GLv; **F**) in hummingbirds and other birds. In each plot, the volume of each region is plotted against the volume of the brain minus the volume of that region. For all scatterplots, the hummingbirds are indicated by the black circles, transiently hovering species by the gray circles, and other birds by the white circles. The solid line indicates the least-squares linear regression line and the dotted lines are the 95% confidence interval around the regression line.

DISCUSSION

These data suggest that, in addition to metabolic (Suarez and Gass, 2002) and morphological (Schuchmann, 1999) specializations, the hovering flight of hummingbirds is enabled by at least one neural specialization: the enlargement of LM, a brain region that mediates the OKR. This is further supported by a less extreme enlargement of LM in three transiently hovering species.

It is important to note that our results could have been affected by significant tissue shrinkage in the museum specimens. Long-term ethanol storage results in tissue dehydration and substantially smaller brain volumes in fluid-preserved museum specimens. Although the brain volumes presented in Table 1 are underestimates of fresh brain volumes, this significant shrinkage is unlikely to have significantly affected our results for two reasons. First, we related the size of each brain region to that of the brain volume of each individual specimen. In doing so, the confounding effect of tissue shrinkage of the museum specimens is significantly less than if we were to compare brain region volumes with the brain volumes of fresh specimens or body masses. Second, the relative size of LM and the other brain regions in the Anna's hummingbird (*Calypte anna*), the only hummingbird specimen that was not procured from a museum, was within the range reported for the museum specimens. If museum preparation had significantly affected the relative size of LM, then the Anna's hummingbird should have been an outlier in our analyses, but it is not. In fact, when expressed as a percentage of total brain volume, the relative volume of LM in the Anna's hummingbird (0.23%) is the same as the average of all hummingbirds (range = 0.19–0.33%; mean = 0.23%). Thus, the potentially confounding effects of including data from both freshly fixed brains and museum specimens appear to be minimal in our analysis.

Importance of stabilization

The OKR is designed to minimize the speed of motion across the retina such that it approaches zero and the retinal image is stabilized. A stable retinal image is important for several reasons. For example, both visual acuity (Westheimer and McKee, 1973) and velocity discrimination (Nakayama, 1985) are superior when the image is stabilized. Owen and Lee (1986) emphasize that visually linking the head to the environment is important for establishing a stable base for the execution of visuomotor behaviors. They cite several examples, including the "spotting" of ballet dancers and high-divers and the hovering flight of pied kingfishers (*Ceryle rudis*) while hunting for fish. Likewise, the unique feeding strategy of hummingbirds, feeding from flowers on the wing, is yet another example of a complex visuomotor behavior that is dependent on stabilization; the hummingbirds must maintain a stable retinal image to feed effectively.

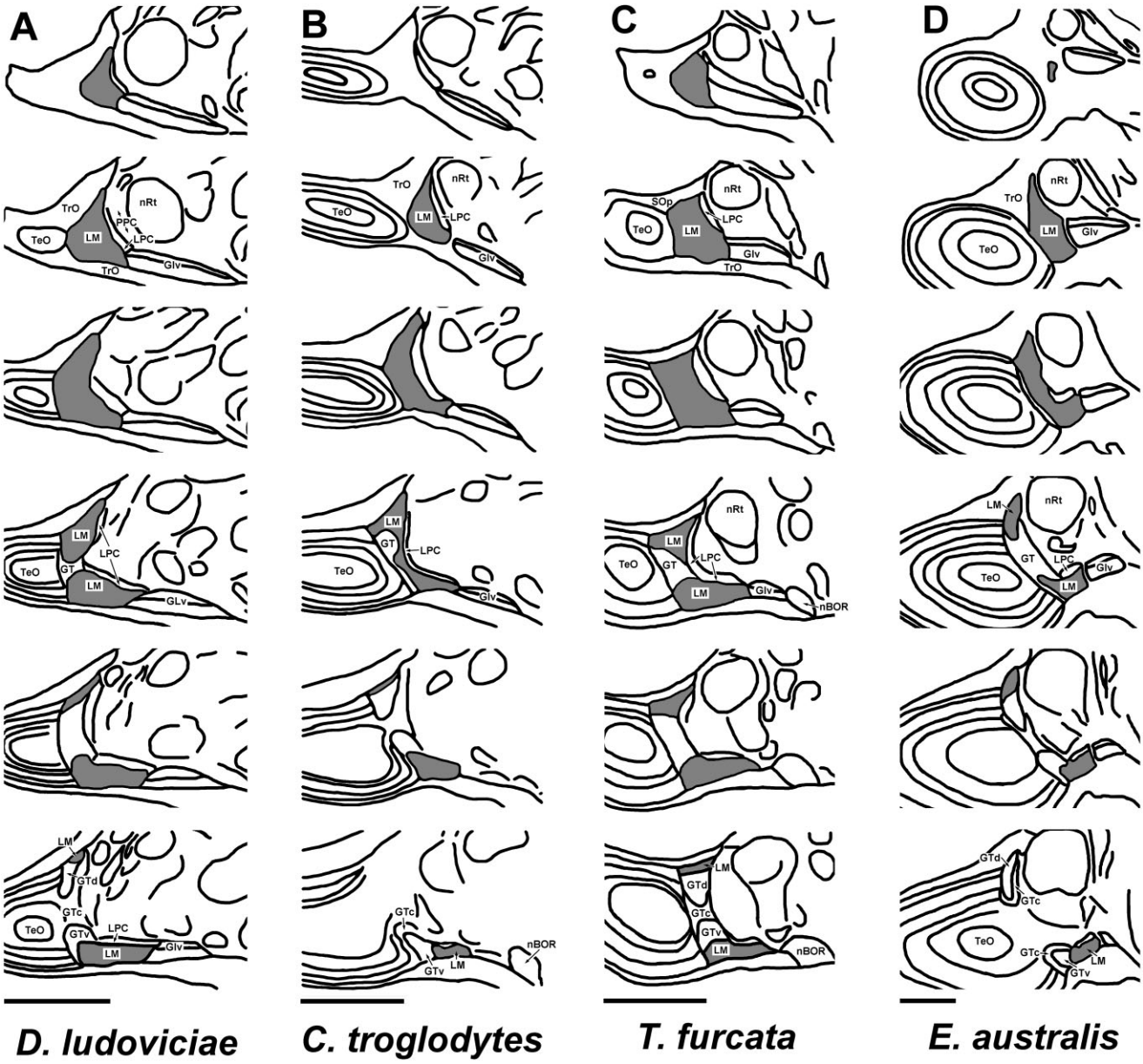

Fig. 3. Line drawings are shown for coronal sections throughout the rostrocaudal extent of the nucleus lentiformis mesencephali (LM) for four species. **A:** Green-fronted lancebill (*Doryfera ludoviciae*, FMNH 320498). **B:** Glossy swiftlet (*Collocalia troglodytes*, FMNH SEA133). **C:** Fork-tailed woodnymph (*Thalurania furcata*, LSUMZ 123339). **D:** Eastern yellow robin (*Eopsaltria australis*). In each section, LM is indicated by the shaded region. The two hummingbirds and the swiftlet are all drawn to the same scale, and the sections shown are 80 μm apart. For the songbird (**D**), the sections shown are 160 μm apart. For other abbreviations see list. Scale bars = 0.5 mm.

LM and the OKR

Hypertrophy of the LM was selective and was not observed in the other visual nuclei measured: GLv, TeO, and nBOR. Although all four structures receive retinal projections (Cowan et al., 1961; Karten et al., 1977; Crossland and Uchwat, 1979; Gamlin and Cohen, 1988a), they differ with respect to visual function. TeO responds primarily to small moving stimuli and is thought to be important for analyzing moving objects in the environment (Frost, 1985). The function of GLv is uncertain. The responses are tectal-like (Pateromichlakis, 1979), but GLv has also been implicated in color vision (Maturana and Varela, 1982; Wakita et al., 1992) and pupillary reflexes (Gamlin et al., 1984). The nuclei of the AOS, LM, and nBOR are visual structures that are highly conserved among vertebrates with respect to function, physiology, and organization of efferent projections (Fite, 1985; Simpson et al., 1988; Ibbotson and Price, 2001; Voogd and Wylie, 2004). It is well established that nBOR and LM, and their mammalian homologs (Simpson, 1984; McKenna and Wallman, 1985a), are critical for the OKR (Simpson, 1984; Simpson et al., 1988). As mentioned previously, lesions to LM and

nBOR dramatically impair or abolish the OKR (Fite et al., 1981; Gioanni et al., 1983a,b). LM and nBOR neurons respond to large-field visual stimuli moving in a particular direction in the contralateral eye (Burns and Wallman, 1981; Winterson and Brauth, 1985). With respect to stimulus speed, studies of the pretectum in wallabies (*Macropus eugenii*) and the LM in pigeons (*Columba livia*) showed that there are two groups of neurons: fast neurons and slow neurons. The fast and slow neurons prefer stimulus speeds on the order of 50°/second and 1°/second, respectively (Ibbotson et al., 1994; Wylie and Crowder, 2000; Crowder and Wylie, 2001; Crowder et al., 2003). The fast neurons are active primarily at the onset of OKR, when retinal image motion is fast (Ibbotson et al., 1994), or during ongoing locomotion. We presume that, during hovering, the slow neurons would be active and responding to extremely slow speeds on the order of a fraction of a degree per second, thereby providing the error signal to maintain a stable position in space. Even when the OKR is at peak efficiency, there is some image motion, but at an extremely slow speed. This serves as the error signal that continues to drive the OKR (Burns and Wallman, 1981; Simpson, 1984; Miles and Wallman, 1993). The slow neurons in the LM and nBOR project to an olivocerebellar pathway (Winship and Wylie, 2003) where neurons have panoramic receptive fields and respond best to optic flow patterns resulting from either self-rotation or self-translation (Wylie et al., 1998). Hummingbirds, and to a lesser extent transient hoverers, may have a greater need for slow cells to allow gaze stabilization while hovering, which could lead to the LM hypertrophy observed.

The selective hypertrophy of LM, but not nBOR, in hummingbirds is somewhat surprising given that both nuclei are critical to the OKR (Gioanni et al., 1983a,b) and have similar response properties (Burns and Wallman, 1981; Morgan and Frost, 1981; Winterson and Brauth, 1985). The major difference between LM and nBOR is their directional preference. Neurons preferring temporal-to-nasal (T-N) motion are rare in the nBOR (Gioanni et al., 1984; Wylie et al., 1998), but most neurons in LM prefer T-N motion (McKenna and Wallman, 1985b; Winterson and Brauth, 1985; Wylie and Frost, 1996; Wylie and Crowder, 2000). In terms of the hypertrophy of LM, this could reflect an increased need to detect T-N optic flow resulting from hummingbirds drifting backward during hovering. Hummingbirds also fly backwards, unlike any other birds, and engage in other complex and rapid flight maneuvers (e.g., mating displays; Schuchmann, 1999), resulting in image motion that would be detected by the fast T-N neurons. This raises the possibility that LM hypertrophy reflects the processing requirements of other visuomotor behaviors in addition to hovering. In insects, the analysis of optic flow has also been linked not only to hovering (Kern, 1998; Kern and Varju, 1998) but also to several other visuomotor behaviors. such as flight stabilization guidance and speed, landing, odometry, and collision avoidance (Egelhaaf et al., 2002; Srinivasan and Zhang, 2004). An increase in the processing requirements of similar behaviors in hummingbirds might have driven the enlargement of LM. The fact that LM was also hypertrophied in the transiently hovering species, however, reinforces the proposed link between LM size and hovering. A comparison of the giant hummingbird (*Patagona gigas*) and the eastern spinebill illustrates this point. The two species share similar brain volumes (~400 mm^3) and feeding strategies, but the LM of the giant hummingbird is about one-third larger than that of the spinebill. Even taking the confounding effect of tissue shrinkage of the giant hummingbird specimen into account, this is a substantial difference and reflects significant differences in hovering flight between the two species; the spinebill hovers only occasionally while feeding (Higgins et al., 2001), whereas the giant hummingbird almost always hovers. We therefore conclude that sustained hovering is a behavior that is dependent not only on metabolic (Suarez and Gass, 2002) and aerodynamic adaptations (Schuchmann, 1999) but also on the neural control of gaze stabilization.

Aside from LM hypertrophy, one might expect that the physiology of the AOS and eye anatomy has also changed in hummingbirds. In other species, the OKR and AOS have become specialized for specific visuomotor strategies. For example, in frontal-eyed animals, the physiology of the AOS and the dynamics of the OKR have changed to promote stabilization of the central visual field (Grasse and Cynader, 1988; Wylie et al., 1994). In pigeons, the dynamics of the OKR are different depending on whether the animal is flying or walking (Bilo and Bilo, 1978; Gioanni and Sansonetti, 1999). With respect to hummingbirds, one might expect that the neurons are sensitive to slower speeds than those reported for other species. Also, given that the task is often to stabilize in front of a flower that may be swaying in the wind, one might also expect a higher density of ganglion cells projecting to the LM and nBOR to be found in the temporal part of the retina. Whether these additional neural specializations have accompanied the hypertrophy of LM in hummingbirds remains to be seen.

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