Brain, Behavior and Evolution

Relative Size of Auditory Pathways in Symmetrically and Asymmetrically Eared Owls

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Abstract

Owls are highly efficient predators with a specialized auditory system designed to aid in the localization of prey. One of the most unique anatomical features of the owl auditory system is the evolution of vertically asymmetrical ears in some species, which improves their ability to localize the elevational component of a sound stimulus. In the asymmetrically eared barn owl, interaural time differences (ITD) are used to localize sounds in azimuth, whereas interaural level differences (ILD) are used to localize sounds in elevation. These two features are processed independently in two separate neural pathways that converge in the external nucleus of the inferior colliculus to form an auditory map of space. Here, we present a comparison of the relative volume of 11 auditory nuclei in both the ITD and the ILD pathways of 8 species of symmetrically and asymmetrically eared owls in order to investigate evolutionary changes in the auditory pathways in relation to ear asymmetry. Overall, our results indicate that asymmetrically eared owls have much larger auditory nuclei than owls with symmetrical ears. In asymmetrically eared owls we found that both the ITD and the ILD pathways are equally enlarged, and other auditory nuclei, not directly involved in binaural comparisons, are also enlarged. We suggest that the hypertrophy of auditory nuclei in asymmetrically eared owls likely reflects both an improved ability to precisely locate sounds in space and an expansion of the hearing range. Additionally, our results suggest that the hypertrophy of nuclei that compute space may have preceded that of the expansion of the hearing range and evolutionary changes in the size of the auditory system occurred independently of phylogeny.

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Introduction

It is well known that owls have extremely sophisticated auditory systems that enable them to hunt, such that some species can accurately localize sounds in complete darkness [Payne and Drury, 1958; Payne, 1971]. In fact, their ability to precisely localize sounds, combined with the developmental plasticity of the underlying neural mechanisms, has made owls, especially the barn owl *(Tyto alba)*, a model for studying the neural mechanisms of sound localization and, more generally, the plasticity of sensory systems [reviewed in Knudsen, 1999; Takahashi, 2010]. To facilitate their auditory abilities, owls possess a suite of
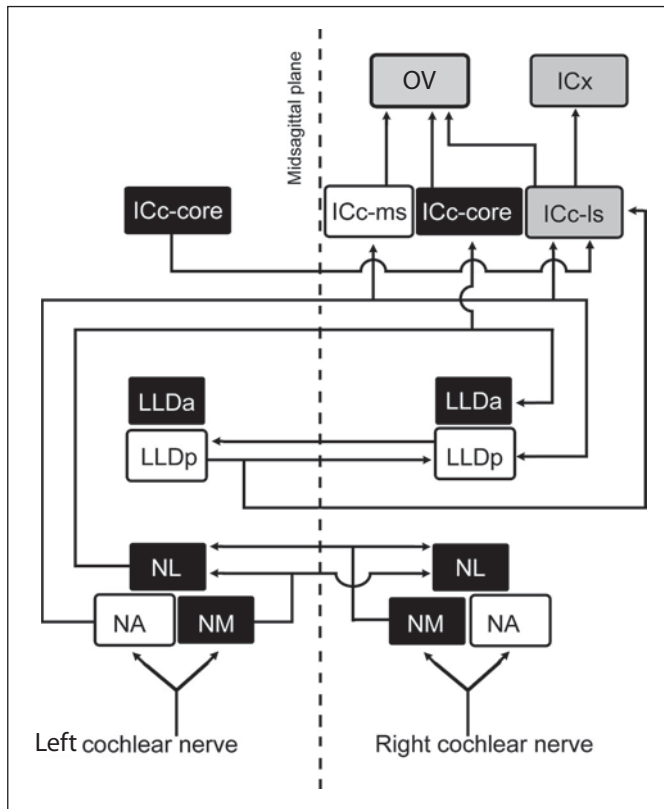
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Fig. 1. Parallel neural pathways in owls for the processing of ITD (black) and ILD (white). ITD is first computed at the NL and ILD at the LLDp. A second level of coincidence detection exists in the LLDa. Information in the ITD and ILD pathways is combined at the level of the ICc-ls (grey). ICc-ls projects to the external nucleus of the ICx. ICc-ls, ICc-core and the medial shell of the central nucleus of the IC project to the ipsilateral OV.

anatomical specializations. Externally, the feathers in the preaural skin folds are sparse and modified to be 'acoustically transparent', while in the postaural flaps, the feathers are densely packed and form a concave surface that helps to direct sound to the ears and increase the intensity of sound [Norberg, 1977, 2002]. The peripheral auditory system is characterized by a unique columella footplate morphology, a long cochlea, a long interaural canal and a relatively large tympanic membrane [Schwartzkopf, 1955, 1968; Schwartzkopf and Winter, 1960; Payne, 1971]. Perhaps the most unique anatomical feature of the owl auditory system is the presence, in some species, of vertically asymmetrical ears. These ear asymmetries have evolved independently several times and are based on a variety of anatomical adaptations [Kelso, 1940; Norberg, 1977, 1978]. In some species the asymmetry is due to differences in the soft tissue. For example, in the eagle owl

(*Bubo bubo*), the genus *Cicabba* and some *Strix* species, the differences between the two ears are mostly in the size of the ear openings in the skin. In the genus *Asio*, the ear asymmetry is caused entirely by differences in the orientation of an intra-aural septum in the skin of the two ears, which results in different shapes and vertical positions of the ear openings [Norberg, 2002]. In contrast, in the genus *Aegolius,* ear asymmetry does not arise from the soft tissues. Instead, the ear openings in the skulls of these species are dramatically different in both shape and vertical position [Norberg, 1977, 1978].

Most of what we know of the neural mechanisms underlying auditory localization comes from the extensive research on the barn owl *(T. alba)*. Several studies have shown that the external ear morphology provides directional cues in azimuth and elevation [Payne, 1971; Coles and Guppy, 1988; Moiseff, 1989; Keller et al., 1998]. Behavioural studies have shown that barn owls can localize sounds with great precision both in azimuth and elevation [Knudsen et al., 1979; Bala et al., 2003; Whitchurch and Takahashi, 2006] and electrophysiological studies revealed that there is a map of auditory space in the external nucleus of the inferior colliculus (ICx) where neurons have spatial receptive fields that are restricted in both azimuth and elevation [Knudsen et al., 1977; Knudsen and Konishi, 1978a, b].

Other asymmetrically eared owls including the northern saw-whet owl *(Aegolius acadicus)* and the long-eared owl *(Asio otus)* have ICx neurons with receptive fields restricted in elevation [Wise et al., 1988; Volman and Konishi, 1990]. However, in symmetrically eared owls, such as the great horned owl *(Bubo virginianus)* and the burrowing owl *(Athene cunicularia)*, the receptive fields of ICx neurons are much less restricted in elevation [Volman and Konishi, 1990]. Thus, vertical asymmetry of the ear openings facilitates localization in elevation.

In barn owls, azimuth and elevation are computed using interaural time differences (ITDs) and interaural level differences (ILDs), respectively [Knudsen and Konishi, 1979, 1980; Moiseff and Konishi, 1981; Moiseff, 1989]. Moreover, ITDs and ILDs are processed independently along two separate pathways from the cochlear nuclei to the ICx [Moiseff and Konishi, 1983; Takahashi et al., 1984; Takahashi and Konishi, 1988a, b; Adolphs, 1993; Mazer, 1998]. The time and intensity pathways are shown in figure 1. The cochlear nerve projects directly to 2 nuclei in the brainstem: nucleus angularis (NA) and nucleus magnocellularis (NM) [Carr and Boudreau, 1991]. Cells in NA are mainly sensitive to stimulus intensity and this nucleus is the starting point of the ILD pathway [Sullivan

and Konishi, 1984; Sullivan, 1985]. NA projects to the contralateral dorsal lateral lemniscus (LLDp) and the medial shell of the central IC and the lateral shell of the central inferior colliculus (ICc-ls) [Takahashi and Konishi, 1988a, b; Takahashi and Keller, 1992; Adolphs, 1993]. LLDp receives an inhibitory projection from the contralateral LLDp and is the first place where ILDs are computed [Manley et al., 1988]. NM cells show phase locking properties [Sullivan and Konishi, 1984; Sullivan, 1985] and represent the start of the ITD pathway. NM projects bilaterally to the nucleus laminaris (NL) [Carr and Konishi, 1988, 1990] where interaural differences in the phase of each spectral component are computed by a binaural cross-correlation-like mechanism [Jeffress, 1948; Carr and Konishi, 1990; Yin and Chan, 1990]. NL cells project to the contralateral anterior dorsal lateral lemniscus (LLDa) and the core of the central nucleus of the inferior colliculus (ICc-core) [Takahashi and Konishi, 1988b]. Information from both the ILD and ITD pathways are combined in ICc-ls, as it receives input from NA, LLDp, and the ICc-core [Knudsen, 1983; Takahashi and Konishi, 1988a, b; Takahashi et al., 1989]. ICc-ls projects to ICx, the site of an auditory space map [Knudsen and Konishi, 1978a; Knudsen, 1983]. All divisions of ICc project to the nucleus ovoidalis (OV) [Proctor and Konishi, 1997; Cohen et al., 1998; Arthur, 2005], which in turn projects to field L in the telencephalon [Cohen et al., 1998] where auditory space is also processed [Pérez et al., 2009].

Previous work has shown that the relative size of some of these auditory nuclei is not only larger in owls, but also differs between asymmetrically and symmetrically eared owls. For example, the asymmetrically eared barn owl and long-eared owl *(A. otus)* have a larger number of cells in the auditory brainstem nuclei than species with symmetrical ears *(B. bubo* and *Athene noctua)* [Winter, 1963; Kubke et al., 2004]. The inferior colliculus (IC) is also enlarged in owls compared to other birds and is much larger in asymmetrically eared owls than symmetrically eared owls [Cobb, 1964; Wagner and Luksch, 1998; Iwaniuk et al., 2006]. While these previous studies suggest a hypertrophy (i.e. enlargement) of the auditory system associated with ear asymmetry, they fail to reveal several aspects of evolution of the auditory system relative to ear asymmetry.

First, as mentioned above, vertical ear asymmetry allows for sound localization in elevation, and the system has evolved such that ILD varies with elevation and not azimuth [Coles and Guppy, 1988]. Even though symmetrically eared owls use ILD in addition to ITD to locate sounds in azimuth [Volman and Konishi, 1989], the increased use of ILD in asymmetrically eared owls to localize sounds in elevation could result in a greater hypertrophy of the ILD pathway. Second, ear asymmetry has evolved independently many times in owls [Norberg, 1977] and arises from various changes in ear morphology (see above). Given that there are differences in the manner in which the external auditory apparatus has evolved, one might expect central differences as well. Finally, there is a great degree of variation in activity patterns within asymmetrically eared owls. In this paper, we present a comparison of the relative volume of eleven auditory nuclei in both the ITD and the ILD pathways of 8 species of symmetrically and asymmetrically eared owls. This includes 5 species from 4 different genera that vary in ear asymmetry (table 1). Based on previous studies and the functional organization of the ITD and ILD pathways, we predict that both the ILD and ITD auditory pathways will be enlarged in asymmetrically eared owls compared to symmetrically eared owls, with an emphasis on the enlargement of the ILD pathway. Moreover, highly nocturnal asymmetrically eared species, such as the barn owl and the northern saw-whet owl, will have larger auditory pathways than more diurnal asymmetrically eared owls, such as the short-eared owl *(Asio flammeus)* and the great grey owl *(Strix nebulosa).*

Materials and Methods

We measured the relative volume of 11 auditory nuclei in 12 specimens representing 8 species (table 1), including 4 species that appear to have a marked ear asymmetry. Within the asymmetrically eared species, each species differs in how the asymmetry is manifested. In the barn owl the ear asymmetry is due to soft tissue, the ear openings are the same shape, but are at different vertical levels (i.e., left is higher than right). Also, the skin flaps in front of the ears are of a different shape and the left ear is higher than the right [Konishi, 1973; Norberg, 1977]. In the short-eared owl, ear asymmetry is also caused by differences in the soft tissue. A horizontal intra-aural septum is oriented in a different manner in the left versus the right ear, which results in a different shape, and the left ear opening being higher than the right [Norberg, 1977, 2002]. As described above, the ear asymmetry in the northern saw-whet owl is inherent in the skull, as the auditory canals differ in shape and position such that the right ear is much higher than the left ear [Norberg, 1977, 1978]. Together these three species are classified as having a high degree of ear asymmetry.

We examined two *Strix* species that appear to have different degrees of ear asymmetry. The great grey owl *(S. nebulosa)* has an obvious asymmetry that is present in both the soft tissue and the skull. The right ear opening in the skin is larger than the left and the preaural skin flaps are asymmetrical [Voous, 1964; Norberg, 1977]. In the skull, the asymmetry is dramatic: the postorbital process on the right side extends further laterally than on the left side. On the right side, the postorbital process is connected to the squa-

Table 1. List of the owl species surveyed and a description of the different structures that contribute to the ear asymmetry in each species

Species	Common name	Ear asymmetry
T. alba	Barn owl	Only soft anatomy Left ear opening in the skin higher Left preaural flap different shape than right and higher
A. acadicus	Saw-whet owl	Only skull structure Ear openings in the skull of different shape; right opening higher
A. flammeus	Short-eared owl	Only soft anatomy Different orientation of skin septum in the ear openings
S. nebulosa	Great grey owl	Soft anatomy and skull structures Right ear opening in the skin bigger Preaural flaps different shape Slightly different position of a horizontal skin fold above the ear openings in the skull Ear openings in the skull are of different shape Left ear canal is directed more upward than the right
S. varia	Barred owl	Only soft structures Right skin ear opening bigger
B. virginianus	Great horned owl	None
B. scandiacus	Snowy owl	None
S. ulula	Hawk owl	None

mo-occipital wing, but not on the left side. Together the asymmetry in soft tissue and skull results in the left external auditory meatus being directed more dorsally than the right (fig. 2a–d) [Collett, 1881; Norberg, 1977]. In the barred owl *(Strix varia)*, the ear asymmetry is quite subtle. There is no asymmetry in the skull, but with respect to soft tissue, the right ear opening in the skin is larger than the left and is a few millimeters higher [Voous, 1964; Norberg, 1977]. Because of the more apparent aural asymmetry in the great grey owl, for convenience it has been grouped with the barn owl, northern saw-whet owl, and short-eared owl, which are labelled in bold letters in figures 5 and 6. The barred owl is labelled in italics, while the 3 species with symmetrical ears, the snowy owl *(Bubo scandiacus)*, the great horned owl *(B. virginianus)* and the northern hawk owl *(Surnia ulula)*, are labeled in plain letters.

For all specimens, the head was immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was then extracted, weighed to the nearest milligram, cryoprotected in 30% sucrose in phosphate buffer, embedded in gelatin and sectioned in the coronal or sagittal plane on a freezing stage microtome at a thickness of 40 μm. Sections were collected in 0.1 M phosphate-buffered saline, mounted onto gelatinized slides, stained with thionin and coverslipped with Permount. The olfactory bulbs were intact in all of the specimens that we collected and sectioned. All brains were cut following bird brain atlases [e.g. Karten and Hodos, 1967; Puelles et al., 2007], in which the brainstem ends at the same rostrocaudal point as the cerebellum. In this manner, brain measurements were consistent among our specimens. Photomicrographs of every fourth section were taken throughout the rostrocaudal extent of each nucleus using a Retiga EXi *FAST* Cooled

mono 12-bit camera (Qimaging, Burnaby, B.C., Canada) and OPENLAB Imaging system (Improvision, Lexington, Mass., USA) attached to a compound light microscope (Leica DMRE, Richmond Hill, Ont., Canada). Measurements of all the nuclei were taken directly from these photos with ImageJ (NIH, Bethesda, Md., USA; http://rsb.info.nih.gov/ij/) and volumes were calculated by multiplying the area in each section by the thickness of the section (40 μm) and the sampling interval. For those species represented by more than one specimen (table 1), the average of the measurements was taken as the species' given value.

Borders of Nuclei in the Auditory System
We measured nuclei in the time and intensity pathways as indicated in figure 1, as well as other auditory nuclei not explicitly associated with sound localization including the superior olive (SO), which receives input from both NA and NL and projects back to NA, NL and NM [Takahashi and Konishi, 1988a; Carr et al., 1989; Carr and Boudreau, 1993; Lachica et al., 1994], and 3 lemniscal subnuclei: the ventral part of the lateral lemniscus (LLv), the caudal part of the intermediate lateral lemniscus (LLIc) and the rostral part of the intermediate lateral lemniscus (LLIr). All three receive input primarily from NA but do not analyze ILDs [Moiseff and Konishi, 1983; Takahashi and Konishi, 1988a; Wild et al., 2001].

Borders for NA, NM and NL were established using the descriptions of Takahashi and Konishi [1988a, b] and Köppl and Carr [1997]. Cells in these 3 nuclei are surrounded by thick bundles of fibers and therefore the borders are easily distinguished by the presence of cells (fig. 3a–d). In the case of the lemniscal com-
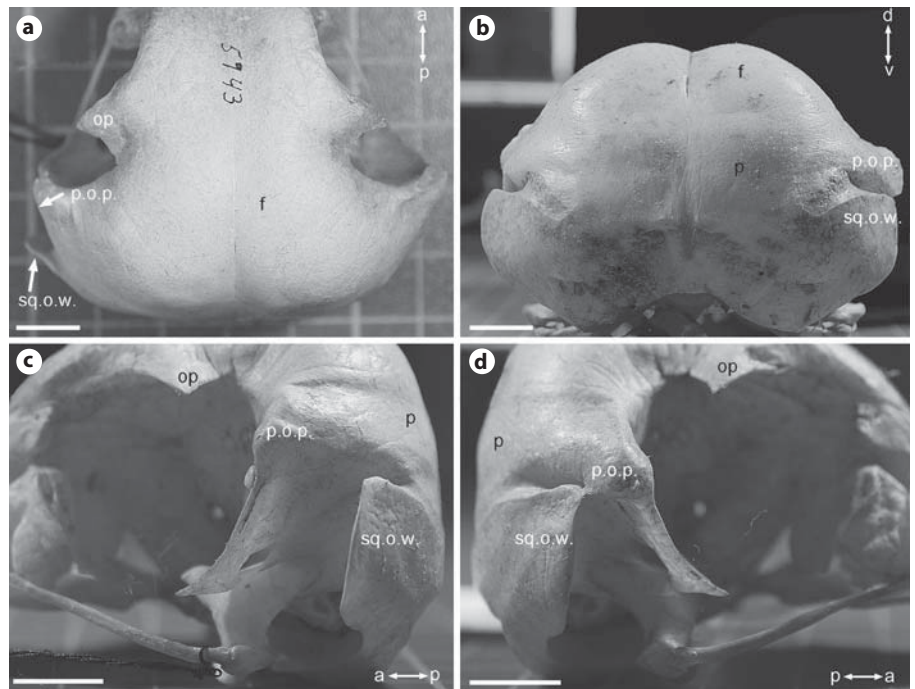
Fig. 2. Dorsal (**a**), posterior (**b**), left (**c**) and right (**d**) views of the skull of the great grey owl *(S. nebulosa).* Specimen number: 5943 (Museum of Zoology, University of Alberta). Scale bars = 1 cm. p.o.p. = Postorbital process; sq.o.w. = squamo-occipital wing; op = orbital process; f = frontal; p = parietal.

plex and SO, we followed the descriptions and nomenclature of Wild et al. [2001]. Even though the nucleus pontine externus (PE) does not receive auditory projections [Wild et al., 2001], it was included in the measurement of the volume of the LLIr because it was impossible to distinguish the border between these 2 nuclei in Nissl-stained material (fig. 3g, h). The LLIc can be identified as a group of cells lateral to the principal sensory nucleus of the trigeminal nerve. The anterior part of LLIc is surrounded by the faciculus uncinatus [Karten and Hodos, 1967] and it lies ventral to a fiber tract, the brachium conjunctivum (fig. 3e, f). LLv was easily distinguished as a group of darkly stained cells anterior to SO and dorsal to the lateral pontine nucleus (fig. 3h). In all species, LLDa could be followed from its anterior border as an oval group of cells ventral and lateral to the nucleus semilunaris (fig. 3g, h). LLDp could be identified as the group of cells dorsal and lateral to LLDa. The borders of the SO were clearly delineated (fig. 4a, b).

In most studies of the avian auditory system, the IC is named the nucleus mesencephalicus lateral pars dorsalis (MLd) after Karten [1967]. Because MLd is homologous to the IC in mammals [Karten, 1967], Knudsen [1983] recommended that the term IC be applied to refer to the MLd in birds. Since then, this terminology has been used in most owl studies [Wagner et al., 2003] and will be adopted here. In the IC, the caudal and rostral poles were defined as the regions ventral to the third ventricle that had larger, darker and more densely packed cells than adjacent regions. The ventral and lateral borders were defined by the presence of a distinct lamina that forms a fibre bundle surrounding the IC [Knudsen, 1983] and the dorsal and lateral borders were defined by the tectal ventricle (fig. 4c, d). Although IC has several subdivisions [Knudsen, 1983; Wagner et al., 2003], the border between the central and external nuclei is very faint in Nissl preparations and we were unable to distinguish the subdivisions, and therefore our measurements are restricted to the entire volume of the IC. We attempted to define the different subdivision of IC by using immunohistochemistry against a calcium-binding protein, calretinin, which is expressed at higher levels in ICx and ICc-core [Wagner et al., 2003]. Unfortunately, because of the various states of fixation and time that the brains have been stored in fixative we could not reliably discern the subdivisions across all species.

OV is a well-defined group of dark-stained cells in the posterior part of the dorsal thalamus, lateral and dorsal to nucleus rotundus (fig. 4e, f). Finally, we were unable to reliably measure field L, the telencephalic target of OV, due to the diffuse borders of this nucleus in Nissl stain preparations.

Cell Counts

We counted cells in the 2 cochlear nuclei (NA and NM) and NL for comparison with previous studies (table 3) [Winter, 1963; Kubke et al., 2004]. Cells were counted in the same sections used for volume estimation. The cells were counted using an unbiased stereological method, the optical fractionator [West et al., 1991; Howard and Reed, 2005]. An unbiased counting frame [Gundersen, 1977] with an area of 0.0088 mm^2 was positioned on the coordinates of a rectangular lattice randomly superimposed on the section. The distance between the coordinates was 282 µm along each axis of the lattice. At each sampling point, the thickness of the sections was determined as the distance between that of the first particle coming into focus and the last particle coming out of focus [West et al., 1991]. An unbiased brick-counting rule [Gundersen and Osterby, 1981; Howard et al., 1985] was used. That is, an unbiased counting frame was projected onto the thickness of the section resulting in a cube with the upper, top and left planes as acceptable surfaces and all others as nonacceptable surfaces. Thus, if a cell contacted the lower, bottom or right planes, it was not
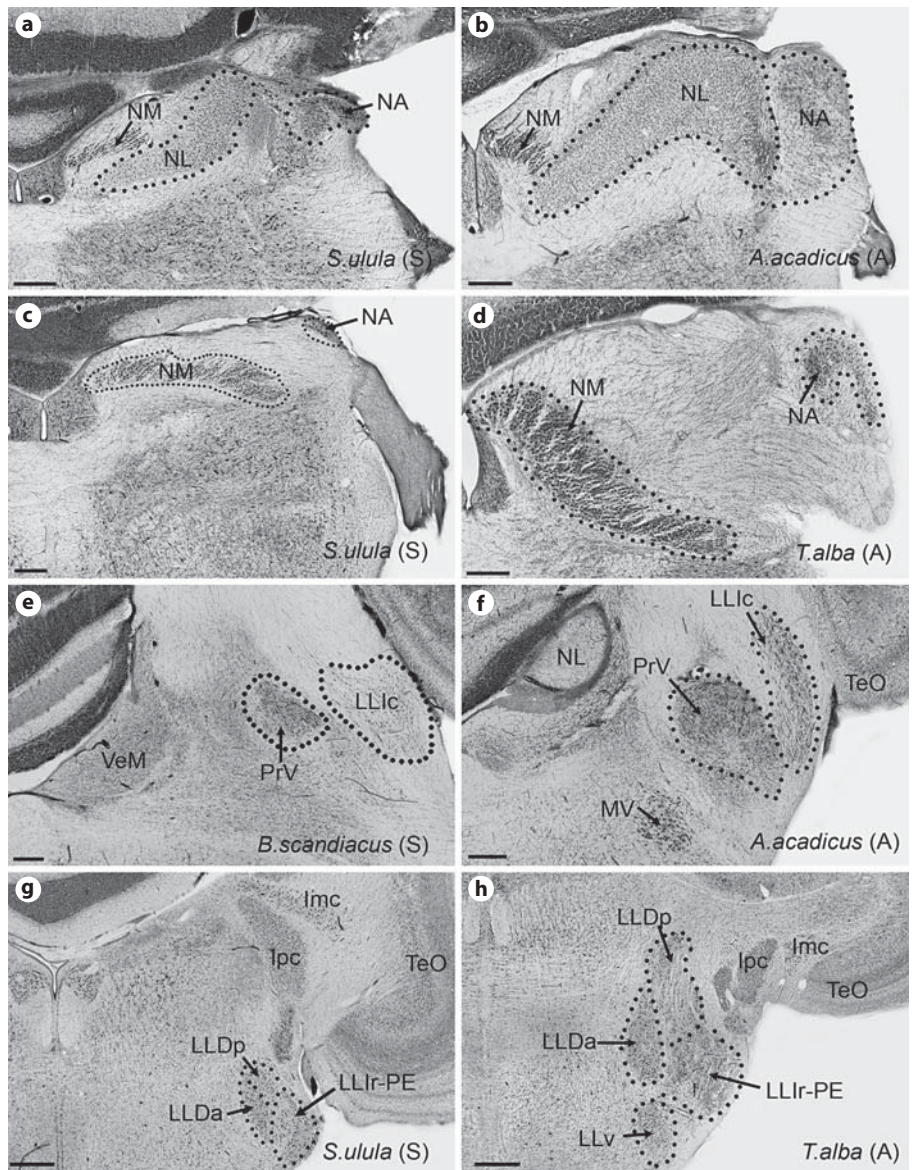
Fig. 3. Photomicrographs of coronal section through the following: NA, NM and NL of a symmetrically eared owl, the hawk owl *(S. ulula)* (**a**) and an asymmetrically eared owl, the northern saw-whet owl *(A. acadicus)* (**b**). NM and NA in the hawk owl (**c**) and an asymmetrically eared owl, the barn owl *(T. alba)* (**d**). Caudal part of the LLIc in a symmetrically eared owl, the snowy owl *(B. scandiacus)* (**e**) and the northern saw-whet owl (**f**). Rostral part of the LLIr, the PE, the LLDp and the LLDa in the hawk owl (**g**) and the barn owl (**h**). Letter in brackets next to the scientific name of the species indicate symmetric (S) or asymmetric (A) ears. TeO = Optic tectum; Ipc = parvocellular part of the nucleus isthmi; Imc = magnocellular part of the nucleus isthmi; PrV = motor nucleus of the trigeminal nerve; MV = motor nucleus of the trigeminal nerve; VeM = nucleus vestibularis medialis. Scale bars = 400 μm.

counted. The upper plane refers to the first section in the plane of focus and the lower plane to the last. Top, bottom, right and left refer the sides on the counting frame. The height of the counting brick was two thirds of the total thickness. Nuclear profiles containing a nucleolus were counted using a 100× oil immersion objective. At least 100 cells were counted per cochlear nucleus across all specimens. Coefficients of error were calculated with the quadratic approximation formula [Gundersen and Hensen, 1986; West et al., 1991]. As with the volumetric measurements, for those species represented by more than one individual, we used the average of the measurements as the species' given value.

Statistical Analyses

In most comparative studies dealing with relative size of brain structures, allometric effects are accounted by comparing residuals from least-squares linear regressions between the structure and body mass or brain volume [e.g. Iwaniuk et al., 2005, 2006; Iwaniuk and Wylie, 2007]. With a relatively small number of species, such comparisons become problematic because a single data point can have a huge influence on the slope and intercept of an allometric line. Instead, we have taken a qualitative approach by examining the relative size of each nucleus as a percentage of overall brain volume.

Also, in recent years comparative analyses have used phylogenetically corrected statistics [e.g. Garland et al., 1992, 2005] to account for possible phylogenetic effects. The small number of species examined herein has low statistical power that would be even further reduced with such a correction. The sample size of our subgroups (e.g. asymmetrical vs. symmetrical) further constrains our statistical power, therefore making such phylogenetic correc-

Fig. 4. Photomicrographs of coronal sections through: the SO of a symmetrically eared owl, the hawk owl *(S. ulula)* (**a**) and an asymmetrically eared owl, the northern saw-whet owl *(A. acadicus)* (**b**). IC of the hawk owl (**c**) and the northern saw-whet owl (**d**). The OV of the symmetrically eared owl, the great horned owl *(B. virginianus)* (**e**) and the northern saw-whet owl *(A. acadicus)* (**f**). Letter in brackets next to the scientific name of the species indicate symmetric (S) or asymmetric (A) ears. TeO = Optic tectum; Ipc = parvocellular part of the nucleus isthmi; Imc = magnocellular part of the nucleus isthmi; Rt = nucleus rotundus; Tel = telencephalon; Cb = cerebellum; OMd = dorsal part of the oculomotor nucleus; OMv = ventral part of the oculomotor nucleus; nIV = abducens nerve nucleus; TOv = tractus ovoidalis; DLP = posterior part of the dorsolateral thalamic nucleus; RP = nucleus reticularis pontis; TTD = nucleus of the descending trigeminal tract. Scale bars = 400 μm.
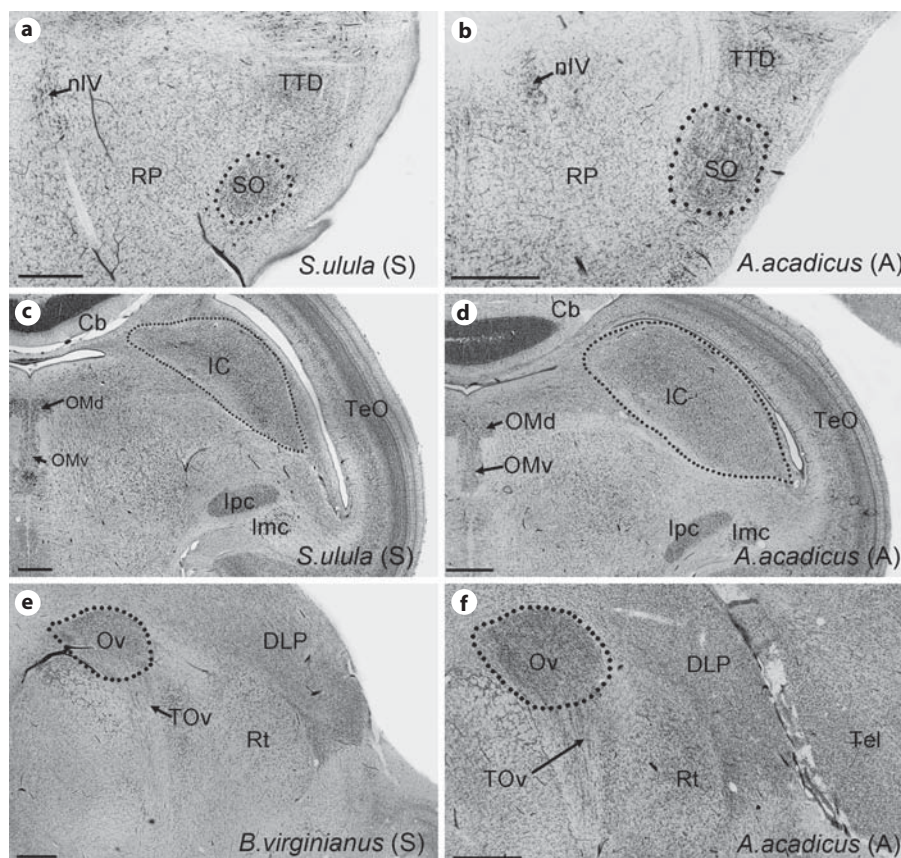
tions impractical. Instead, we compared the results of a hierarchical cluster analysis to the most complete phylogenetic tree available for owls [Wink et al., 2008]. Using a similar approach to Iwaniuk and Hurd [2005], we performed a hierarchical cluster analysis of the proportional size of all auditory nuclei measured, with JMP (Version 7, SAS Institute Inc., Cary, N.C., USA). Although the dendrograms produced by hierarchical cluster analyses are based on similarities among species, comparing the dendrogram with a phylogeny of the species of interest can reveal whether interspecific differences have arisen largely through phylogenetic relatedness or independent evolution [e.g. Iwaniuk and Hurd, 2005]. Here, we show the results generated using an average linkage method, but the dendrograms arising from other linkage methods (e.g. Ward's, UPGMA) shared the same topology.

Results

We found marked differences in the relative size of all auditory nuclei among owl species (fig. 3–6). For illustrative purposes, in figures 5 and 6, nuclei in the intensity pathway and time pathway are shown in white and black, respectively, and nuclei that integrate information from both pathways are shown in grey. Finally, auditory nuclei that have not been explicitly associated with sound localization are indicated with cross-hatching.

Overall, the barn owl, the northern saw-whet owl and the short-eared owl have hypertrophied auditory nuclei when compared to the other species. Both *Strix* species also have auditory nuclei that are somewhat larger than the 3 symmetrically eared species and generally, the great grey owl had relatively larger nuclei than the barred owl (fig. 5, 6). In the great grey owl, for some nuclei, the relative volume approached that of the other asymmetrically eared owls.

Cochlear Nuclei and NL

Shown in figure 5a–c, the volume occupied by NA, NM and NL relative to total brain volume was largest in the barn owl, the northern saw-whet owl and short-eared owl. These values were 4–5 times larger than those of the 3 symmetrically eared species (see fig. 3a–d). The 2 species of *Strix* owls had relative NA, NM and NL volumes that were larger than those of the symmetrically eared
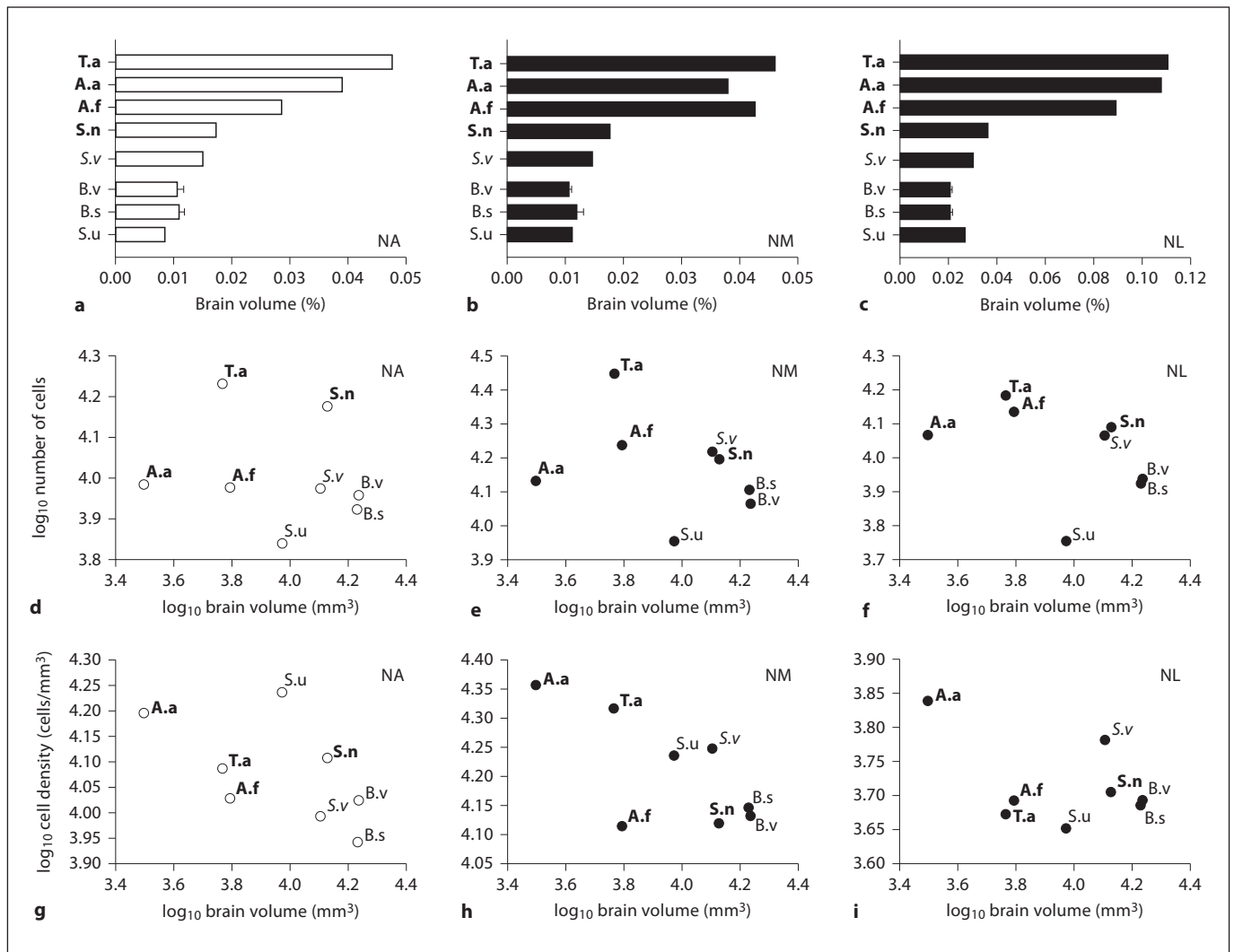
Fig. 5. Bar graphs show the relative size of the NA, NM and NL in 8 species of owls expressed as a percentage of total brain volume. Scatterplots show the number of cells and cell density (cells/mm^2) of NA, NM and NL plotted as a function of the logarithms of the brain volume for all species examined. White bars and dots indicate the nucleus belongs to the ILD pathway. Black bars and dots indicate the nucleus belongs to the ITD pathway. Bold letters indicate a high degree of ear asymmetry, italic letters a moderate degree of ear asymmetry, and plain text symmetrical ears. T.a = Barn owl *(T. alba)*; A.a = northern saw-whet owl *(A. acadicus)*; A.f = short-eared owl *(A. flammeus)*; S.n = great grey owl *(S. nebulosa)*; S.v = barred owl *(S. varia)*; B.v = great horned owl *(B. virginianus)*; B.s = snowy owl *(B. scandiacus)*; S.u = hawk owl *(S. ulula)*.

species, but by a factor of less than two (see also table 2). The hypertrophy of these nuclei in asymmetrically eared owls is readily evident in coronal sections through the brainstem. When compared to symmetrically eared owls (fig. 3a–d) the dorsal part of brainstem of the barn owl, the northern saw-whet owl and the short-eared owl is greatly expanded dorsoventrally, and all 3 nuclei extend much further rostrally.

Figure 5d–i shows a scatterplot of the logarithm of the total cell numbers (fig. 5d–f) and cell densities (fig. 5g–i) of NA, NM and NL plotted against the logarithm of the brain volume. Overall, the barn owl has the highest total number of cells for the 3 nuclei, although for NL, there was little difference between the barn owl and short-eared owl (table 3). The great grey owl (fig. 5) has a large number of cells in NA, especially when compared to the barred owl, which has both a similar relative volume of
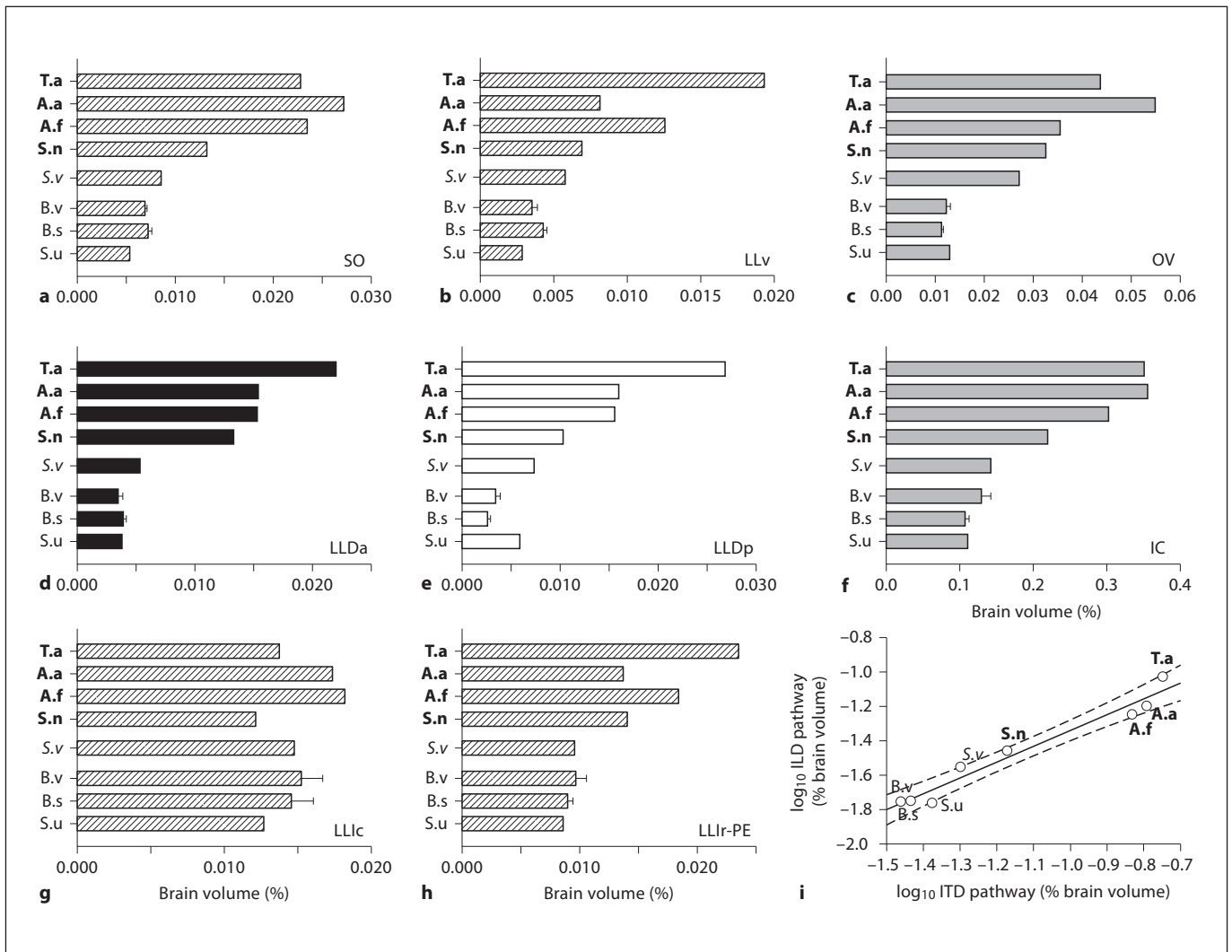
Fig. 6. Bar graphs show the relative size of the SO (**a**), the LLv (**b**), the OV (**c**), the LLDa (**d**), the LLDp (**e**), the IC (**f**), and the caudal part of the intermediate lateral lemniscus (LLIc) (**g**). **h** The LLIr-PE expressed as a percentage of total brain volume for all species examined (see table 2). White bars indicate the nucleus belongs to the ILD pathway. Black bars indicate the nucleus belongs to the ITD pathway. Striped bars indicate the nucleus is not directly involved in binaural comparisons. Grey bars indicate the nucleus receives projections from both the ILD and the ITD pathways (see text and fig. 1). **i** Scatterplot of the total volume of the ILD pathway plotted as a function of the total volume of the ITD pathway in 8 species of owls. The solid lines indicate the least squares linear regression line for all species, and the dotted lines are the 95% confidence interval around the regression line. Bold letters indicate a high degree of ear asymmetry, italic letters a moderate degree of ear asymmetry, and plain text symmetrical ears. T.a = Barn owl *(T. alba)*; A.a = northern saw-whet owl *(A. acadicus)*; A.f = short-eared owl *(A. flammeus)*; S.n = great grey owl *(S. nebulosa)*; S.v = barred owl *(S. varia)*; B.v = great horned owl *(B. virginianus)*; B.s = snowy owl *(B. scandiacus)*; S.u = hawk owl *(S. ulula)*.

NA (fig. 5) and overall brain size (table 2). When we examined cell density within the cochlear nuclei and NL, it was clear that the barn owl and the northern saw-whet owl have the highest cell densities in NA and NM, almost twice those of the short-eared owl. The northern saw-whet owl also had the highest cell density for NL (table 3).

Lemniscal and Midbrain Nuclei in the ITD and ILD Pathways

In all other nuclei of both auditory pathways, the results were similar to those of the cochlear nuclei in that they were hypertrophied in the barn owl, short-eared owl and northern saw-whet owl. However, some nuclei in the

Table 2. List of the owl species surveyed, sample size and volumes (in mm³) of the brain and all nuclei measured: NA, NM, NL, superior olive (SO), LLv, caudal part of the intermediate lateral lemniscus (LLIc), rostral part of the intermediate lateral lemniscus and pontine externus (LLIr-PE), LLDp, LLDa, IC and OV

Common name	Species	n	Brain volume mm³	NA mm³	NM mm³	NL mm³	SO mm³	LLv mm³	LLIc mm³	LLIr-PE mm³	LLDp mm³	LLDa mm³	IC mm³	OV mm³
Barn owl	*T. alba*	1	5,849.81	2.781	2.695	6.464	1.334	1.131	0.802	1.374	1.571	1.287	19.623	2.557
Saw-whet owl	*A. acadicus*	1	3,142.86	1.228	1.193	3.389	0.854	0.256	0.546	0.431	0.503	0.484	11.172	1.726
Short-eared owl	*A. flammeus*	1	6,221.04	1.779	2.654	5.541	1.460	0.780	1.132	1.144	0.969	0.951	18.815	2.206
Great grey owl	*S. nebulosa*	1	13,433.40	2.334	2.380	4.863	1.780	0.929	1.633	1.889	1.385	1.788	29.508	4.381
Barred owl	*S. varia*	1	12,727.12	1.913	1.869	3.844	1.094	0.732	1.878	1.214	0.930	0.679	18.040	3.450
Great horned owl	*B. virginianus*	3	16,323.47	1.740	1.714	3.370	1.127	0.571	2.486	1.577	0.559	0.571	21.200	2.008
Snowy owl	*B. scandiacus*	3	17,065.09	1.869	2.040	3.558	1.231	0.728	2.484	1.523	0.448	0.663	18.272	1.931
Hawk owl	*S. ulula*	1	9,408.30	0.802	1.048	2.554	0.506	0.266	1.192	0.801	0.556	0.357	10.414	1.216

Table 3. List of the owl species surveyed, sample size, number of cells, and cell density (in cells/mm³) in the three cochlear nuclei: NA, NM and NL

Common name	Species	n	NA number of cells	NM number of cells	NL number of cells	NA density cells/mm³	NM density cells/mm³	NL density cells/mm³
Barn owl	*T. alba*	1	17,005.01 (0.020)	27,915 (0.052)	15,199 (0.046)	12,227.49	20,716.31	4,703.10
Saw-whet owl	*A. acadicus*	1	9,627.36 (0.059)	13,550.41 (0.064)	11,666.55 (0.033)	15,685.88	22,711.20	6,884.06
Short-eared owl	*A. flammeus*	1	9,480.21 (0.047)	17,246.55 (0.034)	13,612.34 (0.082)	10,656.71	12,994.69	4,913.49
Great grey owl	*S. nebulosa*	1	14,973.19 (0.049)	15,654.30 (0.064)	12,294.43 (0.066)	12,828.30	13,153.99	5,056.61
Barred owl	*S. varia*	1	9,418.23	16,484.95	11,612.90	9,848.41	17,642.28	6,041.84
Great horned owl	*B. virginianus*	2	9,058.79 (0.093)	11,578.98 (0.088)	8,646.08 (0.079)	10,563.95	13,569.97	4,941.75
Snowy owl	*B. scandiacus*	2	8,351.89 (0.065)	12,743.58 (0.059)	8,369.54 (0.060)	8,767.10	13,983.04	4,839.45
Hawk owl	*S. ulula*	1	6,909.80 (0.098)	8,999.81 (0.088)	5,717.71 (0.102)	17,233.14	17,180.46	4,478.16

Figures in parentheses represent coefficient of error for the estimation of the cell numbers.

Table 4. Degree of ear asymmetry and audiogram parameters of 13 species of owls

Species	Ear asymmetry	Best frequency kHz	Low-frequency sensitivity, dB SPL	High-sensitivity cutoff, kHz	High-frequency cutoff, kHz	Source
Tyto alba guttata	Yes	6.3	7.0	10.6	13.8	1
Tyto alba pratincola	Yes	4	4.8	10.6	12.9	2
Asio otus	Yes	6	−6.5	8.5	11.1	3
Strix virgata	Yes	0.5	−7.5	6.9	11.3	3
Strix seloputo	Yes	2	−7.5	6.6	9.4	3
Strix aluco	Yes	6	−1	8	10.3	3
Strix woodfordii	Yes	6	−9.5	6.7	10.0	5
Bubo bubo	Yes	2	−1.5	6.3	8.6	3
Otus scops	None	4	−0.5	6.3	9.5	3
Otus leucotis	None	2	−9.5	6.3	9.3	3
Bubo scandiacus	None	4	−8.0	6.3	8.5	3
Bubo virginianus	None	1	−1.6	2.3	7.0	4
Bubo nipalensis	None	0.5	−5	3.2	7.7	3
Ketupa zeylonensis	None	1	7.5	1.5	6	3

High-frequency cutoff was defined as where threshold rises to ≥30 dB above the lowest threshold. High-sensitivity cutoff as where the threshold is below 0 dB. Low-frequency sensitivity was defined as the hearing threshold at 500 Hz [Trainer, 1946; Konishi, 1973; Van Dijk, 1973; Nieboer and Van der Paardt, 1977; Dyson et al., 1998].

great grey owl were also hypertrophied to a similar degree (fig. 6). For both the LLDa and LLDp, the relative sizes were largest in the barn owl, on the order of 5 times larger than those in the symmetrically eared owls. The LLDa was also large in the northern saw-whet owl, short-eared owl and great grey owl, 4 times larger than that in the symmetrically eared species. Similarly, compared to the symmetrically eared owls LLDp was about 3.5 times larger in the northern saw-whet and short-eared owls, and 2.5 times larger in the great grey owl. The LLDa and LLDp in the barred owl were only slightly larger compared to the symmetrically eared species (fig. 6d, e). This difference in the relative size of LLDp and LLda are reflected in the organization of both nuclei. In the asymmetrically eared owl and the barred owl, both nuclei appear as two very distinct, independent cell groups all along the anteroposterior axis. Furthermore, in all these species LLDp extends dorsally to lie lateral to the nucleus semilunaris (fig. 3h). In contrast, in symmetrically eared owls both nuclei appear as a one group of cells, ventral to the nucleus semilunaris (fig. 3g).

The IC and OV showed a similar pattern. The relative sizes of both of these nuclei were largest in the northern saw-whet owl, but also larger in the barn owl, short-eared owl, and great grey owl. Compared to the symmetrically eared owls, the IC was 2–3.5 times larger in these 4 species, and the OV was 3–5 times larger (fig. 6c, f). For the barred owl, the IC was only slightly larger compared to the symmetrically eared species, but the OV was almost as large as that of the great grey owl and 2.5 times larger than that of the symmetrically eared species. In asymmetrically eared species IC appears much larger along the dorsoventral axis than in symmetrically eared owls (fig. 4c, d) and it extends further rostrally.

The auditory nuclei not explicitly associated with sound localization also showed some degree of hypertrophy in the asymmetrically eared owls. Compared to the symmetrically eared owls, the relative size of SO was 4–5 times larger in the northern saw-whet owl, barn owl and short-eared owl, and 2 times larger in the great grey owl (fig. 6a). The LLv and LLlr-PE were largest in the barn owl and short-eared owl and about the same size in the great grey owl and the northern saw-whet owl (fig. 6b, g). Nonetheless, these were still larger than those of the symmetrically eared owls. The LLIc was the only nucleus to be approximately the same relative size in all species (fig. 6h).

In addition to examining the proportional sizes of all of the individual auditory nuclei, we calculated the proportional sizes of the entire ITD and ILD pathways (fig. 6i). The relative volume of the ITD pathway, calcu-
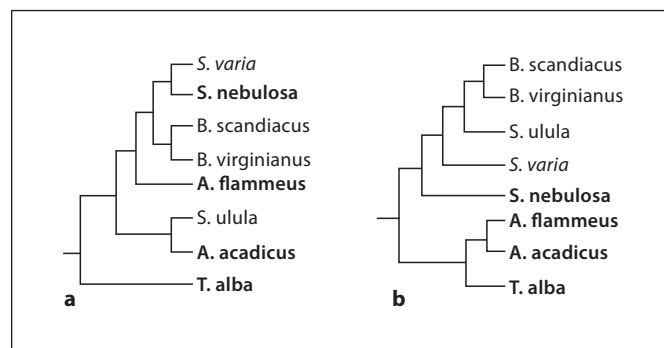

Fig. 7. a Phylogenetic relations among the 8 species used in this study based on Wink et al. [2008]. **b** Phenogram based on a hierarchical cluster analysis of the relative size of all auditory nuclei. Bold letters indicate a high degree of ear asymmetry, italic letters a moderate degree of ear asymmetry, and plain text symmetrical ears.

lated as the sum of the volume of NM, NL and LLDa, is correlated with the total volume of the ILD pathway, calculated as the sum of NA and LLDp (fig. 6i, $r^2 = 0.958$; p <0.001). Note that the barn owl, northern saw-whet owl and short-eared owl have the largest ILD and ITD pathways, followed by the great grey owl, the barred owl, and the three symmetrically eared owls in that order. The slope of the regression line describing the relationship between the volumes of the ITD and the ILD pathways is not statistically different from 1 (one-tailed t test, t = 0.986, p = 0.181), which indicates that both pathways are equally enlarged in the asymmetrically eared owls.

Lastly, we compared a dendrogram resulting from a hierarchical cluster analysis with a molecular phylogeny of the species we examined. Figure 7a depicts the phylogenetic relationships among the 8 species used in this study [Wink et al., 2008] and figure 7b illustrates the similarity among the 8 species based on a cluster analysis of the relative size of all auditory nuclei. This dendrogram has two main clusters, but does not separate symmetrically and asymmetrically eared owls completely. The barn owl, northern saw-whet owl and short-eared owl comprise one group and all other species are in a second group. Within this second group, the two *Strix* species come out at a basal position relative to the 3 symmetrically eared species. This dendrogram contrasts greatly with the phylogeny where the 3 species with greatly enlarged auditory pathways are not closely related, but are distributed across the phylogenetic tree.

Discussion

Overall, our results indicate that asymmetrically eared owls have much larger auditory nuclei than owls with symmetrical ears. In doing so, this study significantly expands upon previous studies [Winter and Schwartzkopf, 1961; Winter, 1963; Kubke et al., 2004; Iwaniuk et al., 2006], which examined only cochlear nuclei or IC, and a smaller number of species. Our study is therefore the first to compare the relative size of all the auditory nuclei from the brainstem to the thalamus among multiple owl species.

Previously, Kubke et al. [2004] compared the relative number of cells in the cochlear nuclei and NL and found that the barn owl had a larger relative amount of cells than the long-eared owl in NA and NM, but not NL, and that the tawny owl had a relative number of cells just slightly larger that symmetrically eared owls. While we found similar differences among asymmetrically eared species in the relative volume of the cochlear nuclei (fig. 5a–c), our results suggest that the total number of cells in the cochlear nuclei is not entirely related to ear asymmetry. This is well illustrated by the northern saw-whet owl; this species has a similar number of cells in the two cochlear nuclei and NL to both *Bubo* species (table 3), but the relative volume of the nuclei is 5 times larger (fig. 5a–c). We also found that while there is little variation in cell density in the NA, NM and NL between asymmetrically and symmetrically eared owls, there are some exceptions. The northern saw-whet owl has particularly high cell densities in NA, NM and NL, despite having relative volumes similar to that of the barn owl (fig. 5d–f). The northern saw-whet owl has the smallest brain of all species sampled, about half the size of the barn owl and the short-eared owl, and this difference in density could therefore be related to overall brain size. In mammals, cell density is inversely proportional to the cubic root of the brain volume [Shariff, 1953; Tower, 1954; Bok, 1959] and the same rule could apply to birds, although this has not been tested to date. Despite previous suggestions that the total number of cells is important for auditory coding [Kubke et al., 2004; Kubke and Carr, 2006], our results suggest that cell numbers may vary according to some scaling function (see above) or other unknown variables. Further research is necessary to determine if other factors, like cell size or the shape of cells and dendritic trees, play more important roles in auditory coding in asymmetrically eared owls.

Our results show that the hypertrophy of auditory pathways is not equal in all asymmetrically eared owls. In the barn owl, the northern saw-whet owl and the short-eared owl, the difference in the relative size of all auditory nuclei is similar when compared to the symmetrically eared owls (fig. 5, 6). In contrast, both *Strix* species present little difference compared to symmetrically eared owls in the 2 cochlear nuclei and NL (fig 5a–c), but the difference is much more pronounced in nuclei further upstream, especially for the great grey owl (e.g. IC, OV; fig. 5, 6). These species represent at least four independent examples of the evolution of ear asymmetry [Norberg, 1977, 2002], and in each case this has arisen from different morphological adaptations (see Materials and Methods for details). Furthermore, in some cases, like the barred owl, the asymmetry is much more subtle than in other species. While it is possible that the differences in the relative size of the auditory pathways are related to the different ear morphologies, or the degree of ear asymmetry, we currently lack the appropriate data to test this hypothesis. To do so, one would need (1) behavioural studies showing the accuracy of sound localization in azimuth and elevation; (2) acoustical studies showing how (or if) ILD varies with a function of elevation and frequency, and (3) neurophysiological data indicating the spatial precision of cells in ICx. Currently these data are only available for the barn owl [Knudsen et al., 1977, 1979; Knudsen and Konishi, 1978b; Coles and Guppy, 1988]. Indeed, the data for barn owls goes beyond this. Measurement of ILDs in barn owls, where the facial ruff and the preaural flaps are removed (which leaves only a small difference in the vertical level of the ear opening), results in much smaller ILDs than in barn owls with unmodified ears, and the ILDs change much more slowly with elevation, providing half the spatial resolution [Coles and Guppy, 1988]. For the saw-whet owl, it is known that they can precisely localize sound in elevation and the receptive fields in ICx are restricted in elevation [Wise et al., 1988; Frost et al., 1989]. One would predict that acoustical studies would show that ILD varies as a function of elevation in this species with a high degree of spatial resolution. In the long-eared owl (*A. otus*), a close relative of the short-eared owl with very similar ear morphology [Norberg, 1977], the receptive fields are much less restricted in elevation than those of the barn owl [Volman and Konishi, 1990]. One would predict that ILD would vary as a function of elevation, but not affording the same resolution as that of the barn owl. One would also predict that this species would not be as precise in the elevational component of sound localization, but behavioural data is only available for their localization in azimuth (2°) [Rice, 1982]. None of these data are available for the other asymmetrically eared owls in our sample, although it is known that

the long-eared owl can hunt in complete darkness [Payne, 1971] and the great grey owl often hunts by hovering over a spot and then plunging into deep snow to capture prey [Nero, 1980]. However, without empirical data it is impossible to assess if different ear morphologies, and especially more subtle ear asymmetries, provide different degrees of spatial resolution. Detailed studies on the variation of ILD and ITD in different asymmetrically eared species, as well as behavioral studies of auditory spatial resolution and electrophysiological studies of the properties of space-specific neurons are needed in order to assess the spatial cues available to each species and whether this explains the differences in the relative size of the auditory pathways.

Because of the increased use of ILDs by asymmetrically eared owls, we expected a greater enlargement of the ILD pathway. In contrast, our results show that the ITD and ILD pathways are equally enlarged in asymmetrically and symmetrically eared owls (fig. 6i). This equal expansion of both auditory pathways might be related to the expansion of hearing range in asymmetrically eared owls. Published audiograms for 13 owl species (table 4) suggest there is an association between ear asymmetry and both a higher range of sensitive hearing (threshold below 0 dB) and a high frequency cutoff (where threshold rises to ≥30 dB above the lowest threshold) [Van Dijk, 1973; Volman and Konishi, 1990; Dyson et al., 1998; Gleich et al., 2005]. In symmetrically eared owls, hearing deteriorates rapidly over 6 kHz and the high-frequency cutoff lies between 7 and 9.5 kHz. By contrast, in asymmetrically eared owls, high-sensitivity hearing goes up to 8–9 kHz and their high-frequency cutoff lies between 10 and 13 kHz [Van Dijk, 1973; Konishi, 1973; Dyson et al., 1998]. This expansion in the hearing range is probably related to the fact that only sounds with short wavelengths can be shadowed enough by the small outer ear structures to produce ILDs that vary with elevation [Norberg, 1978; Volman and Konishi, 1990]. This means that in order to use ILDs to detect sounds in elevation, an asymmetrically eared owl must have high sensitivity at frequencies above 5 kHz [Volman and Konishi, 1990]. In the barn owl, this expansion of the hearing range results in a long cochlea where high frequencies are overrepresented, dedicating more cells per octave than any other bird [Manley et al., 1987; Gleich, 1989; Köppl et al., 1993; Smolders et al., 1995]. It is likely that a similar overrepresentation of high frequencies is present in other asymmetrically eared owls [Kubke and Carr, 2006]. In all birds, each auditory nerve bifurcates as it enters the brain and directly innervates both NM and NA [Whitehead and

Morest, 1981; Carr and Boudreau, 1991]. These projections are organized tonotopically, so an expansion of the hearing range should result in a bigger NA and NM. Furthermore, ITDs in NL and ILDs in LLDp are computed by frequency [Manley et al., 1988; Carr and Konishi, 1990; Yin and Chan, 1990], and other auditory nuclei, like LLDa, LLv, SO and the core and shell of ICc, are also organized tonotopically [Moiseff and Konishi, 1983; Fischer and Konishi, 2008]. Thus, the expansion of the hearing range would explain not only the equal enlargement of the ITD and ILD pathways, but may also explain the hypertrophy of all auditory nuclei.

Unfortunately, the hearing range has not been reported for the great grey owl, but has for other *Strix* species that have only subtle ear asymmetries like the barred owl. These species have a lower high-sensitivity cutoff, very close to that of symmetrically eared owls (table 4). In the great grey owl, there was no hypertrophy of the cochlear nuclei or NL, but there was hypertrophy of the auditory nuclei further upstream. If the hearing range of the great grey owl is close to that of other *Strix* species, then the expansion of lemniscal and midbrain nuclei related to the computation of sound in space may have preceded the expansion of the hearing range and the cochlear nuclei.

The only nucleus in the barred owl that showed a substantial hypertrophy was OV. OV receives projections from ICc and forms part of a sound localization pathway to field L in the telencephalon [Cohen et al., 1998], independent of the ICx-tectal pathway [Knudsen et al., 1993; Wagner, 1993; Cohen and Knudsen, 1999; Pérez et al., 2009]. Cells in OV show spatially selective fields that are as sharp as neurons in ICx [Proctor and Konishi, 1997; Pérez et al., 2009], but their ITD and ILD tunings vary more across frequencies and respond to a much broader frequency range than ICx neurons, especially lower frequencies [Pérez et al., 2009]. The enlargement of OV in both *Strix* species may reflect a greater reliance on the OV-forebrain pathway for sound localization.

We also found differences in the cytoarchitectonic organization of LLDa and LLDp between asymmetrically and symmetrically eared owls. In all the asymmetrically eared owls, LLDa and LLDp are clearly distinguishable in a Nissl's stain preparation, but in symmetrically eared owls they appear as one group of cells throughout the anteroposterior axis (fig 3g, h). Takahashi and Konishi [1988a] had previously reported that in the barn owl these 2 nuclei are clearly distinguishable cytoarchitectonically and hodologically in the posterior region, where high frequencies are represented, but not in the most anterior part where low frequencies are found. As mentioned be-

fore, ILDs vary with elevation in asymmetrically eared owls at high frequencies, but vary with azimuth at low ones. This would suggest that in owls a distinct LLDa and LLDp are characteristic of a functional ear asymmetry and the use of ILD to detect sounds in elevation.

Additionally, we found LLDa is differentially hypertrophied in the barn owl. This nucleus appears as particularly large when compared to other asymmetrically eared owls, especially the northern saw-whet owl and the short-eared owl, even though the relative size of NL (the main afferent to LLDa) [Takahashi and Konishi, 1988a] in these species is very similar to the barn owl (fig. 5c). In the barn owl, LLDa is involved in noise reduction of coincidence detector responses to ITDs [Fischer and Konishi, 2008]. It is possible that the larger relative size of LLDa in the barn owl reflects higher noise reduction capabilities in the ITD pathway compared to the other two species.

We also found hypertrophy in auditory nuclei not directly involved in binaural comparisons, like SO, LLv and LLIr-PE. SO receives projections from NA and NM [Takahashi and Konishi, 1988a] and sends inhibitory projections to the cochlear nuclei, NL, and the contralateral SO [Conlee and Parks, 1986; Monsivais et al., 2000; Burger et al., 2005]. This inhibitory projection is involved in enhanced phase locking to the waveform in NM and NL, improved coincidence detection in NL, and offsetting of intensity levels in the ITD pathway [Reyes et al., 1996; Funabiki et al., 1998; Yang et al., 1999; Monsivais et al., 2000; Burger et al., 2005]. This tight correlation with the ITD pathway suggests that the expansion of SO is related to the hypertrophy of NM and NL. Unfortunately, much less information is available for LLv and LLIr. LLv receives bilateral projections from NA [Takahashi and Konishi, 1988a], but cells respond only to monoaural stimuli [Moiseff and Konishi, 1983], and it projects to the contralateral ICc-core [Takahashi and Konishi, 1988b; Adolphs, 1993]. This suggests that LLv is associated with the ILD pathway, and therefore the higher relative volume in asymmetrically eared owls is probably associated with the increase in relative size of NA. This is probably also the case for LLIr, which receives projections from NA too [Wild et al., 2001]. It should be noted that we included PE when measuring this nucleus (see Materials and Methods), even though it is not an auditory nucleus [Wild et al., 2001], and this may have affected the smaller difference in size between asymmetrically eared and symmetrically eared owls in this nucleus. Finally, the only auditory nucleus where we found no marked difference in relative size between asymmetrically and symmetrically

eared owls was LLIc. This nucleus receives projections from the ipsilateral NA, but also somatosensory information from the sciatic and radial nerves, and projects to nucleus basalis [Wild et al., 2001]. The lack of hypertrophy of LLIc in asymmetrically eared owls would suggest this nucleus is not related to sound localization.

Evolution of Ear Asymmetry

The cluster analysis of the relative size of all auditory nuclei revealed that the barn owl, northern saw-whet owl and short-eared owl share a similar expansion of their auditory pathways (fig. 7). These species represent three independent events of the evolution of ear asymmetry and therefore three independent expansions of the auditory pathways. At least two studies suggest that this independent enlargement of auditory pathway associated with ear asymmetry could be facilitated by adaptation already present in the auditory pathways of symmetrically eared owls. Kubke and Carr [2006] showed that in both symmetrically and asymmetrically eared owls, NL is organized differently than most other birds and this is related to the ability to detect ITDs above 2 kHz. Second, the neural circuitry that underlies ILD selectivity is already present in symmetrically eared owls, but because ILDs vary with azimuth in these species, it serves as an additional cue to detect sounds in azimuth [Volman and Konishi, 1989]. Because ILD is not essential to sound localization in azimuth in symmetrically eared owls, the ILD pathways can be co-opted to detect differences in elevation in asymmetrically eared owls [Volman and Konishi, 1989]. Therefore, the independent enlargement of auditory pathways in asymmetrically eared owls and the accompanying increase in the ability to detect sounds do not depend on the evolution of novel neural circuitry, but rather the exaptation of preexisting traits in the auditory pathways.

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