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Morphological Variation in the Nucleus Laminaris of Birds

M. F. Kubke
University of Auckland, New Zealand

C. E. Carr
University of Maryland, U.S.A.

Interaural time differences (ITDs) are one of the cues used for binaural sound localisation. In birds, ITDs are computed in nucleus laminaris (NL), where a place code of azimuthal location first emerges. In chickens, NL consists of a monolayer of bitufted cells that receive segregated inputs from ipsi- and contralateral nucleus magnocellularis (NM). In barn owls, the monolayer organisation, the bitufted morphology, and the segregation of inputs have been lost, giving rise to a derived organisation that is accompanied by a reorganisation of the auditory place code. Although chickens and barn owls have been the traditional experimental models in which to study ITD coding, they represent distant evolutionary lineages with very different auditory specialisations. Here we examined the structure of NL in several bird lineages. We have found only two NL morphotypes, one of which appears to have emerged in association with high frequency hearing.

The structure of the adult auditory system is shaped by two different processes. At the species level, evolution can select for the characteristics of the circuit to make it suitable to the species' particular behavioural niche. At the individual level, in addition, experience-mediated plasticity modifies this basic Bauplan to adapt the circuit to the characteristics and experience of each individual (Kubke & Carr, 2005). The avian auditory system is an ideal model in which to study what neuronal specialisations are associated with specific features of neuronal coding. Since the auditory sensory epithelium does not contain a topographic map of auditory space, the emergence of an auditory space map in the central nervous system relies exclusively on precise neuronal computations that make use of spectral and temporal cues contained in the auditory stimulus. Neuronal circuits that require precise temporal coding exhibit morphological specialisations that are associated with the accurate transmission of temporal information (Carr & Soares, 2002). Some of these specialisations are found in different and unrelated vertebrate taxa, reflecting their suitability for particular aspects of neuronal coding. One example is the almost ubiquitous presence of endbulb-like synapses in neuronal circuits that rely on precise temporal coding (such as the auditory and electrosensory systems; Carr, 1986; Carr & Soares, 2002; Matsushita & Kawasaki, 2004). These large terminals are particularly suited for efficient temporal coding and can provide a reliable mechanism for synaptic transmission whereby a presynaptic action potential

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elicits a suprathreshold response in the postsynaptic cell (Ryugo & Parks, 2003). Thus, the examination of morphological features of brain structures can be informative since they may reflect the coding requirements of particular neuronal ensembles. We will discuss the morphological variation of the nucleus laminaris (NL) in different avian lineages and hypothesise about the role of auditory coding in the evolution of this variation.

Basic Plan for Sound Localisation

Interaural time differences (ITDs) in birds and mammals serve as one of the main cues used for binaural sound localisation. The circuit responsible for ITD computation in birds has been extensively studied in a nonauditory specialist (chicken) and in an auditory specialist (barn owl, *Tyto alba*; Kubke & Carr, 2000). The auditory nerve enters the brainstem and bifurcates to innervate the two cochlear nuclei magnocellularis (NM) and angularis (NA), which process time and intensity parameters of the auditory stimulus, respectively (Takahashi, Moiseff, & Konishi, 1984). The third order NL is bilaterally innervated by NM generating a topographic representation of ITDs that translates into a place code of azimuthal sound source location (Carr & Konishi, 1990; Overholt, Rubel, & Hyson, 1992; Sullivan & Konishi, 1986). The circuit that gives rise to this place code in NL conforms to the Jeffress model (Jeffress, 1948). Jeffress proposed that time differences could be computed with the use of two elements: delay lines and coincidence detectors (Figure 1). In birds, the auditory nerve conveys phase locked inputs to neurons in NM, via highly efficient calyceal synapses or endbulbs of Held, and this phase locked information is transmitted to ipsilateral and contralateral NL. NM axons innervating NL act as delay lines, synapsing on different coincidence detectors (NL cells; Konishi, 2003). NL cells show maximum response when the ipsilateral and contralateral inputs arrive simultaneously. Thus, a coincidence detector (NL cell) will fire when the delay imposed by the separation of the ears is equal and opposite to that imposed by the delay lines (NM axons; Figure 1). This circuit arrangement results in the formation of a place code that is used for sound localisation (Knudsen & Konishi, 1978; Konishi, 2003). Although the organisation of both the chicken and barn owl NL conforms to this basic model, the organisation of the circuit, and thus of the emergent place code, is quite different (Kubke & Carr, 2000).

In the chicken, NL forms a crescent shaped mass of cells medial and ventral to NM (Rubel & Parks, 1988). Most of NL appears as a flat, oblique plate of clear grey matter containing a single row of small bitufted cells along the centre of the lamina, with a more caudolateral region containing cells with longer dendrites that are not organised in a monolayer. This organisation appears to conform to the plesiomorphic morphotype (Kubke & Carr, 2000; Kubke, Massoglia, & Carr, 2002b). NL is tonotopically organised and the length of the dendritic tufts varies systematically along the tonotopic axis, with higher best-frequency cells exhibiting shorter dendrites than low best-frequency cells (Smith & Rubel, 1979). Inputs from ipsi- and contralateral NM are segregated such that dorsal dendrites receive inputs exclusively from ispilateral NM, whereas the ventral dendritic tuft receives input exclusively from contralateral NM. Axons from the contralateral NM provide delayed inputs to NL cells in such a way that space becomes mapped along the me-

diolateral dimension (Figure 1A, B; Overholt, Rubel & Hyson, 1992). As a result, more laterally positioned cells respond maximally to sounds originating from far contralateral space, whereas cells in more medial positions respond maximally to sounds originating from the front.

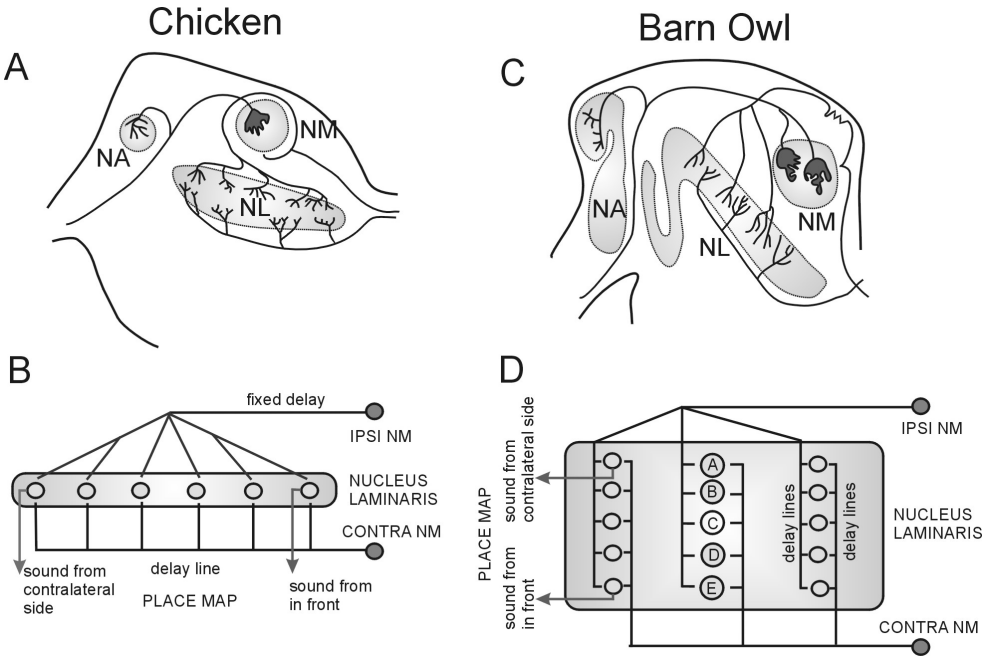

Figure 1. Schematic showing the Jeffress model for sound localization as applied to chicken and barn owl. **A:** Schematic cross section through a chicken brainstem showing the organization of the projections from NM to NL. This organization conforms to a modified Jeffress model (**B**) with delay lines formed by the contralateral NM axons that run ventral to NL. This results in a space map oriented in the mediolateral dimension with cells in more lateral positions responding maximally to sounds originating from far contralateral space, and cells in a more medial position responding maximally to sounds originating from the front. **C:** Schematic cross section through a barn owl brainstem showing the organization of the projections from NM to NL. The organization of the delay lines conforms to the Jeffress model (**D**). NM axons enter NL and traverse it making contact with NL neurons along their way. This results in multiple maps of ITD (**D**) with space mapped in a dorsoventral dimension. Neurons located in the dorsal edge of NL respond maximally to sounds originating from far contralateral space, and neurons located in more ventral position respond maximally to sounds originating from the front (reproduced with permission from Elsevier).

In the barn owl, the organisation of NL is quite different and constitutes an apomorphic morphotype (Kubke & Carr, 2000; Kubke, Massoglia, & Carr, 2002). Instead of being organised in a monolayer, NL cells are sparsely distributed in a 1-mm thick neuropil rich in myelinated fibres (Figure 1C; Carr & Boudreau, 1993). Throughout most of the extent of NL, and unlike the case for chicken, the dendritic trees are not polarised, but instead NL cells are round in appearance and exhibit numerous short dendrites distributed throughout the soma. A more caudolateral region of NL is characterised by cells with morphology similar to that seen in chickens (Carr & Köppl, 2004; Köppl & Carr, 1997). NM axons enter and traverse the entire NL, interdigitating in the dorsoventral axis (Figure 1C; Carr & Konishi, 1988). Unlike the NM-NL circuit of chickens, inputs from both ipsi- and contralateral NM contribute to the delay lines. This appears to be achieved by regulating the axonal conduction velocity through changes in internodal distance of the NM mye-

linated axons, so that they can provide sufficient delay to allow for the entire contralateral space to be mapped within the dorsoventral dimension (Carr, 1995). Thus, a place map emerges in which cells in a more dorsal position respond maximally to sounds originating from far contralateral space, and cells in a more ventral position respond maximally to sounds originating from the front (Figure 1D).

Method

The structure of the nucleus laminaris in 20 different species was determined from direct examination of material from private collections or from data already available in the literature (Table 1). The majority of the material consisted of coronal sections although horizontal and/or sagittal sections were also used to analyse the NL of the chicken, quail, budgerigar, and zebra finch. Most materials were stained with cresyl violet, with the exception of the American krestel, which was stained with a silver method. Material stained by immunocytochemistry using antibodies against calcium binding proteins was obtained from our laboratory (barn owl, chicken, budgerigar, zebra finch, and canary) using standard methods that have already been reported in detail (Kubke, Basu, Gauger, Wagner, & Carr, 1999). The criterion for classification of NL into a given morphotype was based on the presence of a region of NL lacking the monolayer organisation and the presence of bitufted dendrites. Because the material available for different species could not constitute a homogeneous data set suitable for quantification, no measurements were made with respect to the proportion of NL occupied by the each of the two morphotypes. The classification schemes used for phylogenetic analysis was based on Sibley and Ahlquist (1995) and of van Tuinen, Sibley and Hedges (2000). Cell number data were obtained from Winter (1963) and Winter and Schwartzkopf (1961), as previously reported in Kubke et al. (2004). Auditory data was obtained from Fay (1988), Volman (1994), and Dooling, Lohr, and Dent (2000).

Table 1
The Nucleus Laminaris of 18 Species of Birds Belonging to 10 Orders (Sibley & Ahlquist, 1995) were Analysed with Light Microscopy.

Order	Common name	Source
Struthioniformes	Emu	C. E. Carr (p.c.)
	Kiwi	(Craigie, 1930)
Galliformes	Chicken	(Rubel & Parks, 1975)
	Turkey	W. Hodos (p.c.)
	Quail	(Marin & Puelles, 1995)
Piciformes	Downy woodpecker	S. Volman (p.c.)
Psittaciformes	Conure	R. J. Dooling (p.c.)
	Budgerigar	C. E. Carr (p.c.)
	Galah	J. M. Wild (p.c.)
Apodiformes	Hummingbird	S. Brauth (p.c.)
Strigiformes	Great horned owl	C. E. Carr (p.c.)
	Burrowing owl	S. Volman (p.c.)
	Barn owl	C. E. Carr (p.c.) (Carr & Konishi, 1990)
	Oilbird	M. Konishi (p.c.)
	Screech owl	S. Volman (p.c.)
Columbiformes	Pigeon	W. Hodos, J. M. Wild (p.c.)
Gruiformes	Crane	W. Hodos (p.c.)
Ciconiiformes	American krestel	W. Hodos (p.c.)
Passeriformes	Zebra finch	C. E. Carr (p.c.)
	Canary	C. E. Carr (p.c.)

Note. p.c., personal collection.

Results

The NL of birds is typically described as a crescent shaped lamina that lies ventral and lateral to NM. It extends rostrally, where it is found beneath the floor of the fourth ventricle in more rostral positions where NM can no longer be seen (Ariëns Kappers, Huber, & Crosby, 1936). This organisation of NL has been described in the chicken (Rubel & Parks, 1975) and kiwi (Craigie, 1930), and is also characteristic of the organisation of the NL of crocodilians (Carr & Soares, 2002), suggesting that this represents the primitive condition in the bird lineage. We therefore refer to this organisation as the plesiomorphic morphotype. In barn owls, NL cells have lost their bitufted morphology and are not found organised in a monolayer, except during an early developmental phase (Kubke, Massogila, & Carr, 2002b). We therefore refer to this organisation as the apomorphic morphotype. We examined the histology of NL in several species of birds and found many instances in which this laminar arrangement of NL cells was lost, giving rise to a general organisation reminiscent of that found in barn owls (Kubke, Dent, Hodos, Carr, & Dooling, 2002).

NL Morphotypes in Birds

Basal birds, such as emus, exhibited a histological organisation throughout the rostrocaudal extent of NL where cells were arranged in a monolayer in the centre of the nucleus, which was surrounded by a clear neuropil. NL cells were typically bitufted in shape, with their two dendritic tufts projecting in opposite directions occupying the neuropil on both sides of the NL monolayer (Figure 2A). This organisation of NL conforms to the plesiomorphic morphotype. The apomorphic morphotype is best exemplified by the histological appearance of NL in barn owls (Figure 2B). The majority of cells have lost their bitufted morphology, exhibit short dendrites on the entire soma surface, and the monolayer arrangement of cells has been lost, resulting in an expansion of the nucleus in the dorsoventral dimension. Examination of NL histology revealed that this loss of monolayer arrangement was also found in a number of birds where, as in barn owls, cells were found sparsely distributed throughout the neuropil (Figure 2C, arrowhead). This loss of the monolayer arrangement was also accompanied by the loss of the cell's bitufted morphology, with short dendrites distributed instead throughout the soma (Figure 2C, E). In most cases where the apomorphic morphotype could be found, we were able to identify a more caudolateral region of NL which maintained the classic appearance of the plesiomorphic morphotype (Figure 2C, arrow). In these more caudolateral regions, NL cells were typically bitufted with dendrites extending to the abutting neuropil, with the apomorphic morphotype restricted to more rostromedial regions of NL. The extent of NL occupied by each of the morphotypes also appeared to vary between species.

Evolution of NL Morphotypes in Birds

Since the apomorphic morphotype was not confined to owls, we hypothesised that this reorganisation may be linked to phylogenetic history. The classification schemes of Sibley and Ahlquist (1995) and of van Tuinen, Sibley, and Hedges (2000) were followed (Figure 3A), and the prevalence of each morphotype was

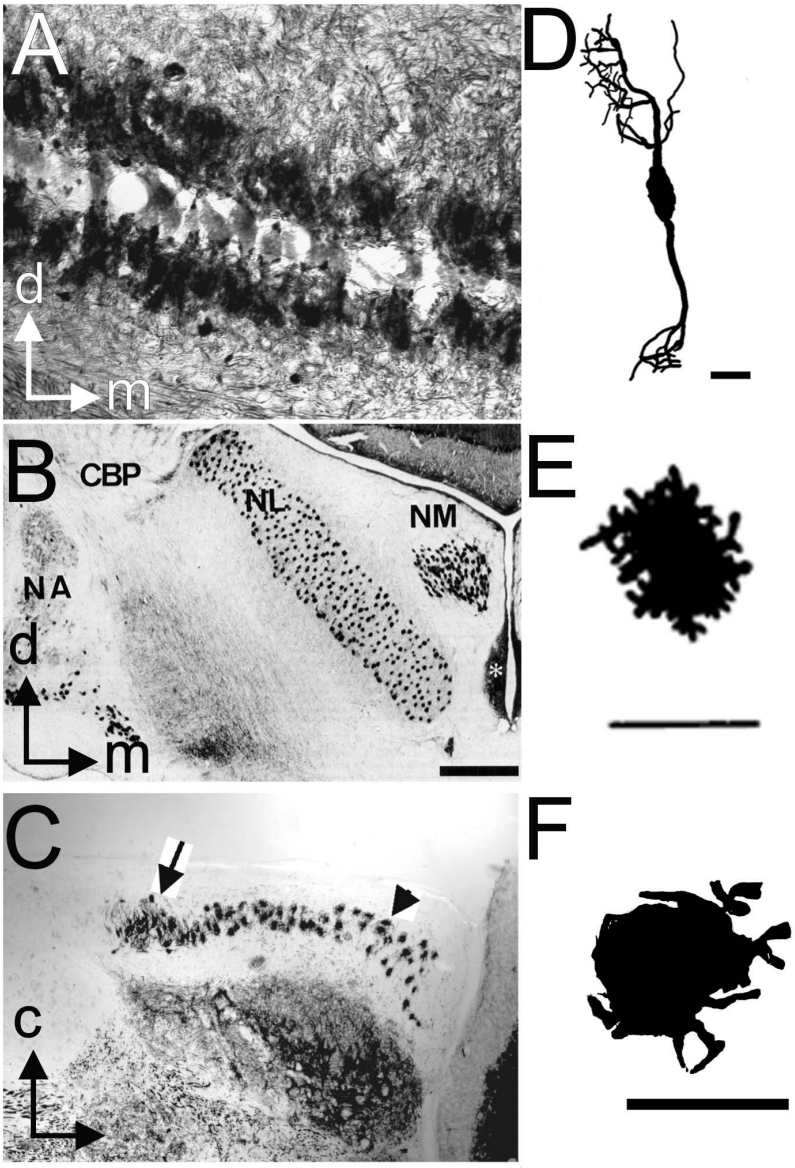

Figure 2. Examples of the different NL morphotypes found in birds. A: Coronal section through an emu NL showing its characteristic apomorphic morphotype. Cells are arranged in a monolayer, and exhibit two dendritic tufts projecting in the dorsoventral dimension. Sections were stained with an anti-calretinin antibody following standard procedures (Kubke, Gauger, Basu, Wagner, & Carr, 1999). B: Coronal section through a barn owl NL showing the apomorphic morphotype. Neurons are no longer found in a monolayer and have lost the polarisation of the dendrites. Sections were stained with an antibody against calcium binding proteins using standard procedures (Takahashi, Carr, Brecha, & Konishi, 1987). C: Horizontal section through the NL of a budgerigar. More medial NL (higher best frequency) exhibits the apomorphic morphotype (arrow head) whereas more lateral NL (lower best frequency) exhibits the typical plesiomorphic morphotype (arrow). Sections were stained with an antibody against calbindin using standard procedures (Kubke, Basu, Gauger, Wagner & Carr, 1999). D, E and F: camera lucida drawings of cells in the three morphotypes. While the emu NL cells exhibit the typical bitufted morphology throughout NL (D), cells in the high frequency region of the owl (E) and the budgerigar (F) do not have any apparent polarisation of dendrites. Scale bar: 20 µm. d: dorsal; m: medial, c: caudal NM: nucleus magnocellularis; NA: nucleus angularis. (B from McLeod, Soares & Carr, 2006, used by permission).

determined in different orders of birds (Figure 3B). Ratites and gallinaceous birds exhibited exclusively the typical plesiomorphic histological organisation of NL. Among the Neognaths, the emergence of the apomorphic morphotype appeared to be exclusive to Neoaves, but was not limited to a particular phylogenetic lineage. Of particular interest was the structure of NL found in the oilbird, a sister species to the owls. Although all owls examined exhibited a typical apomorphic morphotype, the oilbird exhibits a typical plesiomorphic morphotype throughout the rostrocaudal extent of NL (not shown). This suggests that the apomorphic morphotype may not be a basal trait in the order strigiformes. Furthermore, the examination of the phylogenetic relationship between birds exhibiting different morphotypes suggests that the occurrence of one or the other morphotype may not be related to phylogenetic history, since it is equally parsimonious to propose that the apomorphic morphotype emerged independently several times as it is to propose that it was lost independently several times in avian evolution (Figure 3B).

Neuronal Specialisations in Auditory Specialists and Behavioural Correlates

Previous studies have shown that auditory specialists show enlarged auditory nuclei. Barn owls have a large number of cells in their auditory nuclei, and it has always been maintained that they have enlarged auditory nuclei (Winter, 1963; Winter & Schwartzkopf, 1961). This claim was recently verified (Kubke, Massoglia, & Carr, 2004). It was determined that the number of cells in the auditory nuclei of barn owls is much larger than that which one would expect from scaling to brain or brainstem size alone, suggesting that the size of auditory structures may be regulated independently of changes in the size of the brainstem as a whole (mosaic evolution). The hyperplasia of auditory nuclei seen in owls is also seen in oscine passerines (songbirds), another auditory specialist group (albeit showing a different auditory specialisation; Kubke, Massoglia, & Carr, 2004), suggesting that cell number may be an important feature of auditory coding.

Since the histology of NL also shows variation among birds, and since this variation did not appear to be linked to phylogenetic lineage, we hypothesised that it may accompany particular aspects of auditory coding. We therefore examined both the variation in cell number in NL and the presence of the apomorphic morphotype with different aspects of auditory function. In particular, we examined the how these two parameters related to hearing frequency range (Figure 4).

We found no clear relationship between the number of cells in NL and frequency bandwidth, nor with high frequency cut-off (Figure 4B). This suggests that increases in cell number do not necessarily accompany an expansion of hearing sensitivity. For example, a given number of cells is able to subserve a wide range of frequency bandwidths (Figure 4B). The appearance of the two morphotypes, however, was related to hearing frequency range. Although the emergence of the apomorphic morphotype did not appear to be related specifically to the bandwidth of the hearing range (Figure 4C), there was a general tendency for the apomorphic morphotype to be associated with high frequency cutoff (Figure 4D). Thus, the apomorphic morphotype appears only to be present in species that have higher frequency cutoffs, suggesting that it may subserve specific aspects of high frequency coding. Thus high frequency hearing appears to be associated with the appearance of the apomorphic morphotype.

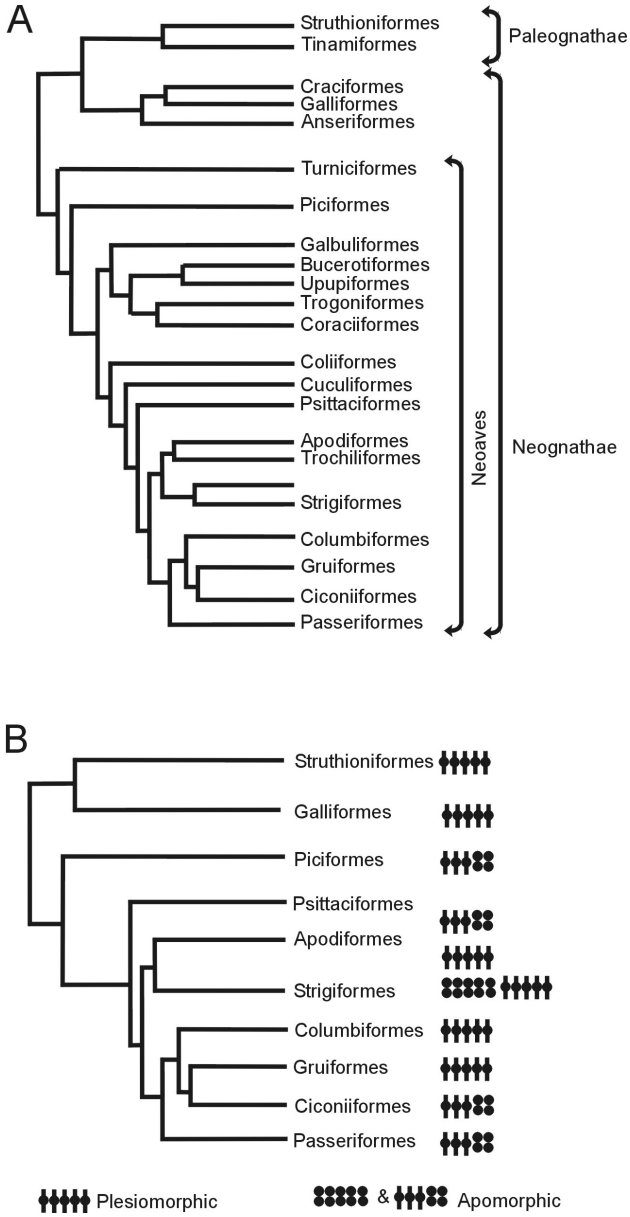

Figure 3. Cladogram showing the distribution of NL morphotypes in different avian orders. The classification scheme of Sibley and Ahlquist (1995) and of van Tuinen, Sibley, and Hedges (2000) were followed (A). B: The plesiomorphic morphotype is typical of basal birds, whereas the apomorphic morphotype may be exclusive to Neoaves. It is equally parsimonious to suggest that the apomorphic morphotype evolved several times independently as it is to suggest that is was lost independently several times.

Discussion

Temporal and intensity features of the auditory stimulus are first extracted in second and third order neurons in the hindbrain. The initial extraction of auditory features that contributes to the map of azimuthal location of sound occurs in NL. This and our previous study (Kubke, Massoglia, & Carr, 2004) suggest that

both total cell number and the appearance of the apomorphic morphotype are features that have been selected for in the auditory system of birds. Enlargement of auditory structures has also been observed in echolocating bats, a mammalian auditory specialist (Hutcheon, Kirsch, & Garland, 2002). Since hypertrophy (or hyperplasia) of neuronal structures has been associated with advantages in neuronal computation (Aboitiz, 1996), we hypothesised that cell number is selected for and that increased cell number is an attribute associated with auditory specialisation. However, the advantage resulting from this enlargement has not yet been unambiguously determined. We can hypothesise, however, that the observed hyperplasia may reflect the overrepresentation of frequencies of biological significance. The barn owl's cochlea shows an overrepresentation of the region that codes the 5-10 kHz frequency range (Köppl, Gleich, & Manley, 1993), and it might be expected that this will be maintained throughout the central auditory pathway. Relative increases in cell number may produce sufficient neurons dedicated to the over-represented frequencies with no detriment to other tonotopic areas. These auditory "foveas" may be a common feature of avian auditory systems.

The morphology of NL varies among birds and the apomorphic morphotype is associated with high frequency hearing. The reorganisation seen in NL may underlie differences in coding ability and/or specialisations associated with high frequency temporal coding, since the plesiomorphic morphotype is inappropriate for temporal computation at high best frequencies (Agmon-Snir, Carr, & Rinzel, 1998). The neurons that detect interaural time differences in low best best frequency region of NL and in those of the mammalian medial superior olive (MSO) share the bitufted organization of their dendrites. Modeling studies suggest that this dendritic organization improves coincidence detection (Agmon-Snir, Carr, & Rinzel, 1998). However, the hypothesized computational advantage achieved by segregating inputs from each ear onto different sets of dendrites breaks down at best frequencies above about 2 kHz (Agmon-Snir, Carr, & Rinzel, 1998; Grau-Serrat, Carr, & Simon, 2003). It is therefore possible that the reorganisation of the dendritic arbours may be associated with the development of the ability to detect interaural time differences above 2 kHz in birds. Similar changes in dendrites with increasing best frequency have not been observed in mammals, which do not detect ITDs much above 2 kHz (Hancock & Delgutte, 2004). Therefore, and unlike mammals, birds appear to have evolved morphological specialisations that allow them to detect ITDs at high frequencies.

Binaural sound localisation is achieved by computing two features of the incoming auditory stimuli: interaural time difference (ITDs) and interaural intensity (level) differences (ILDs). In vertebrates, the separation of the ears creates ITDs that are a function of the location of a sound source in the horizontal plane (azimuth). Generally, these ITDs are used for sound localisation at low frequency ranges. Higher frequency sounds, originating off the midline (with respect to the listener), become "shadowed" by the head, therefore producing ILDs, providing a useful cue for sound localisation in the azimuth. Most vertebrates localise sound in the azimuth by using ITDs at low frequencies whereas ILDs are used by mammals for localisation in the horizontal plane of high frequency sounds.

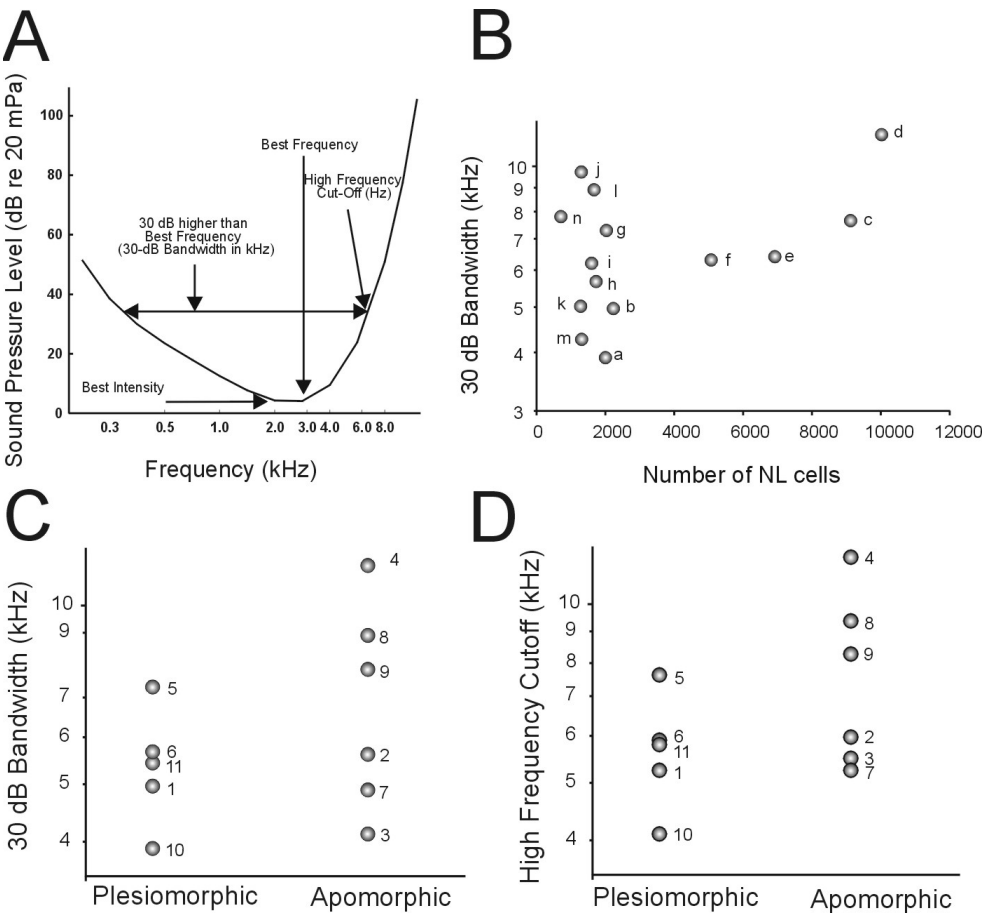

Figure 4. Relationship of cell number and morphotype with hearing range. A: Diagram showing the different auditory parameters used for this analysis. B: Total number of cells does not appear to be associated with frequency range, since a similar number of cells are capable of serving a wide range of frequency bandwidths. C and D: Relationship of morphotypes with hearing frequency range (C) and with high frequency cut-offs (D). High frequency hearing, therefore, may be a condition that has become associated with the apomorphic morphotype. Cell number data was obtained from Winter (1963) and Winter and Schwartzkopf (1961). Auditory data was obtained from Fay (1988), Volman (1994) and Dooling, Lohr, and Dent (2000). Species for B: a: chicken; b: turkey; c: long eared owl; d: barn owl; e: tawny owl; f: eurasian bubo; g: oilbird; h: pigeon; I: starling; j: Eurasian bullfinch; k: great tit; l: island canary; m: house sparrow; n: zebra finch. Species for C and D: 1: turkey; 2: budgerigar; 3: great horned owl; 4 barn owl; 5: oilbird; 6: pigeon; 7: American kestrel; 8: canary; 9 zebra finch; 10: chicken; 11: quail.

There are two models to explain the way in which ITDs are computed. A model based on the response of high best frequency neurons in barn owls suggests that coincidence detection is based on maximal firing to specific ITDs using a peak or rate code mechanism (Konishi, 2003). In small mammals sensitive to low best frequency sounds, however, the slope of the ITD plot in the MSO appears to provide the principal cue for ITD detection (McAlpine, Jiang, & Palmer, 2001). Grothe (2000) suggested that the MSO may exhibit different functional adaptations in mammals with high frequency hearing, such as bats, which do not use ITD information for sound localisation.

One surprising result is the existence of only two NL morphotypes in birds. Because the apomorphic morphotype is not present in all Neognaths, it may have evolved independently or been lost independently several times in evolution, both proposals being equally parsimonious. Embryological data, however, support the second hypothesis. Developmental studies in barn owls have shown that the morphogenesis of NL follows the same sequence described for chickens at early stages, where the characteristics of the circuit are very similar to those of the plesiomorphic pattern. A secondary morphogenetic phase leads to further differentiation of the circuit giving rise to the characteristic apomorphic pattern (Kubke, Massoglia, & Carr, 2002). Thus, the developmental programs responsible for the formation of the plesiomorphic morphotype are retained even in owls, which show one of the more extreme modifications of NL. It is possible that the apomorphic pattern arose early in the evolution of Neognathans, and that the secondary morphogenetic phase was eliminated several times within this lineage. This scenario does not entail the de novo emergence of the secondary morphogenetic phase multiple times, and may therefore be more parsimonious.

Developmental constraints may also be responsible for limits on how this circuit may be modified. Data obtained in barn owls, however, argue against this possibility. The secondary morphogenetic phase includes two major changes: The loss of the monolayer organisation and the loss of dendritic polarisation into dorsal and ventral tufts. It might therefore be expected that some birds undergo only one of these changes. This would result in either a monolayer of cells with nonpolarised dendrites or, alternatively, bitufted cells not organised in a monolayer. In none of the species examined did we find any of these two organisations. We must therefore conclude that both the loss of polarisation and the loss of monolayer structure are selected for in conjunction with each other. Furthermore, we have found no morphological organisation that cannot be accounted for by developmental sequences similar to that seen in barn owls. Thus, the highly consistent appearance of the apomorphic morphotype suggests that it is the combination of these two features (loss of cell polarisation and loss of monolayer organisation with the concomitant rearrangement of NM axons) that provides the NL with the ability to compute temporal parameters at high frequencies.

Differences in the organisation of the NM-NL circuit have been hypothesised to underlie the owl's ability to compute ITDs at high frequencies, since the morphological features of the plesiomorphic morphotype are inappropriate for the same computation at higher frequencies. A number of owls (primarily nocturnal hunters) have independently evolved an asymmetry of their external ears (Norberg, 1977). This asymmetry results in ILDs that are a function of the position of a sound source in the vertical plane, providing a mechanism whereby ILDs can become associated with particular positions of the sound source in elevation (Knudsen & Konishi, 1978). Barn owls have in addition evolved the ability to preserve temporal information of the auditory stimulus at high frequencies. Barn owl auditory nerves can phase lock up to 9 kHz, above what has been described for other species (Carr & Konishi, 1990; Köppl, 1997; Sullivan & Konishi, 1984). These two features have therefore allowed barn owls to evolve a

bicoordinate system for sound localisation in which ITDs are used for localisation in the horizontal plane and ILDs are used for localisation in the vertical plane (Moiseff, 1989; Takahashi & Konishi, 2002; Volman, 1990).

It is unlikely that the apomorphic morphotype has evolved in association with this bicoordinate system of sound localisation. If this were the case, birds with an apomorphic morphotype would also be expected to show the ability to phase lock beyond that which is found in mammals. Owls with asymmetrical ears, which may be able to use a bicoordinate system for sound localisation, are able to phase lock at frequencies higher than what is seen in mammals (barn owl 9 kHz, long eared owl 6 kHz; Volman, 1990). The apomorphic morphotype, however, appears to be common to all owls, including those with symmetrical ears that may not use such a bicoordinate system for sound localisation (Volman, 1994). It is not clear whether the ability to phase lock at high frequencies may have evolved in association with ear asymmetry, since owls with symmetrical ears also do not exhibit high frequency hearing (Volman, 1990, 1994). The only studies performed in owls with symmetrical ears suggest that the range of frequencies used for ITD coding may not be different from what is found in mammals (up to 5.3 kHz in the great horned owl and up to 4.8 kHz in the burrowing owl; Volman, 1990, 1994). The presence of the apomorphic morphotype in songbirds also argues against this possibility, since they do not appear to show phase-locked responses above what is seen in mammals. For example, in pigeons, phase locking occurs to about 3.5 kHz (Hill, Stange, & Mo, 1989), in starlings phase locking occurs to at least 4 kHz (Gleich & Narins, 1988), and in red-winged blackbirds to about 4-5 kHz (Sachs, Woolf, & Sinnott, 1980), which puts them within the mammalian range (4-5 kHz; Köppl, 1997). Thus, different studies suggest that the limits of phase locking in birds other than owls with asymmetrical ears is similar to that of mammals. Since the apomorphic morphotype appears to be a common feature of at least owls and songbirds, its emergence may not be associated with high frequency phase locking, but with the ability to detect ITDs above 2 kHz.

Conclusions

The reorganisation of NL appears to have emerged in several orders of birds associated with high frequency hearing. Studies in barn owls suggest that the new morphotypes result from modifications of developmental programs that occur against a backdrop of conserved features. In barn owls, the initial development of the NL is very similar to that of chickens. A secondary morphogenetic phase transforms the embryonic NL to give rise to the apomorphic morphotype. Studies of species that exhibit both the plesiomorphic and the apomorphic morphotypes will be useful in determining the differences in responses of these two morphotypes to auditory stimuli and elucidate the role that each plays in auditory processing.

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