Bigger Brains or Bigger Nuclei? Regulating the Size of Auditory Structures in Birds

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Key Words

Evolution · Auditory · Neuronal computation · Birds · Allometry

Abstract

Increases in the size of the neuronal structures that mediate specific behaviors are believed to be related to enhanced computational performance. It is not clear, however, what developmental and evolutionary mechanisms mediate these changes, nor whether an increase in the size of a given neuronal population is a general mechanism to achieve enhanced computational ability. We addressed the issue of size by analyzing the variation in the relative number of cells of auditory structures in auditory specialists and generalists. We show that bird species with different auditory specializations exhibit variation in the relative size of their hindbrain auditory nuclei. In the barn owl, an auditory specialist, the hindbrain auditory nuclei involved in the computation of sound location show hyperplasia. This hyperplasia was also found in songbirds, but not in non-auditory specialists. The hyperplasia of auditory nuclei was also not seen in birds with large body weight suggesting that the total number of cells is selected for in auditory specialists. In barn owls, differences observed in the relative size of the auditory nuclei might be attributed to modifications in

neurogenesis and cell death. Thus, hyperplasia of circuits used for auditory computation accompanies auditory specialization in different orders of birds.

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Introduction

Variation in relative brain size has been reported within each vertebrate class. Principally, brain size varies with body size in a way that can be described by the allometric function: brain weight = $a \times \text{body weight}^b$, where a is a normalization constant and b is the scaling exponent [Jerison, 1973; Northcutt, 1985; Harvey and Krebs, 1990; Finlay and Darlington, 1995]. Brain size is determined by two main factors: changes related to differences in body size and increases in size due to encephalization [Jerison, 1985; Aboitiz, 1996; Finlay et al., 2001]. Encephalization can arise through two types of brain evolution, referred to as passive (easy) and active (difficult) modes [Finlay and Darlington, 1995; Aboitiz, 1996; Finlay et al., 2001]. The passive mode refers to global increases in brain size [Finlay and Darlington, 1995; Aboitiz, 1996]. In contrast, the active mode refers to enlargement of specific cell groups not accompanied by an enlargement of the surrounding brain region. Recent studies in mammals suggest that components of functional systems can evolve together

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and independently of size changes in the rest of the brain [mosaic evolution, Glendenning and Masterton, 1998; Barton and Harvey, 2000; Clack et al., 2001]. These changes can thus be best described as belonging to the active mode.

Variations of the passive mode have been extensively reported in the avian literature [Jerison, 1973; Bennett and Harvey, 1985]. Active mode variation in the size of specific structures has also been explored in birds [Cobb, 1964; Bennett and Harvey, 1985] in an attempt to correlate the enlargement of individual brain regions with the expression of specific behavioral repertoires. Bennett and Harvey [1985] failed to see a correlation between specific brain structures and the specific niche occupied by the species. For example, they found no correlation between the size of the optic tectum and diurnal vs. nocturnal habits. By contrast, differences in the size of telencephalic structures have been correlated with specific behaviors [Devoogd et al., 1993; Healy and Krebs, 1996; Reboreda et al., 1996; Brenowitz, 1997; Volman et al., 1997; Clayton, 1998]. These differences can be best attributed to the active mode.

Sound is important to birds, and the role of the auditory brainstem in auditory processing is well understood [Konishi, 1999]. The auditory brainstem is therefore one of the few systems where it is possible to compare the relative sizes of the neuronal structures responsible for a specific neuronal computation within the context of the behavior they mediate. In the avian hindbrain, the cochlear nucleus magnocellularis (NM) encodes and processes temporal information. It projects bilaterally to nucleus laminaris (NL) where interaural time differences are computed [Parks and Rubel, 1975; Takahashi et al., 1984; Carr and Konishi, 1990]. Nucleus laminaris shows considerable histological variation among birds [Ariëns Kappers et al., 1936; Kubke and Carr, 2000; Kubke, unpublished] as well as variability in the number of neurons devoted to computation of sound location [Winter and Schwartzkopf, 1961; Winter, 1963].

We have used the barn owl as a model to show that the auditory NM-NL circuits used for computation of location are larger than would be expected from the passive mode of brain development. There were several reasons to suspect that these auditory nuclei might be larger than expected. First, in the auditory system of barn owls, the number of neurons per octave (calculated as the total number of cells/octaves of hearing range) shows an increase with respect to that of chickens [Winter and Schwartzkopf, 1961; Winter, 1963; Rubel et al., 1976; Parks, 1979; Fay, 1988; Köppl et al., 1993; Solum et al.,

1997]. Second, there is an auditory fovea in the barn owl cochlea, with an overrepresentation of the higher frequency region (5–10 kHz), which covers more than half of the basilar papilla [Köppl et al., 1993]. Third, there is considerable morphological variation in the organization of the NM-NL circuit in different species of birds [Kubke et al., 2000]. These factors, taken together, suggest that these nuclei can respond to selective pressure associated with the emergence of auditory specialization.

In addition to the work on the barn owl, we have also analyzed data from different bird species to examine the variation in size of auditory hindbrain structures in different avian orders. Enlargement of auditory nuclei was mainly restricted to auditory specialists. The hyperplasia of auditory structures in barn owls can be accounted for by changes in cell birth and cell death. This variation in auditory nuclei provides new evidence for mosaic evolution in non-telencephalic structures in birds.

Materials and Methods

Brain size data and brainstem indices were obtained from Portmann [1947]. Cell numbers for the auditory nuclei were obtained from Winter [1963]. Although data obtained from private collections were also examined, they were excluded from the quantification, whenever possible, in order to maintain the homogeneity of the data population. Although this limits the number of species used for our analysis, the homogeneity of the data pool increases the confidence of the interpretation of our results.

Owl embryos were obtained from a breeding colony and maintained in a forced draft incubator at 37°C [Rich and Carr, 1999]. Embryos were staged according to Hamburger and Hamilton [1951], Wagner staging criteria [personal communication] and Meyer and Wagner [1995].

³H-Methyl Thymidine Injections and Autoradiography Analysis

We used timed single injections of ³H-thymidine in a total of 16 barn owl embryos to determine when the cells of nucleus angularis (NA), NM and NL underwent their final mitotic division. Embryos were sacrificed at around E17, when the hindbrain nuclei could be readily identified [Kubke et al., 2002]. Embryos were injected at ages from 4 to 11 days of incubation. Each owl embryo received a single 100-μCi injection of ³H-methyl thymidine (1 mCi/ml) into the yolk sac. Eleven out of the 16 injected embryos developed normally. Embryos were embedded in paraffin, sectioned at 10 μm and mounted on subbed slides. Slides were then dipped in NTB-19 emulsion and stored for 4 weeks at 4°C. Slides were developed with Kodak D-19 for 5 min at 15°C, fixed and counterstained. Analysis of thymidine uptake was done by alternately using a dark field and a bright field condenser. Two independent analyzers using a blind experimental paradigm observed selected sections of each of the injected cases, rating the presence of silver grains as positive (+), negative (–) or indeterminate (?). The onset of neurogenesis was determined by the appearance of unlabeled cells, which underwent their

final division prior to the injection [Hamburger and Hamilton, 1951].

Cell Number

Cell death was estimated by calculating the numbers of neurons in NM and NL from E17, when cells could first be counted in 10- μ m sections [Kubke et al., 2002] until hatching. NM and NL cells could be readily identified by their size and location and by the presence of a clear nucleolus. Barn owl embryos were sacrificed by decapitation and fixed in AFA (70% EtOH/formalin/glacial acetic acid). Fixed brains were embedded in paraffin, sectioned at 10 μ m thickness in the coronal plane and stained with cresyl violet. Estimates of cell number were made from serial paraffin sections at different embryonic ages. Cell counts were done using the assumption-based method [Coggeshall, 1992]. Cells in NM and NL were identified by their position and size. Nuclear profiles containing a nucleolus were counted in every other section with a 40 $\times$ objective. Total cell number was estimated by considering the number of cells in the uncounted section equal to the average of the two adjacent sections, and was not corrected for split nucleoli. Cell counts were performed throughout the nucleus.

Data Analysis

Allometric relationships become linear after log transformation. Analysis was performed using model I linear regressions on log transformed data, based on the data set available in Portmann [1947] which includes data for 141 species in 14 different orders. Brain regions were regressed against the weight of the rest of the brain (total brain weight – weight of the area under consideration, referred to as ‘brain rest’) in order to avoid the whole part fallacy discussed by Deacon [1990]. This consists of false strong correlations that occur when the structure under examination is a significant part of the structure that it is being regressed against, and thus regresses partly against itself. The weight of the brain and the different brain regions was calculated by multiplying Portmann’s intra-cerebral indices by his ‘chiffre basal’. In Portmann’s dataset, indices were calculated by dividing the weight of the brain region to be examined by the mass of the brainstem of a Gallinaceous bird of similar body size, the value of which he defines and provides as ‘chiffre basal’. Thus, multiplying the intracerebral index by the chiffre basal, yields the original weight value for the region in question. Adding the weight of the different regions results in the total brain weight. Hyperplasia of auditory nuclei was examined in 24 species of birds comprising 7 orders (for a complete list of orders and species see legend fig. 4) from data obtained from Winter [1963]. Only those species for which brain weight data was available in Portmann [1947] were used. Hyperplasia was determined by analysis of residuals obtained after regressing the data to the log of brainstem weight, or to relative changes with respect to the mean value in all birds. This is a somewhat arbitrary value because it will be affected by the number of species used in the study. However, because it is impossible to obtain cell number values for all species of living birds, we consider this value as a good approximation because seven orders of birds are represented in our data pool. Although the brainstem index is not available for the oilbird in Portmann’s original data set, we assigned it a value of 1.26, which is the smallest index in Strigiformes. This conservative choice underestimates the differences observed between the oilbird and owls. Phylogenetic relationships were obtained from the Sibley and Ahlquist data based on DNA-DNA hybridization [1995].

Fig. 1. Allometric functions describing the variation of brainstem size with body size (**A**) and with brain weight (**B**) in 141 species of birds. Each data point represents an individual species, and the species used in this study are identified by black circles. Data was obtained from Portmann [1947]. Exponential curves were fitted with Model I linear regression. The exponential values (a), scaling exponent (b) and coefficient of determination (r^2) are as follow: **A:** $a = 0.5487$; $b = -1.6878$; $r^2 = 0.94$; **B:** $a = 0.9000$; $b = -0.7433$; $r^2 = 0.94$. The linear relationship shows a strong dependency of the weight of the brainstem with both the body weight (**A**) and brain weight (**B**). Brain rest weight was obtained by subtracting the weight of the brainstem to that of the total brain to account for the whole-part fallacy discussed by Deacon [1990].

Results

The number of cells in each of the auditory nuclei in birds shows considerable variation [Winter, 1963]. However, increases in cell number per se in a specific neuronal group might simply result from global increases in the surrounding brain structure rather than from changes associ-

Fig. 2. Brainstem indices for different species of owls obtained from Portmann [1947]. **A:** Owl phylogeny was obtained from Sibley and Ahlquist [1995]. Although *Strix aluco* is absent in the original cladogram, other members of the *Strix* family are shown. **B:** Brainstem indices for owls obtained from Portmann [1947]. The brainstem index of a gallinaceous bird is 1, as this is the reference against which indices are obtained. Brainstem indices in owls are large compared to other birds [Bennett and Harvey, 1985], and were largest in owls with nocturnal habits and with asymmetrical ears. *Tyto alba* had the largest brainstem index.

ated with functional specializations [Finlay et al., 2001]. We therefore sought to determine if there were changes in cell number in the hindbrain auditory nuclei that could not be accounted for solely by an increase in the size of the brainstem.

Changes in the Size of Brain Structures

The avian brainstem showed a marked dependency on body weight and brain weight (fig. 1) [Portmann, 1946; Jerison, 1985]. Because the size of the brainstem varies as a result of increases in body size and brain size, the actual weight of the brainstem cannot be compared directly in birds with different body weights. The brainstem weight in birds with different body weights can instead be compared using the intra-cerebral indices of Portmann [1947]. These indices are obtained by dividing the weight of a structure under study by the weight of the brainstem of a gallinaceous bird of similar body size, and thus represent the deviation of a given brainstem size to the smallest found in birds with similar body weight. Portmann's brainstem indices for owls were large compared to those of other birds, with that of *Tyto alba* being the largest (fig. 2B). In addition, the brainstem indices of owls were larger in those with nocturnal habits, asymmetrical ears,

and well developed sound localization (fig. 2) [Portmann, 1947; Volman, 1990, 1994]. Relative brainstem size did not, however, appear to be linked to the phylogenetic history of each species. For example, *Strix aluco* (see *Strix* genus in fig. 2) and *Tyto alba*, two species that do not belong to sister groups, exhibit similar brainstem indices, and share similar nocturnal habits and asymmetric ears (fig. 2).

Hyperplasia of Auditory Nuclei in Auditory Specialists

The brainstem index appeared to reflect the owl's auditory niche. Because barn owls show specializations associated with sound localization and have many neurons in their auditory nuclei, we sought to determine whether the observed increase in cell number could be accounted for by the global increase in relative brainstem size. The same analysis was performed in oscine Passeriformes. This avian order is characterized by different auditory specializations that are associated with their vocal repertoire rather than with sound localization. Auditory generalists including falcons and doves, two visual specialist groups, were used for the purpose of comparison, and were referred to as 'non-specialists' in our analysis (see list of species in fig. 4).

Fig. 3. A: Cell numbers in NA, NM and NL as a function of brainstem weight in 23 species of birds. Note that cell number in these auditory nuclei cannot be predicted by the size of the brainstem alone. The data were fit using a model I linear regression analysis, and the regression line drawn. The exponential values (a), scaling exponent (b) and coefficient of determination (r^2) are as follow: NA: $a = 2,705.21$, $b = 4,713.86$, $r^2 = 0.345$; NM: $a = 4,411.04$, $b = 7,150.36$, $r^2 = 0.336$; NL: $a = 3,135.07$, $b = 4,328.11$, $r^2 = 0.322$. Although there is a general increase in the number of cells with increases in brainstem size, there is significant variation that cannot be accounted for by changes in the brainstem size alone. The largest

deviation to the regression line is found in owls (three largest values). **B:** Residuals for the data presented in the top panels for auditory non-specialists and for Strigiformes and Passeriformes. Residuals are measures of the deviation of each individual point to the plotted regression line, and thus represent the deviation of each individual value from the value expected by the regression analysis. Except for NA in one auditory non-specialist, only *Strigiformes* and *Passeriformes* show residuals above 0. Thus, the data points that lie above the estimated mean function in **A** correspond to auditory specialists.

The increased number of cells in the auditory nuclei of auditory specialists could not be accounted for solely by the global increase in brainstem size (fig. 3). The number of cells in the cochlear nucleus angularis (NA) and NM and in NL could not be predicted by variation in either body weight or brainstem weight alone (fig. 3A), and the determination coefficients (r^2) for these functions were generally low (see legend for fig. 3). For example, birds with brainstem weights that are a log unit apart might have a similar number of neurons in their auditory nuclei (fig. 3A, top panels). There also might be great variation in the number of cells in an auditory structure between birds with comparable brainstem weight. This finding was in contrast to brainstem size, which varied systemati-

cally with either body weight or total brain weight (fig. 1A, B). We analyzed the residuals of these relationships, that is, the deviation of individual data points from the model regression curve, to examine the variation in relative size of auditory hindbrain structures in birds with different auditory specializations (fig. 3B). This analysis showed that the larger relative sizes in auditory structures were common in Strigiformes and also Passeriformes (although to different degrees; fig. 3B), and that non-auditory specialists did not show a relative increase in the size of auditory structures. Thus, the number of neurons in the auditory nuclei was not determined solely by variation in brainstem size, and relative increases in cell number in auditory structures was found almost exclusively in audi-

tory specialists, with *Tyto alba* showing the largest auditory nuclei among birds (*Ta* in fig. 3). This increase in relative cell number was not solely an adaptation to the wider frequency range of the auditory system because in owls the number of neurons per octave is larger than, for example, that of chickens [calculated from Winter and Schwartzkopf, 1961; Winter, 1963; Rubel et al., 1976; Parks, 1979; Fay, 1988; Solum et al., 1997].

We used the brainstem index of Portmann to normalize our data (fig. 4). We chose the index, rather than the actual weight of the brainstem, because it is a direct measure of the deviation of a given brainstem size from the minimum value in the data pool within a given body size range. These brainstem indices are thus a measure of the increases in brainstem size that cannot be accounted for by the increase in body weight itself. We defined hyperplasia of an auditory nucleus as a relative increase with respect to the mean size of the structure in all birds analyzed (plotted as a horizontal line in fig. 4, see comment in Materials and Methods). There was considerable variation in the relative number of cells in the hindbrain auditory nuclei between and within avian orders. Our analysis showed an increase in cell number in owls (Strigiformes) and songbirds (Passeriformes; fig. 4). This demonstrates a hyperplasia of auditory nuclei in auditory specialists.

Fig. 4. Relative cell number in different auditory nuclei (NA, NM and NL) in different orders of birds. The number of cells was normalized to brainstem index and divided by the log of body weight. There is great variation in the relative size of the different auditory nuclei. Large auditory nuclei are only found in Passeriformes and owls, whereas doves and falcons tend to have the smallest. In addition, birds with larger body sizes exhibit relatively smaller auditory nuclei. The horizontal line in each plot represents the mean number of cells in all birds studied. Some species are identified by initials in the plot to show the dependency of hyperplasia with auditory specialization and with body size. The species included in each group are as follows (initials and body weights between brackets when applicable): **Galliformes:** *Melagris gallopavo*, **Anseriformes:** *Anas crecca*, *Anser anser*; **Apodiformes:** *Micropus apus* (*Apus apus*); **Strigiformes:** *Athene noctua* (An, 165 g), *Asio otus* (Ao, 250 g), *Tyto alba* (Ta, 290 g), *Strix aluco* (Sa, 450 g), *Bubo bubo* (Bbb, 2000 g), *Steatornis caripensis* (oil-bird Sc, 450 g); **Columbiformes:** *Columba livia* (Cl); **Ciconiiformes:** *Falco tinnunculus* (Ft), *Buteo buteo* (Bbt); **Passeriformes:** *Motacilla alba* (Ma, 23 g), *Turdus merula*, *Erithacus rubecula*, *Sturnus vulgaris*, *Garrulus glandarius* (Gg, 160 g), *Pica pica* (Pp, 220 g), *Corvus corone* (Cc, 520 g), *Parus major* (Pm, 17.5 g), *Carduelis carduelis*, *Fringilla coelebs* (Fc, 21.6 g), *Passer domesticus*.

Fig. 5. **A** Correlation between the number of cells in NM and number of cells in NA; $r = 0.89$, $p < 0.0001$, $n = 35$. **B** Correlation between the number of cells in NM and the number of cells in NL; $r = 0.964$, $p < 0.0001$, $n = 36$.

The oilbird (*Sc* in fig. 4) and non-nocturnal owls such as *Athene noctua* (*An* in fig. 4) did not appear to follow the trends seen in the nocturnal owls (Strigidae and *Tyto alba*). Oilbirds belong with the owls to the order Strigiformes, but show distinct auditory specializations [Konishi and Knudsen, 1979]. Oilbirds appeared to show a small relative size of both NM and NL, whereas the size of the NA was relatively enlarged. Because the oilbird belongs to a sister group to the owls, the general increases in the relative size of auditory nuclei might not be common to all Strigiformes, and not be linked to phylogenetic lineage.

In both Strigiformes and Passeriformes the relative changes in size of the auditory nuclei were also influenced by body size. In both taxa, hyperplasia of the auditory nuclei was absent in species with large body size. Among owls, *Bubo bubo* (*Bb* in fig. 4), the owl with the largest body weight included in this analysis, did not show hyperplasia of its auditory nuclei (see legend to fig. 4). Among Passeriformes, large-bodied birds such as the crow *Corvus corone*, and the magpie *Pica pica*, also did not show hyperplasia of their auditory nuclei, whereas the largest hyperplasia was found in birds of small body weight (the white wagtail *Motacilla alba* and the chaffinch *Fringilla coelebs*; see legend to fig. 4).

In order to analyze whether the sizes of auditory structures were regulated as a whole, we analyzed the correlation between the numbers of cells in the different auditory nuclei (fig. 5). On average, there was a high degree of correlation between the number of cells in the two cochlear nuclei, NM and NA (fig. 5A) and between the number of cells in NM and NL (fig. 5B). This is in contrast to the relatively low dependency of the size of the auditory structures to brainstem size (fig. 3A). However, variation in the relative enlargement of each auditory nucleus can be seen in individual species (for example in the oilbird), and this variation is somewhat masked by the effects of averaging across species. This suggests that the increase in size of auditory nuclei is regulated by mechanisms that differ from those which regulate the increase of the brainstem as a whole.

Mechanisms That Underlie Hyperplasia: Cell Birth and Cell Death

In order to understand the mechanisms underlying hyperplasia, we examined neurogenesis and cell death in barn owls and compared it to that of chickens. These two species show marked differences in the relative size of their auditory nuclei, and have been models for the study of auditory function [Kubke and Carr, 2000; Rubel and Fritzsch, 2002]. Cell death and cell birth have been previously described in the chicken [Rubel et al., 1976; Parks, 1979; Solum et al., 1997; Wadhwa et al., 1997]. We used timed single injections of ^3H -thymidine in barn owl embryos to determine when the progenitor cells of NA, NM and NL underwent their final mitotic division (fig. 6). The onset of neurogenesis was determined by the appearance of unlabeled cells, which underwent their final division prior to the injection [Hamburger and Hamilton, 1951].

Neurons of the hindbrain auditory nuclei NA, NM and NL were born during distinct but partially overlapping

	E4	E5	E6	E7	E8	E9	E10	E11
NA	+++	+++	+++	+++	+++	+++	++	+
NM	+++	++	++	+				
NL	+++	+++	+++	+++	+++	++	+	

Fig. 6. Cell birth in the auditory nuclei of the barn owl. **A–D:** Micrographs showing coronal sections from ^3H -thymidine injections in barn owl embryos. **A:** E6; **B:** E8; **C, D:** E9+. Bar = 100 μm , all figures at same magnification. Embryos received a single injection of ^3H -thymidine. The beginning of the cell birth period was established as the first appearance of unlabeled cells, and the end of the cell birth period was defined by the last appearance of labeled cells. **A** After injections at E6, all three auditory nuclei were labeled with ^3H -thymidine. Note that at E6, only some NM neurons were labeled. **B** After injections at E8, NM cells were clearly not labeled when there was strong label in NL, showing that NM progenitors underwent their final mitotic division before NL. **C, D** Cell birth followed a medial to

lateral gradient that persists along the rostro-caudal axis, and does not appear to be associated with the tonotopic axis. Rhombomere boundaries in chicken embryos of similar stages of development are oblique to the embryonic AP axis. Thus, in coronal sections such as **C** and **D**, cells originating from more caudal rhombomeres would be positioned in a more medial location throughout the AP axis. Table: Time line summarizing the results obtained from the thymidine injections NA: nucleus angularis; NM: nucleus magnocellularis; NL: nucleus laminaris. The number of crosses signify the density of label: +++ all cells labeled; ++ appearance of unlabeled cells; + few cells labeled.

time periods (fig. 6, table). Embryos injected after E12 contained no labeled cells in the auditory hindbrain, whereas an E4 embryo injection produced labeled cells throughout the auditory hindbrain. Neurogenesis in NA was delayed with respect to NM and NL (note well-labeled NA in fig. 6A, B, D). Neurogenesis in the auditory

hindbrain nuclei was complete by E12 and late injections showed heavy label in the auditory midbrain. NM cells were born first between E4–7, in a medio-lateral gradient. NL cells were born between E8 and E10, also in a medio-lateral gradient (fig. 6C, D). This medio-lateral gradient is clear in figure 6D, where medial cells in rostral NL were

Fig. 7. Cell death in nucleus magnocellularis (NM) and nucleus laminaris (NL) in the barn owl embryo. Cell death was evident in both NM and NL in barn owls, with the number of cells reduced by 32% in NM and 39% in NL.

unlabeled after an E9 ^3H -thymidine injection, and had thus undergone their final mitotic division before that time. More lateral neurons had not been born by E9. This same gradient was visible in a more caudal section through NL (fig. 6C, arrow marks unlabeled medial region), where only the most medial NL neurons were unlabeled. Tonotopy in NL is mapped along a rostral-medial (higher frequency) to caudal-lateral (lower frequency) axis. It does not appear that cell birth progresses along this tonotopic axis. Instead, the persistence of the medio-lateral differences in the appearance of unlabeled cells along the rostral-caudal axis suggests that the rhombomeric position of the progenitor cells in the neuroepithelium was the primary determinant of the date of birth, with cells in more caudal rhombomeres being born first (see legend to fig. 6). A similar rhombomere-dependent cell birth has been described for chickens [Puelles and Martinez de la Torre, 1987; Cambrero and Puelles, 2000]. This is consistent with the pattern of ^3H -thymidine labeling in NA. Here, frequency is mapped in a dorsoventral axis. NA did not show any gradients of cell birth along the dorsoventral axis, whereas these gradients were evident in other devel-

opmental processes that do progress along the frequency axis [Kubke et al., 1999].

We examined the period of naturally occurring cell death in NM and NL to determine to what extent this process contributed to the regulation of the final cell number in the auditory nuclei. At the earliest stage studied (E16), NM contained about 22,000 neurons. Cell death was determined to begin at around E22 and progressed until E27 to show a reduction to about 15,000 neurons by hatching (fig. 7), similar to that seen in the adult [15,760; Winter and Schwartzkopf, 1961]. At E17 and E19, NL contained 21,000 neurons. Cell death in NL began around E21 with a reduction of 39% by the time of hatching from the initial neuronal population (from 21,000 to 13,000 neurons). Thus, the final number of cells in the auditory nuclei of barn owls is in part modified by developmental cell death.

Discussion

The large relative size of auditory structures in owls and songbirds shows that mechanisms other than those that underlie the general increases in brain size can produce a hyperplastic auditory system. It is possible that the enlargement of the auditory structures seen in the hind-brain might be seen throughout the auditory system, similar to the mosaic evolution of functional units described in mammals [Barton and Harvey, 2000; Clack et al., 2001]. Several authors have provided evidence for mosaic evolution. In primates and insectivores, size changes can occur in functional systems independent of size changes in other structures [Barton and Harvey, 2000]. In birds, size changes in the hippocampus have been reported in relation to spatial behavior [Healy and Krebs, 1996; Rebores et al., 1996; Volman et al., 1997; Clayton, 1998] and in song nuclei in relation to vocal repertoire [Devoogd et al., 1993; Brenowitz, 1997].

Relative enlargement of auditory structures could be selected for in auditory specialists. Hyperplasia of auditory structures was found in Strigiformes and Passeriformes, but not in non-auditory specialists. In addition, our analysis suggests that the size of the different nuclei of the auditory brainstem might be regulated differentially. The albeit small differences in the relative enlargement of NA, NM and NL (fig. 5), for example, argues in favor of a differential regulation of the size of the different components within the auditory system. Although this might not be a general trend in birds, it is evident in some individual species and exemplified by the relative size of the three

auditory structures in the oilbird. Oilbirds produce echolocating clicks [Konishi and Knudsen, 1979], and can thus be expected to show different auditory specializations than those seen in owls. Unlike most other birds, the oilbird shows a relative enlargement of NA, and a reduction in the relative size of NM and NL. It is interesting to note that in barn owls, NA has been shown to process the intensity parameters of the auditory stimulus, whereas NM and NL have been shown to process temporal information [Takahashi et al., 1984; Köppl and Carr, 2003]. It is possible that a similar functional separation between these parallel pathways could also be present in other species. This segregation of information processing might allow for the differential selective pressure on the size of one information channel over another. Therefore variation in the relative size of the neuronal assemblies can occur in the absence of a parallel variation in relative size of the entire functional unit.

The variation seen in the relative number of neurons in the auditory nuclei of auditory specialists also appeared to be dependent on body size in addition to niche. The hyperplasia of auditory nuclei was not generally found in song bird auditory specialists of relatively larger body size. However, in these larger birds the total number of cells in the auditory nuclei was comparable to that of smaller bodied birds. For example, *Motacilla alba* (white wagtail, *Ma* in fig. 4) has 1,360 cells in NL, whereas *Garrulus glandarius* (eurasian jay, *Gg* in fig. 4) has 1,820 cells in NL. If auditory specialization requires a minimum number of neurons devoted to auditory computation, larger birds might achieve sufficiently large auditory nuclei simply as a result of scaling of the brain to body size. Smaller sized birds have limits on brain weight. A relative increase in cell number (hyperplasia) would provide an equally enlarged auditory nucleus. The hypothesis that the hyperplasia of auditory nuclei is driven by auditory specialization, and by the constraints of brain weight, is strengthened by the observation that the highly visual falcons and doves had the smallest relative auditory nuclei.

Mechanisms That Underlie Hyperplasia: Cell Birth and Cell Death

An increase in the number of neurons might be achieved by increasing the rate at which neuronal precursors are produced, increasing the length of time over which they are produced, decreasing the extent of neuronal death or a combination thereof [Dehay et al., 1993; Kornack and Racik, 1998]. For example, the increase in neurogenesis associated with altricial development in birds could contribute to the relative enlargement of the

brain [Starck and Ricklefs, 1998]. In mammals, protracted neurogenesis underlies the increase in the neuronal precursor population and an overall increase in the number of neurons [Finlay and Darlington, 1995]. Although relative increases in neurogenesis must underlie relative increases in the size of the auditory nuclei, cell death could in turn mask such increases within a nucleus by a secondary neuronal loss.

In the barn owl, the cells of the auditory hindbrain are born later and over a longer period of time than their chicken counterparts [Rubel et al., 1976]. Because barn owls have considerably larger brainstems than chickens, these differences in the length of neurogenesis might be related to the increase in size of the brainstem per se and not underlie the relative increase in size of the auditory nuclei. We have demonstrated that the barn owl NL is hyperplastic, thus increased neurogenesis must at least contribute to the enlargement of NL. Mechanisms such as the rate of cell division might also contribute to an increase in the progenitor pool. Increases in cell number from an increase in rate of cell division does not require a lengthening of the cell birth period, and could be differentially regulated in the precursors of each auditory nucleus [Dehay et al., 1993; Kornack and Racik, 1998].

Cell death in the auditory nuclei also affects final cell number. Cell death was evident in both NM and NL in barn owls, with the number of cells reduced by 32% in NM and 39% in NL. This is in contrast to what has been reported in chickens, where NL shows a 19% reduction in the final number of cells, whereas NM shows no significant cell death [Rubel et al., 1976; Parks, 1979; Solum et al., 1997]. A more recent report using stereological counting methods to examine cell death in the chicken has found 43% cell death in NM and 52% cell death in NL [Wadhwa et al., 1997]. The discrepancies in the extent of cell death with previously reported data may reside in differences in the strains of chickens, or in the methods used.

Cell death could regulate the degree of hyperplasia of the auditory nuclei in the adult, and lead to the relative size differences observed in specific auditory components. Factors such as afferent input and auditory experience could regulate the differences in the extent of cell death in NM seen between barn owls and chickens. For example, the number of apoptotic profiles in the developing auditory nuclei of chickens is reduced when the embryos are exposed to species-specific vocalizations [Wadhwa et al., 1999]. Otocyst removal in the embryonic chicken results in a reduction of NM cells by cell death [Parks, 1979]. The effect of target-derived trophic factors

in the development of the auditory nuclei has received less attention. It has been known for a long time that soluble trophic factors can reduce the extent of naturally occurring cell death [Levi-Montalcini and Hamburger, 1953]. It was later established that these trophic factors, such as NGF and CNTF, could be provided by the target tissue during development, regulating the number of surviving neurons [for a review on this topic see Bennett et al., 2002]. It has been also established that the extent of neuronal cell death can be proportionally modified by changes in the size of the target population or, alternatively, by changes in the number of synapses that are formed with their target, which in turn are dependent on the level of electrical activity [Dahm and Landmesser, 1991; Tang and Landmesser, 1993; Kubke, 1994; Bennett et al., 2002]. These factors, however, might not play a crucial role in the extent of cell death in the barn owl because the enlarged NL in barn owls should be able to support a large population of NM cells. Thus, both neurogenetic factors and trophic interactions with the periphery could contribute to the final relative size of auditory structures in birds. These regulatory mechanisms might act in concert to create auditory structures whose size conforms to the behavioral repertoire of each species.

Conclusions

Variation in the size of brains and brain structures has been the focus of many studies. The mechanisms by which these modifications in size are achieved are not yet fully understood. It has become clear that size variation can occur at both a global level as well as within functional units [Cobb, 1964; Bennett and Harvey, 1985; Finlay and Darlington, 1995; Barton and Harvey, 2000]. It is also clear that variation in neuronal cell number might contribute to specific behavioral repertoires. We have demonstrated such local variation within the auditory system

of birds. Because of the limited number of species in our studies, we have maintained the homogeneity of the data pool to best interpret our analysis. Although we recognize that a more thorough analysis at the family level would further contribute to our hypothesis, the data presented in this paper clearly shows variation in the number of neurons in auditory structures that cannot easily be attributed to phylogenetic lineage. Instead, our analysis and data suggest that total cell number in auditory structures can be selected for in auditory specialists. In fact, this variation is more evident when individual species are taken into account rather than the averages across taxonomic bird groups. The increase in the number of cells can be acquired by passive increases linked to changes in body size, as well as by active mechanisms that lead to hyperplasia. These increases would then be selected for if they contribute adaptively to enhanced computational performance.

On average, when all species are considered, cell number increases with increases in brainstem size. Individual variation is evident, however, showing that changes in relative cell number can occur. We have also presented evidence for the roles of cell birth and cell death in shaping the final relative size of different components within these functional units. Some of the observed variation appears to be linked to specific behavioral abilities, such as the well-developed sound localization ability of owls, or the requirements of the song system of Passeriformes [Carr et al., 1996; Kubke and Carr, 2000].

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