Introduction

The barn owl is a well-established species in auditory research, showing many adaptations for its nocturnal hunting habits using passive acoustic prey localization. Previous work concentrated mainly on the behavioral demonstration of the owl’s unusual localization accuracy, the underlying brain stem and midbrain circuits, and their development (reviews in Carr 1993; Knudsen and Brainard 1995; Konishi 1993). In a “top-down” approach, a map of auditory space was first demonstrated in the midbrain and its step-by-step synthesis was followed in reverse down to the level of the cochlear nucleus. Two basic brain stem pathways are now known, evaluating interaural time and intensity information, respectively, and combining at the midbrain level to synthesize the auditory space map.

Relatively little attention has been paid so far to the auditory sensory organ, the basilar papilla, of the barn owl, especially its physiology. This is an important gap in knowledge, because what is transduced in the basilar papilla and then relayed to the brain via the auditory nerve forms the basis of all further computations. Without any knowledge about the input, it is impossible to judge, e.g., the contributions of the cochlear nuclei in auditory processing. To date, only one small set of data, describing vector strengths of phase locking in barn owl auditory nerve fibers, has been published (Sullivan and Konishi 1984).

In addition, anatomic studies of the barn owl’s basilar papilla revealed a number of interesting specializations that suggest this species could be a valuable model for investigating cochlear mechanisms in birds. With an average length close to 11 mm, the barn owl has the longest basilar papilla known in birds (Fischer 1994b; Fischer et al. 1988; Köppl et al. 1993; Schwartzkopff and Winter 1960; Smith et al. 1985). Approximately the apical third to apical half can be regarded as the equivalent of a normal bird papilla, showing all the typical morphological features and gradients (Fischer 1994b; Fischer et al. 1988; Smith et al. 1985; review in Fischer 1994a). The basal portion, however, is clearly specialized. There is an unusual basilar membrane thickening, an unusual subtype of short hair cell (Smith et al. 1985), an unusual concentration of afferent fibers on the most neural tall hair cells (Fischer 1994b), and a remarkable constancy of parameters, such as stereovillar height, that normally show a gradual change along the epithelium (Fischer 1994b; Fischer et al. 1988). Corresponding to these morphological specializations, the basal half of the barn owl’s basilar papilla has been shown to contain an extremely expanded representation of frequencies between ~5 and 10 kHz, termed an auditory fovea (Köppl et al. 1993). All lower frequencies are mapped in the apical half and, up to ~2 kHz, are represented with space constants comparable with those of other birds. Above 2 kHz, the length of papilla representing 1 octave rapidly grows, culminating in the fovea (Köppl et al. 1993).

The present study intends to provide the basis of a detailed investigation of auditory nerve physiology in the barn owl. It describes a reliable approach to the auditory nerve at the brain stem level and the criteria developed for identification. Emphasis was placed on obtaining recordings across the whole frequency range, to be able to compare the data with those obtained in other birds whose hearing range does not

Köppl, Christine. Frequency tuning and spontaneous activity in the auditory nerve and cochlear nucleus magnocellularis of the barn owl Tyto alba. J. Neurophysiol. 77: 364–377, 1997. Single-unit recordings were obtained from the brain stem of the barn owl at the level of entrance of the auditory nerve. Auditory nerve and nucleus magnocellularis units were distinguished by physiological criteria, with the use of the response latency to clicks, the spontaneous discharge rate, and the pattern of characteristic frequencies encountered along an electrode track. The response latency to click stimulation decreased in a logarithmic fashion with increasing characteristic frequency for both auditory nerve and nucleus magnocellularis units. The average difference between these populations was 0.4–0.55 ms. The most sensitive thresholds were ~0 dB SPL and varied little between 0.5 and 9 kHz. Frequency-threshold curves showed the simple V shape that is typical for birds, with no indication of a low-frequency tail. Frequency selectivity increased in a gradual, power-law fashion with increasing characteristic frequency. There was no reflection of the unusual and greatly expanded mapping of higher frequencies on the basilar papilla of the owl. This observation is contrary to the equal-distance hypothesis that relates frequency selectivity to the spatial representation in the cochlea. On the basis of spontaneous rates and/or sensitivity there was no evidence for distinct subpopulations of auditory nerve fibers, such as the well-known type I afferent response classes in mammals. On the whole, barn owl auditory nerve physiology conformed entirely to the typical patterns seen in other bird species. The only exception was a remarkably small spread of thresholds at any one frequency, this being only 10–15 dB in individual owls. Average spontaneous rate was 72.2 spikes/s in the auditory nerve and 219.4 spikes/s for nucleus magnocellularis. This large difference, together with the known properties of endbulb-of-Held synapses, suggests a convergence of ~2–4 auditory nerve fibers onto one nucleus magnocellularis neuron. Some auditory nerve fibers as well as nucleus magnocellularis units showed a quasi-periodic spontaneous discharge with preferred intervals in the time-interval histogram. This phenomenon was observed at frequencies as high as 4.7 kHz.
extend as high as the owl’s, and to be able to recognize any possible correlates of the morphological specializations in the owl’s high-frequency range. Frequency tuning of auditory nerve fibers was of special interest, because studies in bats suggested that expanded, foveal representations of certain narrow frequency ranges in the cochlea may be accompanied by a dramatic increase in the sharpness of neural frequency tuning (Kössl and Vater 1990; Suga et al. 1976). The same expectation has been raised by models attributing frequency selectivity of psychophysical masking patterns or single-unit tuning curves to the spatial representation of frequencies along the cochlea (review in Greenwood 1991). Some data are also included on cochlear nucleus magnocellularis (NM) units, which were regularly encountered in the chosen recording area. Neither frequency tuning nor spontaneous activity in the NM of the owl have been investigated in any detail so far (Sullivan and Konishi 1984).

**Methods**

Experiments were performed on seven adult barn owls (*Tyto alba guttata*, 4 females and 3 males) from the department’s own breeding colony. They were 1–3 yr old and weighed between 310 and 390 g. The care and use of these animals was approved by the government of upper Bavaria (license no. 211-2531-64/92). General anesthesia was induced by intramuscular injections of 3 mg/kg xylazine (rompun) and 4 mg/kg ketamine hydrochloride (Ketavet). The heart beat and the (unaided) breathing cycle were monitored via a combined electrocardiogram and muscle potential spikes. After a unit was isolated, the response to ipsilateral condensation clicks (10 or 20 per s, 300–500 repetitions, peak pressure 80–100 dB SPL) was usually recorded first. This was followed by the presentation of a frequency-intensity raster of tone bursts at ±10 frequencies around the cell’s characteristic frequency (CF); the sound pressure level was increased at each frequency in steps of ±5 dB between 100 Hz and 10 kHz. Peak sound pressure levels for pure tones were set at 75–95 dB SPL in different experiments.

**Recordings of cell activity**

Glass microelectrodes filled with 3 M KCl or 2 M NaCl and with impedances mostly between 50 and 100 MΩ were positioned near the tips of the horns, ~10 mm from the eardrums. Sound stimuli were generated alternatively by computer-controllable frequency synthesizers (Wavelet Rockland 5100 or TDT WG2), a white noise source (Bruel & Kjær noise generator 1405) or a 0.1-ms square-wave trigger signal (for click stimuli). All stimuli were passed through an equalizer (Technics SH 8075) and, except for the click stimuli, were gated by a TDT SW2 cosine switch. They could be attenuated both manually and under computer control (TDT PA4 and custom-built attenuators or HP 4436A). The frequency response of the sound system showed sound pressure variations of ±5 dB between 100 Hz and 10 kHz. Peak sound pressure levels for pure tones were set at 75–95 dB SPL in different experiments.

**Data analysis**

Spontaneous discharge rates were averaged over sequences of 1,000–46,000 (mostly 10,000–20,000) spikes recorded in quiet. Time-interval histograms from these recordings were subjected to a fast Fourier transform analysis to check for quasiperiodic activity. If both the fast Fourier transform amplitude spectrum and the interval histogram showed subjectively clear peaks, a unit was classified as showing preferred intervals (Manley 1979).

In addition to measurements in prolonged quiet, which could only be obtained for about half of all units, an estimate of spontaneous rate was derived from the units’ discharges in the silent intervals between stimuli in the raster measurements used for obtaining tuning curves. To minimize possible depressive effects of the stimulation on spontaneous rate, only the time window 90–140 ms...
C. KÖPPL

RESULTS

Classification of recorded units

Electrodes were aimed at the area where the auditory nerve enters the brain stem and forms a thick sheet on its dorsal surface, posterior to the cochlear nucleus angularis (Fig. 2). Although blood vessels provided approximate landmarks, visual identification of the nerve itself was not possible. Histological verification of selected recording sites in three animals by horseradish peroxidase marking confirmed that each of those tracks had indeed penetrated the auditory nerve layer, as intended and as recognized by physiological criteria (Fig. 2). However, output fibers from the NM mix with auditory nerve fibers in the chosen recording area (Köppl and Carr 1997), and the NM lies under part of the auditory nerve (Fig. 2). Fibers leaving the cochlear nucleus angularis run further anteriorly within the brain stem.

Physiological criteria were therefore used to distinguish different types of recorded units. Unit classification was at first performed separately for the data of each animal, by comparing the sequence of CF, click latencies, and estimated spontaneous rate (see METHODS) for every electrode track. Positive direct current was passed through the electrode for 15–20 min. Eight to 12 h later, the owl was killed by a ketamine overdose (~100 mg/kg) and fixed by transcardial perfusion with 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The procedures for subsequent cryostat sectioning of the brain and histological processing to visualize the horseradish peroxidase label with the use of a cobalt-intensified diaminobenzidine reaction have been described in detail by Köppl et al. (1993).

Histology

In three animals, a successful single-unit recording track was immediately repeated with the use of a broken glass electrode (external tip diameter 14–17 µm) filled with 30% horseradish peroxidase in 0.15 M KCl and 0.05 M tris(hydroxymethyl)aminomethane buffer, pH 7.6. Sufficient multiunit activity could be recorded with these electrodes to confirm that the sequence of CFs did reproduce the previous track. At a selected depth, 0.2–0.5 µA

FIG. 1. Peristimulus time histogram of the typical click response of an auditory nerve fiber [characteristic frequency (CF) 7.8 kHz, binwidth 0.05 ms]. Vertical dashed lines: time frame used as a reference window for determining the maximal number of spikes per bin occurring spontaneously (horizontal — — —). The 1st bin after the onset of the click exceeding this spontaneous level, and followed by a bin also meeting this criterion, was defined as the latency, in this case at 0.9 ms (pointed out by the thick arrow).

FIG. 2. Histological verification of the area of recordings. Right: schematic drawing of the right half of a brain stem cross section, including an outline of nucleus magnocellularis (NM). At this level of sectioning, the auditory nerve (nVIII) is seen joining the brain stem. Auditory nerve axons make up most of the tissue that lies dorsal of NM; however, output fibers from NM may also be found anywhere in that area. Dashed square: area covered by each of the photomicrographs at left. Photomicrographs: examples of horseradish peroxidase injections at the top and bottom of an electrode penetration in 2 different brains, respectively. Positions of dense label are also indicated as gray areas in the schematic drawing.
A combination of relatively short click latency and relatively low estimated spontaneous rate identified auditory nerve units. Conversely, relatively long click latencies and relatively high estimated spontaneous rates were interpreted as belonging to NM units. Often, a small jump in CF also accompanied a change from one type of unit to another. Very rarely, units with similar thresholds to both ipsilateral and contralateral stimulation and unusually long click latency were encountered; these were classified as nucleus laminaris units. Examples of two electrode tracks and the classification of units encountered are shown in Fig. 3, A–C and D–F, respectively. The chosen combination of criteria allowed a confident classification; some arbitrariness remained only in rare cases when criteria were in conflict or data for some criterion were missing. A discriminant analysis of the data pooled across animals was subsequently performed with the use of click latency, the log of estimated spontaneous rate, and the log of CF. The statistical discrimination agreed with the subjective classification in 99% of the cases and showed virtually no overlap of the discriminant scores of the two groups, i.e., very good group separation. Figure 4 displays the groups graphically, showing the data as a function of all three parameters used for classification.

It is important to note that neither recording depth (relative to the surface of the brain stem) nor spike waveform correlated with the physiological classification. Both auditory nerve fibers and NM units were encountered anywhere between close to the surface and ~2.5 mm deep into the brain stem (Fig. 5). Even the very few nucleus laminaris units did not cluster at a particular depth. Similarly, spike waveform did not correlate with unit type. Figure 6 shows some examples of the averaged spike forms of units classified as auditory nerve or NM units, respectively. The mean width of the averaged spike waveform, measured at the half-maximal point of its positive excursion, was 0.17 ms for auditory nerve fibers (n = 35) and 0.21 ms for NM units (n = 9); these two values were not significantly different (Mann-Whitney U test). Also, the ratio of the positive and negative excursion did not differ between spikes of the two groups, with a mean value of 2.7 in both cases. Together, the fast time course and nearly monophasic shape of spikes thus suggest that most of the recordings, whether auditory nerve or NM, were from fibers.

**CFs**

The responses from 236 auditory nerve fibers were recorded. Their CFs ranged from 0.53 to 9.0 kHz. The majority (137) had CFs between 4 and 8 kHz and only a handful of fibers were encountered with CFs <1 kHz.

NM units (n = 66) had CFs between 0.54 and 7.2 kHz. Because the primary target was the auditory nerve, no attempt was made to systematically sample NM units. Their CF distribution cannot therefore be regarded as representative.
Click latency

Click latency increased systematically toward lower CFs in all types of units (Fig. 7). The scatter and overlap in the latency values of the auditory nerve and the NM may be partly due to variation in the peak sound pressure of the click (80–100 dB SPL) in different experiments. There was little overlap in data from individual owls.

Both populations were fit reasonably well by logarithmic regressions, which showed that average latency decreased from 1.7 to ~1 ms in the auditory nerve, and from 2.25 to 1.4 ms in the NM (Fig. 7). The average difference between the latencies in the auditory nerve and the NM was thus 0.4–0.55 ms. The few nucleus laminaris units for which latencies were determined indicated a similar or even greater difference between the NM and the nucleus laminaris (Fig. 7).

Thresholds at CF

The most sensitive thresholds of auditory nerve fibers were near 0 dB SPL at CFs between 2.5 and 5.5 kHz (Fig. 8A). Toward both lower and higher frequencies, thresholds
The tuning curves were narrowly tuned even at sound pressure levels much above CF threshold and usually showed no indication of a tail-like opening of either the low- or high-frequency flank (Fig. 10A). This was reflected in generally high $Q_{10dB}$ values, which also increased according to a power law with CF, from 0.8 at 0.5 kHz to 3.4 at 9 kHz (Fig. 11A).

The slopes of the low- and high-frequency flanks of auditory nerve tuning curves had similar values up to a CF of ~6 kHz, i.e., tuning curves were on average symmetrical (Fig. 12B). Both flanks showed an increase in steepness from ~80 dB/octave at low CFs to 120 ± 150 dB/octave at 3 kHz (Fig. 12A). The low-frequency slopes then scattered around this stable value at all higher CFs, whereas the high-frequency flanks showed a dramatic steepening above ~6 kHz, resulting in increasingly asymmetric tuning curves (Fig. 12).

Some examples of tuning curves of NM units are shown in Fig. 10B. Their characteristics were very similar to those of the auditory nerve in the same CF range.

Spontaneous activity

All neurons encountered were spontaneously active. Average spontaneous rate in prolonged quiet was 72.2 spikes/s for auditory nerve fibers ($n = 127$). The range observed was 16.1–145.7 spikes/s, i.e., there were no fibers with extremely low spontaneous rates. The rate distribution was positively skewed (Fig. 13A). Spontaneous activity was correlated with CF, such that fibers with lower CF had higher rates (Fig. 13B).

Time-interval histograms of auditory nerve fibers’ spontaneous activity typically showed the well-known quasi-Poisson distribution with short dead times of, on average, 0.72 ms (example in Fig. 14A). In three animals, a majority of fibers (68%) showed a large early peak in the time-interval...
and $Q_{10\text{db}}$ values (Mann-Whitney $U$ test). In addition, there was no relation between the presence or absence of preferred intervals and the threshold at CF, neither in individual animals nor in pooled data. The peak frequency derived from a fast Fourier transform analysis of time-interval histogram showing preferred intervals (see METHODS) typically corresponded to the CF of the unit. Values for the quotient of CF/fast Fourier transform peak frequency varied from 0.90 to 1.14, the average being 1.00, the median 0.99. In 37 fibers with CFs $>5$ kHz, preferred intervals were never seen.

For NM units, average spontaneous rate in prolonged quiet was 219.4 spikes/s, and rates ranged from 119.3 to 291.7 spikes/s ($n = 30$). Spontaneous rates also fell exponentially with CF; however, the scatter was large (Fig. 13B). A typical interval distribution for spontaneous spikes in NM units is shown in Fig. 14B. Preferred intervals were also seen, with a similar incidence as in auditory nerve fibers (8 of 26, or 31% of units with CFs up to 5 kHz).

Deviations from the quasi-Poisson distribution were also seen in the form of preferred intervals (Manley 1979), however, these occurred only in fibers with CFs up to 5 kHz (example in Fig. 14C). Of 53 fibers evaluated with CFs between 0.53 and 4.9 kHz, 17 (32%) showed preferred intervals. To examine for differences between those units and the group not showing preferred intervals, spontaneous rates and $Q_{10\text{db}}$ values were normalized and expressed as deviation from the linear and power fits shown in Figs. 13B and 11A, respectively, thus removing the known influence of CF on those parameters. There was no significant difference between the two fiber groups in normalized spontaneous rates and $Q_{10\text{db}}$ values expected according to the equal-distance hypothesis (see DISCUSSION).

FIG. 10. Some typical examples of frequency-threshold curves of auditory nerve fibers (A) and NM units (B). Note the simple V shape of the curves and the absence of low-frequency tails.

FIG. 11. Frequency selectivity of auditory nerve fibers, expressed as $Q_{10\text{db}}$ (A) and $Q_{40\text{db}}$ (B), as a function of CF. Solid lines: power fits to the data [$Q_{10\text{db}} = 0.074(CF \text{ in Hz})^{0.036}; r = 0.82, P < 0.0001; Q_{40\text{db}} = 0.036(CF \text{ in Hz})^{0.090}; r = 0.90, P < 0.0001$]. Dashed line in A: $Q_{10\text{db}}$ values expected according to the equal-distance hypothesis (see DISCUSSION).
well-established procedure that has been used in several bird species to record single-unit activity from the auditory nerve or the cochlear nuclei (Hill et al. 1989a; Klump and Gleich 1991; Sachs et al. 1974; Stopp and Whitfield 1961; Sullivan and Konishi 1984; Temchin 1988; Warchol and Dallos 1990). To obtain recordings specifically from the auditory nerve, authors either relied solely on visual identification of the nerve (Hill et al. 1989a; Klump and Gleich 1991; Temchin 1988) and/or used spike waveform to distinguish between recordings from fibers and cell bodies, assuming that these coincided with the auditory nerve and the cochlear nuclei, respectively (Sachs et al. 1974; Sullivan and Konishi 1984).

In the barn owl, the auditory nerve could not be identified easily by visual inspection. In addition, spike waveform is not a suitable criterion for distinguishing auditory nerve fibers from higher-order auditory fibers, which are known to occur in the chosen recording area (Köppl and Carr 1997). The combination of physiological criteria used in the present

Correlations between sensitivity, tuning sharpness, and spontaneous activity

The auditory nerve data were also examined for any correlations between sensitivity, sharpness of frequency tuning, and spontaneous rate. To minimize the known influence of frequency on all these parameters, three CF ranges were selected within which average threshold did not change appreciably (1–2, 4–6, and 6–8 kHz; Fig. 8A). Spontaneous rates and $Q_{10\text{dB}}$ values were normalized and expressed as deviation from the exponential and power fits shown in Figs. 13B and 11A, respectively. In none of the three frequency ranges was a correlation between threshold at CF and spontaneous rate seen (Spearman rank correlation test). At CFs from 4 to 6 kHz, as well as from 6 to 8 kHz, weak correlations between threshold at CF and $Q_{10\text{dB}}$ existed (Fig. 15; Spearman rank correlation test, $r = -0.44, P < 0.001, n = 72$ and $r = -0.34, P = 0.009, n = 57$), but not for the lower frequency range of 1–2 kHz ($r = 0.05, P = 0.82, n = 24$).

DISCUSSION

Reliability of unit classification

The surgical approach taken in the present study, exposing the auditory brain stem by aspirating the cerebellum, is a well-established procedure that has been used in several bird species to record single-unit activity from the auditory nerve or the cochlear nuclei (Hill et al. 1989a; Klump and Gleich 1991; Sachs et al. 1974; Stopp and Whitfield 1961; Sullivan and Konishi 1984; Temchin 1988; Warchol and Dallos 1990). To obtain recordings specifically from the auditory nerve, authors either relied solely on visual identification of the nerve (Hill et al. 1989a; Klump and Gleich 1991; Temchin 1988) and/or used spike waveform to distinguish between recordings from fibers and cell bodies, assuming that these coincided with the auditory nerve and the cochlear nuclei, respectively (Sachs et al. 1974; Sullivan and Konishi 1984).

In the barn owl, the auditory nerve could not be identified easily by visual inspection. In addition, spike waveform is not a suitable criterion for distinguishing auditory nerve fibers from higher-order auditory fibers, which are known to occur in the chosen recording area (Köppl and Carr 1997). The combination of physiological criteria used in the present
Interestingly, latency differences between the auditory nerve and the NM appeared to increase toward low frequencies in the barn owl, which correlates with the absence of endbulb-of-Held-type synapses below ~1 kHz (Köppl 1994).

Representation of CFs

The distribution of the CFs of the auditory nerve fibers recorded was prominently skewed toward high frequencies above ~4 kHz, although no attempt was made to preferentially record from this frequency range. It is known that high frequencies are represented by increasingly longer segments of the barn owl’s basilar papilla (Köppl et al. 1993). However, the number of afferent fibers for a fixed frequency range cannot be directly predicted by the cochlear map, because the number of hair cells per unit length of basilar papilla and their innervation pattern both change substantially from apex to base (Fischer 1994b). Recently, the number of cochlear afferent fibers in the barn owl and their distribution across frequencies have been determined from

![Image](https://example.com/image1)

**Fig. 14.** Typical examples of time-interval histograms of spontaneous activity from an auditory nerve fiber (A, binwidth 0.1 ms) and an NM unit (B, binwidth 0.1 ms). C: distribution of intervals up to 5 ms of an auditory nerve fiber on an expanded scale (binwidth 0.01 ms), revealing preferred intervals. In addition, this fiber showed a sharp, large peak at the shortest intervals, presumably due to slight injury (note the break in the ordinate scale).

study allowed a robust classification of the encountered units that we believe is most reliable, short of labeling every recorded unit. Latency differences are expected between first-, second-, and third-order auditory units, predominantly because of synaptic delays. The average difference of only ~0.5 ms found between the auditory nerve and the NM is in excellent agreement with direct measurements of the synaptic delay in chicken brain slices (Hackett et al. 1982). It is also similar to the difference between auditory nerve fibers and cochlear nucleus cells with a primary-like response pattern in the cat (Rhode and Smith 1986; Young et al. 1988). Most NM cells, as well as the primary-like units in the mammalian cochlear nucleus, receive their auditory nerve input via endbulb-of-Held synapses (Carr 1992; Rhode

![Image](https://example.com/image2)

**Fig. 15.** Normalized $Q_{10db}$ (see text) as a function of threshold at CF, for auditory nerve fibers of 2 different CF ranges, 4–6 kHz (A) and 6–8 kHz (B). Data from individual animals are shown with different symbols and the same code is used as in Fig. 8.
direct counts (Köppl 1996). Those numbers are largely similar to the distribution found in the present recordings, i.e., the sample of auditory nerve fibers is fairly representative across frequencies. Fibers with CFs < 5 kHz appear to be slightly underrepresented, possibly because of their smaller axon diameters (Köppl 1996), which would make them more difficult to record from. An increased difficulty in obtaining stable recordings was indeed noted in fibers with CFs below ~2 kHz.

The CF distribution of NM units was obviously skewed toward frequencies < 4 kHz. This is consistent with the fact that the chosen recording area overlaps only with the caudo-lateral portion of NM, where those frequencies are represented (Köppl 1994; Köppl and Carr 1997; Takahashi and Konishi 1988).

**Latencies across frequency**

The click latencies measured in the present study were onset latencies or signal front delays. These are shorter than group delays, which are commonly derived from phase-versus-frequency functions and correspond to the peak of the response (e.g., Ruggero 1992). Both types of delay increase with decreasing frequency. This increase is commonly thought to reflect the propagation time of a traveling wave from the base to the apex of the cochlea (e.g., Ruggero 1992), but, especially in nonmammals, could also include differential response times of other filter types (Manley et al. 1990; Smolders and Klinke 1986). The latencies of auditory nerve fibers in the barn owl were in the lower range of those reported for a variety of other species (reviews in Manley et al. 1990; Smolders and Klinke 1986); however, the method of latency determination (Fig. 1) would tend to produce the shortest possible values.

**Thresholds**

The audiogram that can be deduced from the best neural thresholds (Fig. 8) showed very sensitive hearing at 0–10 dB SPL across a broad frequency range from 0.5 to 9 kHz. These thresholds are not as sensitive as those of the behavioral audiogram of the barn owl (Konishi 1973). However, our measurements were taken with the use of a closed sound system, thus eliminating the amplifying effect of the owl’s facial mask (Coles and Guppy 1988). If the behavioral thresholds are corrected upward for the frequency-specific effect of the facial mask, they coincide with the lowest values of our neural thresholds for frequencies > 2.5 kHz. Below that, the best neural thresholds still lie ~10 dB higher. Because we have fewer data for the low frequencies, the difference could be due to a sampling error. However, it is also likely that sealing both ears with closed sound systems alters, for example, the resonance properties of the ear canal and/or interaural canal, and thus effectively changes sensitivity. Evidence for such an effective change may also be seen in the differences between thresholds for ipsilateral and contra-lateral stimulation (Fig. 9), a measure for the cross talk across the interaural canal. In a similar set of measurements on cochlear nucleus units in the barn owl, but with the use of only one closed sound system at a time, Moiseff and Konishi (1981) obtained similar threshold differences for neurons with CFs > 5 kHz, but consistently smaller differences for units below a CF of 5 kHz. Their threshold differences approached 0 even at 2–3 kHz, whereas the present data indicated a minimal difference of ~10 dB even at CFs < 1 kHz.

**Frequency tuning and its relation to the cochlear map**

Frequency-threshold curves in the owl were unremarkable. They showed the simple, nearly symmetrical V shape that has long been recognized as typical for birds (Manley et al. 1985; Sachs et al. 1980; Salvi et al. 1992). Also, the frequency tuning of auditory nerve fibers, expressed as $Q_{10dB}$, was very good, but not exceptional among birds. In comparable frequency ranges the values were very similar to or slightly below those reported for the starling (Gleich 1994; Manley et al. 1985), the pigeon (Klinke et al. 1994; Sachs et al. 1974; Smolders et al. 1995; Temchin 1988), the chicken (Manley et al. 1991; Salvi et al. 1992), and the emu (Manley et al. 1997). Even beyond the highest CFs found in auditory nerve recordings in other birds (~5 kHz) (Sachs et al. 1974), the $Q_{10dB}$ values of the owl showed no sudden or unexpected increase.

This is an important point, because it runs contrary to the so-called ‘equal-distance hypothesis’ that relates tuning sharpness to the space available for a particular frequency range on the basilar papilla (review in Greenwood 1991). The frequency map of the barn owl’s basilar papilla is unusual in that frequencies above ~2 kHz are greatly expanded (Köppl et al. 1993). The mapping constants, i.e., the length of papilla devoted to 1 octave, in the low-frequency range are comparable with the values found in other birds, they rise continuously, however, toward the highest frequencies, reaching values 2- to 10-fold above those typical for birds (Köppl et al. 1993). This vast spatial expansion was not reflected at all in the quality of frequency tuning (Fig. 11A). The actual bandwidths observed in high-frequency auditory nerve fibers were 2–3 times wider than expected according to the equal-distance hypothesis. This also differs from the situation in some bat species that have similarly unusual cochlear frequency maps with regions of vast spatial expansion of certain narrow frequency bands (Kössl and Vater 1985; Vater et al. 1985). In those mammals, the spatial increase is correlated with a dramatic increase in the sharpness of neural frequency tuning (Kössl and Vater 1990; Suga et al. 1976). It may be argued that cochlear micromechanics are potentially very different in birds and mammals (e.g., Manley 1995; Smolders et al. 1995) and that therefore such a correlation would not hold across groups. However, in the starling, a songbird, a good correspondence between measures of frequency selectivity and cochlear representation was found (Buus et al. 1995). Conversely, there is also evidence that even in mammals, cochlear representation and frequency tuning are not necessarily linked. In the mustache bat, exceptionally sharp tuning in the 90-kHz region is not mirrored in a high cochlear space constant (Kössl 1994; Kössl and Vater 1990).

Frequency-tuning curves were very similar between auditory nerve fibers and NM units. Our $Q_{10dB}$ values were also in the same range as those reported in an earlier study of the barn owl NM (Sullivan and Konishi 1984). Thus there appears to be no significant frequency convergence in NM.
Spontaneous activity

Spontaneous discharge rates in the owl auditory nerve were in a similar range to that found for other bird species and showed a monomodal distribution that has long been recognized as typical for birds (Gummer 1991; Hill et al. 1989a; Klinke et al. 1994; Manley et al. 1985, 1991; Sachs et al. 1974; Salvi et al. 1992; Smolders et al. 1995). A decrease of spontaneous rate with increasing CF was also observed in all studies in which adult birds were used: in the starling (Manley et al. 1985), the pigeon (Klinke et al. 1994; Richter et al. 1996; Smolders et al. 1995), and the redwing blackbird (M. B. Sachs, cited in Manley et al. 1985). In a recent developmental study on the pigeon, Richter et al. (1996) showed that this relationship develops within the first few weeks after hatching and that spontaneous rates increase during the same time. This is also consistent with the observation of an increase of average spontaneous rate within the first 2 wk of life in the emu (Manley et al. 1997) and differences in average spontaneous rates reported for chickens of different ages (Manley et al. 1991; Salvi et al. 1992). Taken together, an average spontaneous rate of 50–100 spikes/s and a pattern of decreasing spontaneous rate with increasing CF, as was found for the barn owl, appear to be the typical mature situation in birds.

Although far fewer data exist for the avian NM than for the auditory nerve, it appears that spontaneous rates in the NM are generally higher than in the auditory nerve. However, the spontaneous rates found for NM units in the barn owl in the present study, with an average value of 219.4 spikes/s, appear exceptionally high. Sachs and Sinnott (1978) reported an average spontaneous rate of 115.7 spikes/s in the NM of the redwing blackbird. In young chickens, rates of 40 and 94.3 spikes/s were found (Cohen and Saunders 1993; Warchol and Dallos 1990); however, as discussed for the auditory nerve, this spontaneous activity may not yet reflect the mature condition. In an earlier study on the barn owl cochlear nucleus, Sullivan and Konishi (1984) reported an average spontaneous rate of 94.9 spikes/s for NM units (calculated from their values given in spikes per 150 ms), and their highest rates were still below our average. There is no obvious explanation for these discrepancies within the same species. Anesthetic agents have long been suspected of influencing spontaneous activity, as has been shown for vestibular afferents (Anastasio et al. 1985), but the same primary anesthetic agent, Ketamine, was used in both barn owl experimental series. Also, the spontaneous rates of our auditory nerve fibers, recorded in the same individuals, were unremarkable for birds. There is a possibility of a (typographical) error in the paper by Sullivan and Konishi (1984), because their spontaneous spike counts given per 150 ms do not appear to match the data in their Fig. 9, where rate-intensity functions for NM units are shown. From this figure, similar spontaneous spike counts, but per 100-ms stimulus, can be estimated. If the spontaneous spikes were indeed also counted in a 100-ms window, then the mean value would be 141.6 spikes/s. Considering that the majority of the sample of Sullivan and Konishi (1984) consisted of high-frequency units, this value would be close to our measurements.

The data presented here suggest that the spontaneous rate of NM units is about twice that of auditory nerve fibers at any given frequency. In mammals, there is also evidence that the spherical bushy cells in the anteroventral cochlear nucleus, which are generally regarded as analogous to the avian NM (e.g., Rhode and Greenberg 1992), show spontaneous rates that are about twice those found in the auditory nerve (Smith et al. 1993). Most NM cells, as well as the spherical bushy cells in the anteroventral cochlear nucleus, receive their auditory nerve input via large endbulb-of-Held synapses (Cant 1992; Carr 1992; Ryugo 1992). Recordings from spherical bushy cells show a characteristic prepotential preceding each spike, which is assumed to reflect the activity of the large endbulb of Held (Rhode and Greenberg 1992). These prepotentials, together with the fast transmission properties of endbulb-of-Held synapses with little temporal summation (Hackett et al. 1982; Oertel 1985; Raman and Trussell 1992), make it likely that single auditory nerve spikes can induce a spike in NM cells or spherical bushy cells. The ratio of the spontaneous rates in these cells and the auditory nerve, ~2:1, could therefore provide an estimate for the convergence. For spherical bushy cells, this agrees with estimates of two to three converging auditory nerve inputs, derived from labeling experiments (Liberman 1991). Similar numbers of auditory nerve inputs may thus be assumed for NM cells in the barn owl.

Some auditory nerve fibers and NM units showed preferred intervals in the spontaneous discharge. This deviation from the more common quasi-Poisson distribution of intervals has been observed in all bird species investigated, although its significance remains controversial (Gummer 1991; Klinke et al. 1994; Manley et al. 1985, 1991; Salvi et al. 1992; Temchin 1988). In the red-eared turtle, such preferred intervals were shown to reflect spontaneous hair cell membrane potential oscillations associated with an electrical tuning mechanism (Crawford and Fettiplace 1980). Similar voltage oscillations have also been observed in hair cells isolated from the chicken and alligator basilar papilla (Fuchs and Evans 1988; Fuchs and Mann 1985), leading to the assumption that in birds, too, preferred intervals in the spontaneous discharge are indirect evidence for an electrical tuning mechanism. However, care has to be taken to exclude ambient noise unintentionally phase locking the fibers to their CF around threshold levels and thus producing a similar pattern. If no control experiments are performed, such as eliminating the middle ear (Crawford and Fettiplace 1980; Temchin 1988), differences between units showing preferred intervals and those that do not may be suggestive of ambient noise. This is especially true if units showing preferred intervals prove to be the more sensitive ones (Klinke et al. 1994). No such evidence for inadvertent stimulation was found in the present study.

The possible significance of the phenomenon of preferred intervals in the barn owl lies in the comparatively high upper frequency limit of almost 5 kHz. Electrical tuning is generally regarded as a low-frequency mechanism, partly because there is no evidence for it in mammalian hair cells (e.g., Patuzzi and Robertson 1988). The observed limit so far was ~2 kHz, the highest frequency of preferred intervals in the pigeon (Temchin 1988). This, however, approximately coincides with the limit of temporal resolution of the auditory nerve fibers (Gleich and Narins 1988; Hill et al. 1989b).
Barn owl auditory nerve fibers are able to phase lock to much higher frequencies, up to ~9 kHz (Köppel 1995; Sullivan and Konishi 1984), and should thus reflect spontaneous hair cell membrane potential oscillations up to those frequencies, if present. Interestingly, the observed limit at almost 5 kHz is in excellent agreement with model calculations based on the properties of the underlying membrane channels as quantified in the turtle (Wu et al. 1995). Scaled for the higher body temperatures of birds, and with the use of still realistic assumptions about channel numbers, Wu et al. (1995) estimated an upper frequency limit for electrical tuning of ~4 kHz.

Physiological classes of auditory afferents?

In mammals, two fundamentally different types of auditory afferents are well documented, the type I and type II afferents, innervating inner hair cells and outer outer hair cells, respectively. Many morphological differences are known between those two types (review in Ryugo 1992) and the type I afferents appear to be the only physiologically active group, the function of the type II afferents still being obscure (Robertson 1984). Within the type I population, two to three physiological subpopulations are typically recognized in mammals, which (in the cat) are also known to correlate with morphological differences in the terminals on inner hair cells and also have different target areas in the cochlear nucleus (Ryugo 1992). These are units with low spontaneous rates, high thresholds, and straight rate-intensity functions, units with medium spontaneous rates, medium thresholds, and sloping saturating rate-intensity functions, and units with high spontaneous rates, sensitive thresholds, and saturating rate-intensity functions (e.g., Liberman 1978; Sachs and Abbas 1974; Winter et al. 1990).

In birds, there is no anatomic evidence for different populations of auditory afferents comparable with mammalian type I and type II afferents (Fischer 1994a; Fischer et al. 1994; Köppel 1996). However, a significant proportion of hair cells completely lacks an afferent innervation (review in Fischer 1994a). Fischer (1994a) suggested the use of the presence or absence of afferent innervation as the defining criterion for the two types of hair cells in birds (tall and short hair cells), because they grade into each other in every other respect. With the use of this definition, it could be argued that birds have simply abandoned the afferent innervation of one of their hair cell types, leaving only one basic type of afferent neuron.

This afferent population innervates the tall hair cells, many of which may be found in one cross section of the basilar papilla. Physiologically, only gradual differences are seen between afferents in any one frequency range, but no distinct classes. The many studies available on bird auditory afferents have invariably reported a monomodal distribution of spontaneous rates (see above). Also, there is no consistent, strong correlation between spontaneous rate and sensitivity or frequency selectivity (Manley et al. 1985, 1991; Salvi et al. 1992; Smolders et al. 1995). The type of rate-intensity function is similarly unrelated to any of those parameters (Richter et al. 1995). Tracing experiments suggest that fibers innervating hair cells situated at different positions across the width of the basilar papilla differ in sensitivity and possibly frequency selectivity (Gleich 1989; Smolders et al. 1995). This is an entirely different concept than in mammals, where many afferents that likely contact the same inner hair cell differ in their physiology.

It is important to point out that the barn owl data, too, did not show any indication for subpopulations of afferents reminiscent of the situation in mammals. Morphologically, the high-frequency portion of the barn owl’s basilar papilla shows a number of unusual features, including a fairly abrupt change of hair cell shape between the most neural tall hair cell and more abneural hair cells, and a concentration of afferent fibers on this most neural hair cell (Fischer 1994b; Köppel 1993; Köppel et al. 1993). Although this could be interpreted as a trend toward the typical mammalian configuration with a single row of inner hair cells receiving the bulk of afferent fibers, there was no reflection of that in a more mammal-like physiology. A possible correlate of the unusual concentration of afferent fibers on one hair cell row in the barn owl, however, is the rather small spread of thresholds observed (Fig. 8). Birds typically show threshold ranges of 40–50 dB (Manley et al. 1985, 1991; Sachs et al. 1974; Salvi et al. 1992; Smolders et al. 1995) at any one frequency. If it is true across avian species that threshold correlates with the cross-sectional position of the innervated hair cell, with the most sensitive cells sitting near the neural edge (Gleich 1989; Smolders et al. 1995), then a reduction in the number of innervated hair cells to the most neural ones should reduce the threshold range.

Thanks to G. Manley for continuous support and discussion, G. Klump, M. Baumann, and S. Kießlich were responsible for the excellent custom hardware and software used in the experiments. C. Carr, O. Gleich, G. Klump, and G. Manley made valuable comments on an earlier version of the manuscript.

This research was supported by the Deutsche Forschungsgemeinschaft within the SFB 204 “Gehör” and by a research fellowship.

Address for reprint requests: C. Köppel, Institut für Zoologie der Technischen Universität München, Lichtenbergstr. 4, 85747 Garching, Germany.

Received 15 July 1996; accepted in final form 12 September 1996.

REFERENCES


