Improvement of Phase Information at Low Sound Frequency in Nucleus Magnocellularis of the Chicken

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INTRODUCTION

Auditory nerve fibers (ANFs) encode sound intensity and timing as a series of action potentials and fire at a particular phase of the sound stimulus (phase-locking), especially to low-frequency sound signals (Irvine 1986; Kiang 1965). In avian species, the auditory nerve fibers having a single synaptic input from a single ANF and generating a large excitatory postsynaptic current (EPSC) in an all-or-nothing manner (Carr and Boudreau 1991; Parks and Rubel 1978).

The terminals of ANFs differentiate along the tonotopic axis and form a large end-bulb-shaped synapses in the regions encoding middle to high characteristic frequencies (CFs). Each fiber innervates a single NM neuron and generates a large excitatory postsynaptic current (EPSC) in an all-or-nothing manner (Carr and Boudreau 1991; Parks and Rubel 1978).

Each NM neuron in these regions receives input from only two to three ANFs. In contrast, in the low CF region, ANF terminals are smaller, and each NM neuron receives a larger number of ANF inputs (Fukui and Ohmori 2004; Köppl 1994; Köppl and Carr 1997). In the low CF neurons of chicken, EPSCs are small in amplitude and increased gradually with stimulus intensity (Fukui and Ohmori 2004). In the middle to high CF neurons, the temporal jitter of spikes in response to ANF stimulation was 10–20 μs compared with 30 μs in the low CF neurons (Brenowitz and Trussell 2001; Fukui and Ohmori 2004). A large EPSC seems advantageous in high-fidelity transmission, and activation of a single ANF is sufficient to generate action potentials in high to middle CF NM neurons. The small EPSCs of low CF neurons, on the other hand, require summation of a number of synaptic inputs for threshold depolarization. This summation might be effective in improving phase-locking of low-frequency cochlear nucleus neurons compared with their ANF inputs. Evidence for this idea was first recorded in bushy cells of cat cochlear nucleus, the mammalian homologue of NM neurons (Joris et al. 1994).

However, this improved phase-locking was not found in barn owl in NM over ANFs in CF of 1–10 kHz (Köppl 1997), although some comments have been made about systematic improvement of phase-locked firing in NM neurons (Sullivan and Konishi 1984). In chickens, when comparing the previous results for ANF (Salvi et al. 1992) with NM (Warchol and Dallos 1990), there seems to be some improvement of phase responses around 300–400 Hz and a decrease around 1,000–1,600 Hz in NM. However, there are several problems in this comparison because of the differences in the ages, anesthesia, and the rate threshold criteria. Moreover, data points are sparse, especially in NM units of CF lower than 1 kHz. We, therefore recorded unit activities of ANF and NM under identical conditions and compared the nature of phase locking.

METHODS

Animal preparation

Chickens between P3 and 9 were anesthetized with an intramuscular injection of chloral hydrate (160 mg/kg). We used 34 chickens, and in 9 birds, recordings were made both from ANF and NM. Additional chloral hydrate (~50 mg/kg) was injected in some cases to maintain the level of anesthesia. The bird’s body temperature was maintained (40°C) throughout all procedures by using a heating pad. The soft tissue around the ear was removed to expose the tympanic membrane. The skin covering the skull was removed, and

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the skull was opened to expose the cerebellum. A slit was made in the
dura mater for electrode penetration. Care was taken not to injure the
large venous sinus. A metal rod was fastened to the top of the cranium
with dental cement, and the position of the head was fixed to a
stereotaxic stage. The plane formed by the pair of ear bars and the tip
of the beak was tilted 30° downward. These procedures conformed to
the guidelines for the care and use of animals in the field of
physiological sciences set by the Japanese Physiological Society.

In vivo recording

Sound stimuli were presented through a pair of hollow ear bars with a
closed acoustic system from a pair of earphones (EF-1935, Knowles
Electronics). Probe microphones (BT-1751, Knowles Electronics),
calibrated with a standard microphone (Type 2603, B&K), were
positioned near the ear canals, and the sound pressure level was
calibrated at the beginning of each experiment. The maximum sound
intensity was 120 dB and the sound frequency range was 100–6,000
Hz. The sound calibration and electro-physiological recordings were
made in a sound-isolation booth placed within a soundproof, shielded
room. Sound stimulus duration was 80 ms with a 3-ms rise and fall
time for a triangular-shaped envelope.

Recording electrodes were made from quartz glass capillary tubes
(No. 100-70-10, Sutter Instrument), pulled with a micro-electrode
puller (P-2000, Sutter Instrument) and filled with 3 M potassium
acetate (electrode impedance was 8–20 M\(\Omega\)) with Alexa 488 or 568
(10 K dextran, Molecular Probes). The recording electrode was
inserted 1.5–2.5 mm lateral and 0.7 mm caudal to 1.0 mm rostral of
the inion. The penetration was made vertically when recordings were
made from the core region of NM, but was tilted 6° medially for
recording from NM axons near the midline and 6° laterally for
recording from ANFs.

Neuronal action potentials were recorded extracellularly with an
AxoClamp-2B amplifier (Axon Instruments) and band-pass filtered
(2-pole) between 150 Hz and 5 kHz. Data were collected with 12-bit
resolution at 50-kHz sampling frequency. Customized software, writ-

FIG. 2. Latency to the pure tone characteristic frequency (CF). Latency as
a function of CF was plotted for all units. NM units were recorded in NM (\(\bullet\),
NM-nucleus) and from the midline of the brain stem (\(\circ\), NM-midline). ANF
units were recorded from the ANF bundle (\(\oplus\), ANF-fiber). Among units
recorded within NM, those having a latency shorter than the mean \(\pm\) 2 SD
were grouped as ANF unit (\(\ominus\), ANF-nucleus). In units recorded from ANF
bundle, some units had an exceptionally long latency (\(\triangledown\), others). The origin
of these units was not identified. The increased latency at low frequencies
probably reflected the long period of a sound cycle. Note that the latency was
measured by a pure tone stimulus of 90–120 dB, the maximum intensity we
applied, at CF and included a 3-ms rise time.

\[1B\) and from axons near the midline where fibers projecting to the
contralateral side of the brain stem cross (indicated in Fig. 1A, right, \(\ast\)).

Among the units recorded in the core region of NM, we separated
ANF units (37 units, ANF-nucleus) and NM units (148 units, NM-
midline) according to the onset latency when pure tone stimuli of
90–120 dB at the characteristic frequency (CF) were applied (Fig. 2).
The latency was defined as the time to the onset of PSTH histogram
(0.1-ms bin width) after the onset of sound stimulus, which included
the 3 ms of stimulus rise time. ANF units were defined as those having a
latency shorter by 2 times of SD than the mean latency of NM units in
each CF region (Fig. 2, ANF-nucleus). CF regions were defined as
follows; \(<\)200, 201–300, 301–500, 501–800, 801–1,300, 1,301–2,000, 2,001–3,200, and \(>\)3,201 Hz (the number of cells per bin are
given in the legend for Fig. 3). These CF regions were adopted as bins
in creating average plots as a function of CFs (see Figs. 3, \(Bb\) and \(Cb\),
9B, and 10B). Among the units recorded from the bundle of ANF, we
sometimes encountered a unit which had a long latency. Those units
having a latency longer by 2 SD than the mean latency of ANF units
in each CF region were excluded from further analysis (Fig. 2, others).

NM neurons and axons were located by playing a white-noise
stimulus (80 dB, 80-ms duration, 3 pulses/s) while lowering the
electrode into the brain stem. After isolation of a unit from back-
ground noise, a frequency tuning curve to an ascending series of
sound frequencies (12 sub-frequencies per octave, 3 pulses/s, 10-dB
step) was obtained. Firing threshold was defined as the sound intensity
that induced spiking at 40 spikes/s higher than the spontaneous firing
rate (Warchol and Dallos 1990). CF was defined as the frequency with
the lowest threshold. A series of tone stimuli were then presented at
the CF of the neuron: tone pips of 80-ms duration were presented at
levels ranging from 10 to 90 dB (10-dB steps), and in the case of units
having a higher threshold (higher than \(\sim\)50 dB), the sound intensity
was increased to as much as 120 dB. Each stimulus was presented
15–50 times (2–3 tones/s) in a randomized sequence.

Vector strength, an indicator of the phase locking, was calculated
(Goldberg and Brown 1969) using spikes occurring between 10 and
80 ms of the sound stimulus. The phase angle of the stimulus sinusoid
at which each spike occurs is expressed as a vector of unit length. The
vectors of all spikes are then averaged with the mean length indicating
the vector strength. Thus this measure equates to the relative abun-
dance of firing at a particular phase angle. Vector strength varies from
0 (no phase-locking) to 1 (perfect phase-locking). We further calcu-
lated a temporal jitter for each neuron as a measure of the phase-
locking. Temporal jitter was defined as the SD of spike timing in the period histogram.

Small spikes, those with an amplitude of less than twice the noise level (~0.2 mV), were excluded from vector strength calculations. However, these small spikes were sometimes included as unit activity when they were observed immediately following larger spikes (multiple spiking units, Fig. 6). The multiple spiking units were observed only in the low CF region (<401 Hz; Fig. 7).

All data are presented as means ± SE (n = number of cells). Statistical evaluations were made using Student’s unpaired t-test with statistical significance being achieved when P < 0.05. While most summary data of vector strength presented in this paper is obtained at statistical significance being achieved when P < 0.01. *

RESULTS

We recorded a total of 206 ANF units in bundle of ANF fiber (171 units) and in NM (35 units), and a total of 199 NM units in NM (143 units) and from near the midline of the brain stem, where the projection fibers to NL cross (56 units; Fig. 1). CFs ranged from 120 to 4,757 Hz for ANF and from 119 to 3,100 Hz for NM. Exemplary ANF recording sites are shown in Fig. 1A (CF of 169–635 Hz), NM sites in B (CF of 238–378 Hz); †, direction of the electrode penetration track; arrowhead, sites of Alexa 488 injection (Fig. 1A, fluorescence image) or of the blood clot (Fig. 1B, bright field image).

The latency of each unit was plotted in Fig. 2. NM units had systematically larger latency than ANF units. There were some other units of exceptionally longer latency recorded in ANF (Fig. 2, ANF-others). The origin of these units was not identified. Tuning curves were broader for lower CF units, both in ANF and in NM (Supplementary Fig. 1).  

Single-unit activity of ANF and NM

The spontaneous firing rate of NM units was higher than that for ANF units (Fig. 3a). The firing rate for NM-nucleus units was the same as that for NM-midline units, and was significantly higher than that for ANF units (Fig. 3a). The spontaneous firing rates reported here are comparable to those reported previously in the chicken [NM = 94.3 spikes/s (Warchol and Dallos 1990), ANF = 20.5 spikes/s (Manley et al. 1991), ANF = 86 spikes/s (Salvi et al. 1992), ANF = 23.6 spikes/s (Saunders et al. 2002)].

Rate threshold stimulus intensity was determined for each unit. Units with CF ~1–2 kHz were the most sensitive (i.e., had the lowest thresholds), whereas units with lower or higher CFs were less sensitive (Fig. 3b). This tendency was the same as in preceding reports [ANF (Manley et al. 1991; Salvi et al. 1992; Saunders et al. 2002), NM (Warchol and Dallos 1990)]. NM (●) and ANF (○) units had almost similar thresholds across frequencies (Fig. 3b).

The online version of this article contains supplemental data.

**Table 1. Vector strengths of the first spikes calculated from ANF unit and NM units at several CF regions lower than 800 Hz**

<table>
<thead>
<tr>
<th>CF (Hz)</th>
<th>All units</th>
<th>Multiple spiking units</th>
<th>All units</th>
<th>Multiple spiking units</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 200</td>
<td>0.94 ± 0.01 (20)</td>
<td>0.94 ± 0.01 (20)</td>
<td>0.96 ± 0.01 (6)**</td>
<td>0.96 ± 0.01 (5)**</td>
</tr>
<tr>
<td>201–300</td>
<td>0.91 ± 0.01 (21)</td>
<td>0.91 ± 0.01 (18)</td>
<td>0.95 ± 0.01 (10)**</td>
<td>0.95 ± 0.02 (3)*</td>
</tr>
<tr>
<td>301–500</td>
<td>0.87 ± 0.01 (13)</td>
<td>0.87 ± 0.02 (4)</td>
<td>0.92 ± 0.01 (34)**</td>
<td>n.d.</td>
</tr>
<tr>
<td>501–800</td>
<td>0.83 ± 0.01 (31)</td>
<td>n.d.</td>
<td>0.83 ± 0.01 (51)**</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

For all units statistics were calculated from the first spikes from units of multiple-spiking and unitary spiking. Numbers in parentheses are the number of units. ** statistical significance P < 0.01, *P < 0.05. n.s.: not significant. n.d.: not detected. ANF, auditory nerve fibers; NM, nucleus magnocellularis; CF, characteristic frequency.
The firing rate increased with stimulus intensity; in most cases, the rate became saturated at the highest intensity tested (see Figs. 4 and 6). The maximum firing rate was plotted against CF in Fig. 3Ca. The maximum firing rate was the highest in the middle CF region for both ANF and NM units (Fig. 3Cb) and had a tendency to be greater for the ANF units than for the NM units in the high CF ranges (Fig. 3Cb). The range of maximum firing rates was similar to the rates reported previously [NM, 110–480 spikes/s (Warchol and Dallos 1990), ANF, 227.3 spikes/s (Saunders et al. 2002), 207.6 ± 84.7 spikes/s (P21) and 138.9 ± 56.0 spikes/s (P2) (Manley et al. 1991), 327 spikes/s (Salvi et al. 1992)]. In most units, the maximum firing rate was lower than the own CF of units (broken line in Fig. 3Ca). However, some low CF units demonstrated a higher maximum firing rate than CF. These units generated multiple spikes in some stimulus phase cycles. Forty-two of 206 ANF units and 8 of 199 NM units exhibited multiple spiking. The details of multiple spiking units are described in the following text.

Improved phase locking with the increase in stimulus intensity

Figure 4 compares the firing pattern of an ANF unit (CF = 378 Hz) with a NM unit (CF = 360 Hz) to tone stimuli presented at the CF. Raster plots in Fig. 4, A and B, illustrate 30 traces at each sound intensity displaced vertically. As firing rates increased with increasing sound level, greater phase locking in both ANF and NM created clear bands in the raster plot. These bands were spaced at the period of the stimulus tone or 1/CF. Figure 4, C and E, illustrates 10 superimposed raw data traces for the ANF and NM units, showing higher synchronization in the NM unit than in the ANF unit, both generated by an 80-dB stimulus. These 10-ms traces were sampled around 16 ms after the onset of the sound stimulus. In both ANF and NM, the phase locking started at the intensity lower than the rate threshold (Fig. 4, D and F; see also Fig. 5). The maximum vector strength of these units was 0.89 for ANF and 0.95 for NM, indicating a high phase-locking in the NM unit. This ANF unit showed some incidence of multiple spiking at the onset of large sound stimuli (Fig. 4A, 120 dB).

The vector strength relative to the rate threshold was plotted in Fig. 5 for all the units with CFs < 800 Hz. In most units, vector strength reached a plateau level around the intensity that was 20 dB higher than the rate threshold. At the rate threshold level, in units with CFs < 800 Hz, the vector strength was not different between ANF and NM (Fig. 5C). However, at the +20-dB sound level, for units with CFs lower than 500 Hz, there was significantly larger vector strength for NM versus ANF units (Fig. 5C, right). In contrast, at CF > 500 Hz, NM units generally had a smaller vector strength than did the ANF units.

Multiple spikes during one sound cycle in low CF units

At low CFs, some units fired multiple spikes during one sound stimulus phase cycle (Fig. 6). Warchol and Dallos (1989) reported similar multiple spiking units in both NM and nucleus angularis. Figure 6A illustrates a multiple spiking NM unit with a CF of 224 Hz (stimulus intensity: 60 dB). In multiple spiking units, typically a large spike was followed by a smaller spike, apparently at a fixed interval. Figure 6D shows 30 overlaid raw data traces, obtained at 80-dB stimulus intensity, illustrating this phenomenon.
The firing rate of this multiple spiking unit rose with increasing stimulus intensity starting at 40 dB (Fig. 6, B and E). The probability of generating the second spike increased with stimulus intensity; nearly 64% of large spikes were followed by a small spike at 90 dB (Fig. 6G), creating a clear second band in the raster plot (Fig. 6B). Similar to other units, vector strength increased with the stimulus intensity (Fig. 6F). When the vector strength was calculated separately for the first or the second spike, both approached 0.97. However, when vector strength was calculated with both large and small spikes included, it reached only 0.69 (Fig. 6F).

Spike amplitude plotted against interspike interval (ISI) reveals three clusters of spikes (Fig. 6C). These are the ISIs between the first (large) spike and the second (small) spike, the second spike and the next first spike, and the two first spikes. Because spike amplitude was plotted against a preceding ISI in Fig. 6C, the spike amplitude measured for the first cluster was the amplitude of the second spikes and ISI was smallest. The other two clusters of larger-amplitude spikes were the first spikes at two different ISIs (second and third clusters). The third cluster represents the neuron spiking once per phase cycle; ISI was 4.46 ms, equal to 1/CF of the unit.

Occurrence of multiple spiking units

We calculated multiple spiking index (MSI): the number of second spikes divided by the number of first spikes during a sound stimulus. A unit was considered multiple spiking when MSI was >0.05 (i.e., >5% of phase cycles contained 2 spikes) in response to a sound stimulus at the maximum intensity (see also Fig. 6G). All multiple spiking units had a CF <401 Hz, and MSI was largest in units with the lowest CFs (Fig. 7A). ANF units were more likely to exhibit multiple spiking than were NM units of equivalent CF (Fig. 7B). In NM units had a CF <300 Hz, 1 of 2 units recorded in the midline, and 7 of 14 units recorded within the nucleus of NM were multiple spiking.

The ISI between the first and second spikes of multiple spiking units varied between 1 and 2.5 ms with the mean ISI being 1.40 ± 0.05 ms (n = 42) for ANF unit and 1.40 ± 0.10 ms (n = 8) for NM unit. This ISI was larger in lower CF units (Fig. 7C). The mean ISI for ANF and NM units together was 1.52 ± 0.07 ms for ≤200 Hz (n = 25), 1.32 ± 0.04 ms for 201–300 Hz (n = 21), and 1.11 ± 0.02 ms for 301–500 Hz (n = 4). These differences among the three frequency ranges were significant (P < 0.05 in reference to 201–300 Hz).

Difference of synchronization between ANF and NM

Figure 8 illustrates period histograms from five ANF (top) and five NM (bottom) units, across the range of CFs. Period histograms of multiple spiking units contained two peaks, corresponding to the first and second spike fired in each stimulus phase cycle. Units of either type with CFs >3,000 Hz did not show clear peaks, indicating a lack of phase-locking, and vector strengths were correspondingly small (see also Figs. 5C and 9A). In the single spiking units of lower CF, period histograms exhibited a clear peak, indicating a significant phase-locking. Phase-locking by single-spike NM units was systematically higher than by ANF units, reflected by the taller peak and the narrower distribution in the period histograms of NM units. The average peak of period histograms for the
single-spiking NM units was 20 ± 1.7% (n = 7) and ANF units 13.9 ± 0.8% (n = 3) for CF 201–300 Hz (P < 0.05). This is particularly clear in the examples illustrated in the second column of Fig. 8 (CF = 283 Hz for ANF, 224 for NM). The stimulus level was different between ANF and NM units; however, the vector strength was the maximum at this intensity in each unit.

Maximum vector strength was plotted against CF in Fig. 9 for all units. At CFs >500 Hz, the maximum vector strengths of NM were the same or smaller than that of ANF units. At CFs <500 Hz, however, the maximum vector strength was significantly greater for NM units (Fig. 9B). Vector strength was smaller in multiple spiking units of both ANF and NM (■ and □, Fig. 9A; see C for the expanded illustration of a top left region in Fig. 10A), and was 0.81 ± 0.01 for NM (n = 8) and 0.79 ± 0.01 for ANF (n = 42, not significant).

To compare single and multiple spiking units, vector strengths were calculated for the first spike of each doublet from the multiple spiking units (■ and □) and compared with single spiking units (○ and △) in Fig. 9D. First spike vector strength in all units was significantly higher in NM with CFs between 201 and 500 Hz than ANF units of comparable CF (Table 1). When the first spikes of multiple spiking NM units were compared with only the first spikes of multiple spiking ANF units (i.e., excluding single spiking ANF units), vector strength of NM units was also significantly larger between 201 and 300 Hz (Table 1). The Kolmogorov-Smirnov test, to compare the distribution of the first spike vector strength of units with CF <500 Hz, demonstrated a larger fraction of the smaller vector strength units in ANF (P < 0.05). The maximum vector strengths of the second spikes of multiple spiking NM and ANF units were not significantly different (0.96 ± 0.01, n = 8 for NM; 0.89 ± 0.02, n = 42 ANF, P = 0.09).

Temporal jitter is another measure of phase locked firing (defined in METHODS). We plotted the temporal jitter of the first spike for all units (Fig. 10). The temporal jitter was significantly smaller for NM units than for ANF units at CF 201–500 Hz. At higher CFs, the temporal jitter for NM units was significantly larger than for ANF units (Fig. 10B). This corresponds with the larger vector strength for ANF units than NM units in high-frequency region (Fig. 9B). Temporal jitter for
NM units with CF between 301 and 500 Hz was the same level as that at 501–1300 Hz for both ANF and NM units.

DISCUSSION

Is phase information improved in NM?

While Sullivan and Konishi (1984) reported some improvement of phase locking in NM over ANFs of the barn owl, other data suggest that this does not occur in neurons with CFs >1 kHz (Köppl 1997). In the chicken, vector strength seems to be improved around 300–400 Hz and decreased around 1,000–1,600 Hz in NM as opposed to ANF (Salvi et al. 1992; Warchol and Dallos 1990). However, this comparison is made under different conditions: in ages, anesthesia and the rate threshold criteria. Joris et al. (1994) compared the vector strength between ANF and the bushy cells of AVCN in cats, the mammalian homologue of NM, and found that the vector strength of bushy cells was higher than that of ANF at CFs <700 Hz.

We compared the vector strength for ANF and NM and found a small but significant improvement of phase locking in NM versus ANF units at CFs <500 Hz (Fig. 9). The vector strength we observed was almost the same as in previous results for ANF (Salvi et al. 1992) but slightly higher than that for NM at CF 1–2 kHz (Warchol and Dallos 1990), although the vector strength compared was made using different criteria (maximum vector strength in this paper vs. vector strength at stimulus 20 dB higher than the rate threshold in Warchol and Dallos). We also observed a decrease of vector strength in the high CF region (Figs. 5 and 9), which is consistent with the results for the barn owl (Köppl 1997).

Overall, the timing information in low CF NM neurons is likely improved by two factors: a reduced occurrence of multiple spiking units in NM (Fig. 7B) and the increased phase-locked firing of the first spikes in multiple-spiking NM units (Fig. 9D, Table 1). The reduced occurrence of multiple spiking in NM units should improve coincidence detection in NL. However, the functional significance of the presence of multiple-spiking units in low CF units is not clear. These
multiple firings are probably not a developmental artifact, because multiple firings were also observed in P10–28 (War- chol and Dallos 1989).

Multiple spiking units

There is a possibility that the multiple spiking units may represent recordings of unit activities originated from different neurons or activities of pre- and postsynaptic origin; i.e., a sequential spikes of presynaptic ANF and postsynaptic NM neurons (Kopp-Scheinpflug et al. 2002). However, because of the following four reasons, we believe that the spike doublet represents the activity of a single neuron. 1) The smaller spike never appeared as a single spike but always followed a larger spike with a fixed delay; the delay time varied depending on the unit (Fig. 7C) and the spike amplitude was dependent on the preceding ISI (Fig. 6C). 2) Two ANF units (CF = 120 and 140 Hz) generated three spikes per sound cycle, and the amplitude of the third spike was smaller than that of either the first or the second spike. 3) Some ANF or NM units, recorded outside of the nucleus of NM, were multiple spiking. Coupled pre- and postsynaptic spike recordings are not be expected in cells outside the nucleus due to the distance between the recording site and the synapse. 4) CF of all multiple firing units was <40 Hz (Fig. 7A). In low CF NM neurons in vitro, multiple spikes were observed in response to current injections with the amplitude of the second spike being smaller than the first spike (Fukui and Ohmori 2004). We therefore believe that the second spike is likely generated in the same unit as the first one, and the smaller amplitude results from the active K+ conductances and the residual Na+ channel inactivation owing to the short ISI to the first spike. Moreover, the second spikes in NM neurons may be triggered by the second spike of ANF units; in fact, ISI between the first and second spikes shows an overlapping distribution (Fig. 7C), and the mean ISI was the same in ANF (1.40 ± 0.05 ms) and in NM units (1.40 ± 0.10 ms).

The multiple firings called “peak-splitting” were observed in cat ANFs when high-intensity sound stimuli were applied (Kiang 1990). Some peak-split histograms showed a larger number of firings for the second peak, and the splitted-peak merged, accompanied by a displacement of the phase at still higher intensity stimuli; these were not observed in our experiments and the phenomenon is likely different from the multiple spiking reported in this paper.

Mechanisms of improvement of synchronized firing in NM

The previously described results from AVCN bushy cells have been attributed to synaptic convergence; each neuron acts as a monoaural coincidence detector for individual ANF synaptic inputs of small amplitude, which induce subthreshold responses (Joris et al. 1994). A major argument against this hypothesis is that bushy cells are known to be innervated by large and powerful synapses, end-bulbs of Held, and the synapse is reported to generate action potentials regularly in response to single ANF inputs (Liberman 1991; Rhode and Greenberg 1992; Ryugo and Sento 1991). However, this may not be true across the entire tonotopic axis of AVCN. A tonotopic gradient is reported in the size of ANF synapses formed on spherical bushy cells (Liberman 1991; Sento and Ryugo 1989). Synaptic size was quantified with a measure of the bushy cell surface area covered, called the contact index, defined as the ratio of presynaptic area of ANF in contact with the postsynaptic membrane to the somal surface area of postsynaptic neuron. Comparison of contact indices across the tonotopic axis suggests smaller synaptic size for both the lower (150–500 Hz) and higher (10–50 kHz) CF neurons compared with middle CF neurons (1–2 kHz). The smaller contact index on low CF bushy cells could allow for multiple synaptic innervations, allowing the coincidence detector model proposed in the preceding text (Joris et al. 1994). However, more details on the tonotopic variation of synaptic currents or the membrane excitabilities should be investigated in bushy cells before this issue is considered to be resolved.

In the chicken NM, ANF terminals in the low CF region (<500 Hz, estimated from the position in NM) are bouton-shaped, while large end-bulb-shaped terminals are present in higher CF regions (Carr and Boudreau 1991; Fukui and Ohmori 2004; Parks and Rubel 1978). In low CF neurons, most single excitatory postsynaptic potentials (EPSPs) were of sub-threshold amplitude and unable to generate action potentials on their own (Fukui and Ohmori 2004). Therefore summation of synaptic inputs is required for generation of action potentials, indicating that a monaural coincidence detection mechanism is likely operating in the synapse between ANF to NM neurons. The large presynaptic terminal and large EPSC evoked by ANF stimulation means that higher CF NM units likely fire in a high-fidelity, synchronized manner with their ANF inputs (Brenowitz and Trussell 2001; Zhang and Trussell 1994). Thus improvements in phase locking across this synapse are possible in low CF neurons due to convergence but are unlikely to occur in higher CF neurons due to the lack thereof.

In the barn owl, NM neurons are innervated by a few ANFs with large terminal structures at higher CFs (Carr and Boudreau 1991) and by larger numbers of ANFs with small bouton-like terminals in extremely low CF neurons (<0.64 kHz) (Köppl 1994). Based on the results of the present study, we expect that NM neurons in this extreme low CF region of the barn owl will exhibit an improvement in phase locking compared with the ANFs that innervate them.

GABAergic input from superior olivary nucleus (SON) is known to inhibit the activity of NM neurons (Lachica et al. 1994; Monsivais et al. 2000). The low-frequency improvement of vector strength for NM over ANFs observed in the present study was evident at supra- but not peri-threshold intensities (Fig. 5). SON inhibitory feed back mechanisms could contribute to the improvement in phase locking at high intensities. Further studies will be required to understand the potential contribution of GABAergic feedback to the level dependent improvement of phase locking in NM.

Improvement of synchronization is essential in the low CF region

In low CF units, although vector strength is high, temporal jitter is still larger than in the higher CF units (Fig. 10). In high CF neurons, the temporal jitter in ANF is already small, and therefore high-fidelity transmission through a large end-bulb-type of synaptic terminal seems appropriate. For low CF units, the large temporal jitter was improved during transmission from ANF to NM, and this improvement of phase locking may
indicate that NM is not simply relaying the timing information from AN to NL but actually sharpening it.

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REFERENCES


