Tonotopic Specialization of Auditory Coincidence Detection in Nucleus Laminaris of the Chick

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The interaural time difference (ITD) is a cue for localizing a sound source along the horizontal plane and is first determined in the nucleus laminaris (NL) in birds. Neurons in NL are tonotopically organized, such that ITDs are processed separately at each characteristic frequency (CF). Here, we investigated the excitability and coincidence detection of neurons along the tonotopic axis in NL, using a chick brainstem slice preparation. Systematic changes with CF were observed in morphological and electrophysiological properties of NL neurons. These properties included the length of dendrites, the input capacitance, the conductance of hyperpolarization-activated current, and the EPSC time course. In contrast to these gradients, the conductance of low-threshold K^+ current and the expression of Kv1.2 channel protein were maximal in the central (middle-CF) region of NL. As a result, the middle-CF neuron had the smallest input resistance and membrane time constant, and consequently the fastest EPSP, and exhibited the most accurate coincidence detection. The specialization of middle-CF neurons as coincidence detectors may account for the high resolution of sound-source localization in the middle-frequency range observed in avians.

Key words: nucleus laminaris; tonotopy; Kv1.2; coincidence detection; dendrite; sound localization

Introduction

The acuity of azimuthal sound-source localization depends on the frequency of sound, and in some species, the highest acuity is observed in the middle of the audible range of frequencies (Klump, 2000). For example, the highest acuity is ~ 4 kHz for the barn owl (Knudsen and Konishi, 1979), 2 kHz for the great tit (Klump et al., 1986), 4 kHz for the canary (Park and Dooling, 1991), and 1 kHz within the low-frequency band (0.5–1.5 kHz) for humans (Mills, 1958). The interaural time difference (ITD) is a significant cue for the sound-source localization in the azimuth plane (Klump and Eady, 1956; Moiseff and Konishi, 1981; Klump, 2000). The acuity of ITD detection likely depends on several factors: the synchronized firing of auditory nerve fibers and relay neurons at a particular phase of sound (phase-locking) and the number and sensitivity of ITD-sensitive neurons (Fitzpatrick et al., 1997; Fitzpatrick and Kuwada, 2001). The strength of phase-locking (the vector strength) (Goldberg and Brown, 1969) depends on sound frequency, such that vector strength decreases with an increase in characteristic frequency (CF) (Hill et al., 1989; Carr and Konishi, 1990; Warchol and Dallos, 1990). Therefore, it seems unlikely that the ability to phase-lock could by itself determine the improvement of sound-source localization in the middle-frequency range.

In birds, ITDs are first encoded in the nucleus laminaris (NL)

(Carr and Konishi, 1990; Overholt et al., 1992). Neurons in NL are highly sensitive to ITDs, and their intrinsic membrane properties, especially the strong outward rectification, are inevitable for improving the ITD sensitivity (Reyes et al., 1996; Funabiki et al., 1998; Kuba et al., 2002b, 2003). Roles of inhibitory synaptic inputs are also proposed to improve the ITD sensitivity (Fujita and Konishi, 1991; Pena et al., 1996; Bruckner and Hyson, 1998; Funabiki et al., 1998; Yang et al., 1999).

In NL, neurons are arranged tonotopically from the rostromedial high CF to the caudolateral low CF (Rubel and Parks, 1975), and ITDs are determined separately at each CF. Furthermore, NL neurons exhibit a striking systematic change in their morphology along the tonotopic axis; neurons with high CF have a large number of short dendrites, whereas those with low CF have a small number of long dendrites (Smith and Rubel, 1979). These observations suggest the possibility that other features of NL neurons could be specialized along the tonotopic axis and may contribute to the acuity of sound-source localization in a frequency-dependent manner.

In this study, we examined properties of neurons in the chick NL, using whole-cell patch recordings in brain slices. We observed gradients in morphology, certain synaptic properties, and membrane properties of these neurons along the tonotopic axis. Moreover, we found that middle-CF neurons showed a robust expression of Kv1.2, one of the channels responsible for low-threshold K^+ current (I_{LTK}), as well as fast EPSPs, features that would make these cells the accurate coincidence detectors in NL. These cellular specializations may be critical to the frequency dependence of sound-source localization.

Materials and Methods

Slide preparations. Animals were kept and used according to the regulations of the Animal Research Committee, Graduate School of Medicine,

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Kyoto University. Chickens [posthatch day 3 (P3) to P11] were deeply anesthetized with halothane (Fluothan; Takeda, Osaka, Japan) before decapitation, and coronal brain slices (200–250 μm) were obtained as described previously (Kuba et al., 2002b). Slices were mounted on a recording chamber on the stage of an upright microscope (BX50WI; Olympus, Tokyo, Japan) and continuously perfused with artificial CSF (in mM: 125 NaCl, 2.5 KCl, 26 NaHCO_3 , 1.25 NaH_2PO_4 , 2 CaCl_2 , 1 MgCl_2 , and 17 glucose, pH 7.6) at 40°C. Neurons were visualized with an 60 $\times$ objective lens and Nomarski optics equipped with an infrared differential interference contrast CCD camera (C5999; Hamamatsu Photonics, Hamamatsu, Japan).

Electrophysiological recordings and data acquisition. Whole-cell recordings were made with a patch-clamp amplifier (EPC-8; Heka, Lambrecht, Germany) for voltage-clamp experiments or with a current-clamp amplifier (MEZ-8301; Nihon Kohden, Tokyo, Japan) for current-clamp experiments, as described previously (Kuba et al., 2002b, 2003). When both EPSCs and EPSPs were recorded from the same neuron, the patch-clamp amplifier was used (see Fig. 5). Patch pipettes were made from thin-walled borosilicate glass capillaries (GC150TF-100; Harvard, Holliston, MA) and had a resistance of 2–3 M Ω when filled with a KCl-based internal solution (in mM: 160 KCl, 0.2 EGTA, and 10 HEPES-KOH, pH 7.4). Pipettes were coated with a silicone resin (Sylgard; Dow Corning Asia, Tokyo, Japan) and fire polished before use. The electrode capacitance and series resistance (3–6 M Ω) were estimated and compensated electronically by 60–80%. Series resistance errors were evaluated as described previously (Kuba et al., 2003). The liquid-junction potential (3.1 mV) was corrected after the experiment. GABA_A receptors were blocked by adding 20–40 μM bicuculline (Sigma, St. Louis, MO) to the extracellular medium. Miniature EPSCs (mEPSCs) were recorded in the presence of 1 μM TTX (Sankyo, Tokyo, Japan). Data were sampled at 10–50 kHz as described previously (Kuba et al., 2002b).

Electrical stimulation of presynaptic fibers. Electrical stimuli of ipsilateral and contralateral projection fibers from the nucleus magnocellularis (NM) were made using bipolar tungsten electrodes, as described previously (Kuba et al., 2002b). In experiments designed to examine coincidence detection (see Figs. 7 and 8), trains of four stimuli at 10 ms inter-stimulus intervals were applied to projection fibers either unilaterally or bilaterally, changing the time separation between two sides (Δt). Δt was defined as 0 when the firing probability was maximal. Positive and negative values indicate contralateral-leading and ipsilateral-leading stimuli, respectively. The firing probability, defined as the total number of spikes divided by the total number of stimuli, was calculated at each Δt from 10 to 30 trials (40–120 stimuli). The “stimulus intensity” was tuned at the beginning of each experiment so that unilateral stimuli alone evoked EPSPs but were not strong enough to generate a spike (Kuba et al., 2002b). The probabilities of spike generation by unilateral stimuli were 0.01 ± 0.01 ($n = 10$) in high-CF neurons, 0.01 ± 0.01 ($n = 15$) in middle-CF neurons, and 0 ($n = 6$) in low-CF neurons ($p = 0.47$). Once set, the stimulus intensity was maintained throughout the experiment. Effects of stimulus intensity on the coincidence detection were tested previously (Kuba et al., 2003).

Data analysis. The CF of neurons was predicted from the linear relationship between CF and the mediolateral and rostrocaudal location in NL (Rubel and Parks, 1975); five coronal slices containing NL were obtained from each animal. The level of each slice could be confirmed by the arrangement of NL and adjacent NM in each slice. In the rostral-most slice, NL showed a ventromedial to dorsolateral orientation, and NM was not observed. In the second slice, the rostral pole of NM appeared, and NL neurons were still lined in a ventromedial to dorsolateral orientation (see Fig. 6E). In the third and fourth slices, NL gradually tilted toward a mediolateral orientation and significantly increased its length (see Fig. 6F). In the caudal slice, NL was oriented in a mediolateral direction, and neurons were slightly scattered. At this level, NM extended widely in a mediolateral orientation (see Fig. 6G). Each slice was divided into 1–3 sectors depending on their mediolateral position within the nucleus, and 11 sectors were defined on the two-dimensional reconstructed image of the entire nucleus (Fig. 1). We classified these sectors into three CF regions: the rostral sectors 1–4 as the high-CF region (open sectors), the middle sectors 5–8 as the middle-CF region (gray sectors), and the caudal

Figure 1. Planar projection of NL. Two-dimensional image of NL was constructed on a percentile grid (Rubel and Parks, 1975), and 11 sectors were defined depending on caudal-to-rostral and lateral-to-medial positions within the nucleus. From the linear relationship between CF and rostrocaudal and mediolateral position in NL (Rubel and Parks, 1975), these sectors were classified into three regions, and the characteristic frequency of each region was estimated as follows: high-CF region (open sectors, 2.5–3.3 kHz), middle-CF region (gray sectors, 1–2.5 kHz), and low-CF region (filled sectors, 0.4–1 kHz). Dotted lines indicate isofrequency lines corresponding to 2.5 and 1 kHz, respectively.

sectors 9–11 as the low-CF region (filled sectors). These should correspond to a CF of 2.5–3.3 kHz for the high-CF, 1–2.5 kHz for the middle-CF, and 0.4–1 kHz for the low-CF region, respectively.

Analysis of action potentials and synaptic responses were made as described previously (Kuba et al., 2002b). Data are given as mean $\pm$ SE (n = number of cells). Statistical significance was tested with one-way ANOVA and a *post hoc* (Scheffé's *F*) test for comparisons among the CF regions or sectors, unless otherwise stated.

Morphological study. Whole-cell recorded neurons were marked with Lucifer yellow CH (5 mg/ml in the internal solution; Molecular Probes, Eugene, OR); slices were then fixed with 2% (w/v) formaldehyde in PBS, pH 7.4, for ~2–6 h at room temperature (20–25°C). They were then mounted onto glass slides, dehydrated, cleared in xylene, coverslipped, and observed under a confocal laser-scanning microscope with a confocal depth of 1 μm (CSU 10; Yokogawa, Tokyo, Japan). Each primary dendrite was identified on these sequential images, and its width was measured at 2–5 μm away from the soma–dendrite boundary. Somatic surface area and dendritic length were measured from a series of confocal images (IPLab). The soma was represented as an ellipsoid formed by rotating an ellipse along its major axis, and the somatic surface area (A) was calculated using the following formula (Nitzan et al., 1990):

$$A = 2\pi b^2 + \frac{2\pi b}{\epsilon} \arcsin(\epsilon),$$

where $2a$ and $2b$ are the lengths of the major and minor axes, respectively, and

$$\epsilon = \frac{\sqrt{a^2 - b^2}}{a}.$$

The length of dendrite was measured for each primary dendrite, by summing the length of all of the branches. The dendritic length was thus calculated as an average length of all of the primary dendrites in one neuron.

NL neurons were also retrogradely labeled with lipophilic dye [1,1'-diiododecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (Di-I);

Molecular Probes] after fixation, as follows. Chicks (P7–P9) were deeply anesthetized by ether, perfused transcardially with 4% (w/v) formaldehyde in PBS, and postfixed for ~10 h at 4°C. A Di-I crystal was placed in the inferior colliculus, and the brain was incubated for 6 months at 37°C in PBS containing 0.02% (w/v) sodium azide.

Immunohistochemistry. Under deep anesthesia, seven chicks (P3–P8) were perfused transcardially with periodate–lysin–paraformaldehyde fixative (ml/g body weight): 2% (w/v) paraformaldehyde, 2.7% (w/v) lysin HCl, 0.21% (w/v) NaIO₄, and 0.1% (w/v) Na₂HPO₄. This fixation procedure is thought to preserve the antigenicity of proteins better than the conventional fixation procedure using formaldehyde as a result of the mild interaction of fixatives with proteins (McLean and Nakane, 1974). The brainstem was postfixed for 4 h at 4°C. After cryoprotection with 30% (w/w) sucrose in PBS, coronal (four chicks) or transverse (three chicks) sections (20 μ m thickness) were prepared with a cryostat (CM3050S; Leica, Nussloch, Germany).

Sections were incubated overnight with 1 μ g/ml anti-Kv1.2 channel rabbit polyclonal antibody (APC-010; Alomone Labs, Jerusalem, Israel). The antibody was raised against a peptide that had complete homology in the chicken brain Kv1.2 (GenBank accession number AY329361). The staining pattern with this antibody was the same as that obtained with anti-Kv1.2 mouse monoclonal antibody (5 μ g/ml; 05-408; Upstate Biotechnology, Lake Placid, NY). The incubation was performed at room temperature in PBS containing 0.3% (v/v) Triton X-100, 0.25% (w/v) λ -carrageenan, 1% (v/v) donkey serum, and 0.02% (w/v) sodium azide, and then rinsed with PBS containing 0.3% (v/v) Triton X-100. Sections were then incubated for 2 h in PBS with 10 μ g/ml biotinylated anti-rabbit IgG donkey antibody (Chemicon, Temecula, CA), and for 1 h with 1 μ g/ml Alexa 594 conjugated with streptavidin (Molecular Probes). Sections were mounted onto glass slides, coverslipped, and observed under a confocal laser-scanning microscope. Preadsorption of the antibody with the Kv1.2–glutathione S-transferase (GST) fusion protein (3 μ g/ml; Alomone Labs) blocked the staining in the chicken brain sections.

Kv1.2 immunoreactivity of NL neurons in each sector was quantitatively measured using NIH ImageJ; the outline of the cell body was traced, and the fluorescence intensity within the selected area, including the nucleus, was measured for each cell. At least 10 neurons were measured in each sector from four coronal slices (excluding the most rostral slice, the one containing sector 1). After subtracting the baseline intensity corresponding to the dark level of the CCD camera, fluorescence intensities of cells were normalized relative to the fluorescence intensity measured in the medial vestibular nucleus over an area of 7500 μ m². The reference intensities were not different among slices ($p = 0.88$). The normalized intensities of individual cells were averaged for sectors (see Fig. 6I).

Results

Morphology and membrane properties

NL neurons extend their bipolar dendrites in ventral and dorsal directions. The morphology of the dendrites changes systematically along the tonotopic axis (Smith and Rubel, 1979). We confirmed these previous observations by filling neurons with Luci-

Figure 2. Confocal images of NL neurons. A series of pictures were taken with a confocal microscope with a z-depth of 1 μ m and stacked up to one image. **A–C**, Lucifer yellow-labeled neurons: high-CF (**A**), middle-CF (**B**), and low-CF (**C**) neuron. Length of dendrites increased from high- to low-CF neuron. **D, E**, Di-I-labeled neurons: high-CF (**D**) and middle-CF (**E**) neuron. Note the different scale in **C**. LY, Lucifer yellow; V, ventral; L, lateral.

fer yellow during whole-cell recording (Fig. 2, Table 1). In the high-CF region, neurons had a large number of relatively unbranched short dendrites (Fig. 2A, from sector 1). The number of dendrites decreased in the middle-CF neurons, but they became thicker and longer (Fig. 2B, from sector 7). Only a few primary dendrites extended away from the soma in the low-CF neurons, and they had extensive branching (Fig. 2C, from sector 10). The dendritic length and width systematically increased and were greatest in the low-CF neurons (Table 1) (Smith and Rubel, 1979). The somatic surface area also increased slightly toward the low-CF neurons (Table 1) (Deitch and Rubel, 1989). When NL neurons were labeled with Di-I after fixation, dendrites and axon were clearly delimited, and the dendritic width was 40–70% greater than those listed in Table 1 (Fig. 2D,E) (see Discussion).

The resting membrane potential was slightly depolarized in low-CF neurons ($p < 0.05$), and -58.5 ± 0.7 mV ($n = 23$) in high-CF, -58.9 ± 0.4 mV ($n = 33$) in middle-CF, and -56.6 ± 0.9 mV ($n = 19$) in low-CF neurons. Parameters of basic membrane properties were determined under current clamp by injecting small hyperpolarizing currents from the membrane potential

Table 1. Morphology of neurons filled with Lucifer yellow during electrical recordings

	High CF (<i>n</i> = 8) ^a	Middle CF (<i>n</i> = 6)	Low CF (<i>n</i> = 5)
Somatic surface area (μm^2) ^b	581.8 $\pm$ 37.6	620.2 $\pm$ 45.4	813.1 $\pm$ 118.8
Number of primary dendrites ^c	19.5 $\pm$ 1.3	16.7 $\pm$ 1.5	4.2 $\pm$ 0.2
Dendritic length (μm) ^c	8.9 $\pm$ 1.3	20.9 $\pm$ 5.1	155.6 $\pm$ 29.4
Dendritic width (μm) ^b	1.4 $\pm$ 0.1	1.6 $\pm$ 0.1	1.9 $\pm$ 0.1
Simulated capacitance (pF) ^d	13.4	23.7	47.1

^aNumber of cells is indicated in parentheses in this table and in Table 2.

^bDifference was significant between high- and low-CF neurons ($p < 0.05$) but was not significant between the other two pairs ($p > 0.1$).

^cDifference was significant between low-CF neurons and neurons in the other two regions ($p < 0.01$) but was not significant between high- and middle-CF neurons ($p > 0.1$).

^dInput capacitance was simulated from the somatic surface area and the dendritic surface area using the specific capacitance of $1 \mu\text{F}/\text{cm}^2$. The dendritic surface area was estimated as the simple integration of the length and the width times the dendritic number.

maintained at approximately -63 mV. Voltage responses could be fitted by a single-exponential function in the high-CF and the middle-CF neurons and by a double-exponential function in the low-CF neurons. The double-exponential fitting in the low-CF neurons yielded $24.1 \pm 2.0\%$ for the faster (time constant, 0.18 ± 0.01 ms) and $75.9 \pm 2.0\%$ for the slower (1.31 ± 0.13 ms) component ($n = 19$). The faster component probably reflected the contribution of the long dendrites; thus, the slower component was chosen as the membrane time constant of the cell body (Rall, 1969). Figure 3A shows representative records, and the grand averages of these values are presented in Figure 3B–D. The double-exponential fitting in the low-CF neurons yielded a significantly smaller error when evaluated as the sum of squared errors than did the single-exponential fitting ($n = 19$; $p < 0.05$ by paired *t* test).

The time constant and input resistance were smallest in the middle-CF neuron (Fig. 3B,C) (statistical significance was tested in reference to the middle-CF neuron in this and subsequent

figures, unless otherwise stated). The input capacitance gradually increased from high-CF to low-CF neurons (Fig. 3D). Low-CF neurons had significantly greater input capacitance than neurons in the other two CF regions ($p < 0.01$); however, the difference between the high-CF and the middle-CF neuron was not significant ($p = 0.36$). The neurons presented in Figure 2A–C showed similar tendencies. They had a membrane time constant of 0.77 ms and input resistance of $15.7 \text{ M}\Omega$ for the high-CF, 0.46 ms and $8.4 \text{ M}\Omega$ for the middle-CF, and 1.03 ms and $12.7 \text{ M}\Omega$ for the low-CF cell. The input resistance and membrane time constant were relatively small in these cells, but the input capacitance was close to the grand average: 49.4, 54.5, and 81.1 pF , respectively, for the high-CF, middle-CF, and low-CF cells. The input capacitance estimated electrophysiologically (Fig. 3D) was approximately two times larger than the value estimated morphologically (Table 1, simulated capacitance) (see Discussion).

Conductance for I_{LTK} and I_{h}

Neurons in NL have a significant conductance at the resting potential because of the activation of outward-going conductance, attributable to I_{LTK} , and the hyperpolarization-activated cation current (I_{h}) (Reyes et al., 1996; Funabiki et al., 1998; Kuba et al., 2002b, 2003). We investigated the membrane properties along the tonotopic axis with special interest in these two currents. Figure 4A shows voltage responses to both depolarizing (top traces) and hyperpolarizing (bottom traces) currents. With depolarization, NL neurons generated a single action potential at the onset of current injection. During hyperpolarization, all of the neurons showed depolarizing voltage sag, reflecting the activation of I_{h} current. The size of peak hyperpolarization may reflect the input resistance of the neuron and was largest in the high-CF neuron.

The current–voltage relationship measured at the pulse end showed outward rectification (Fig. 4B). The limiting slope conductance for both the depolarizing and hyperpolarizing directions was measured along the three CF regions.

Figure 3. Membrane properties along the tonotopic axis. **A**, Voltage responses to a small hyperpolarizing current injection of 7 ms duration and 0.04 nA amplitude. **a**, High-CF neuron; **b**, middle-CF neuron; **c**, low-CF neuron. Resting membrane potential is indicated near the trace in this and in the following figures. Thinner lines indicate exponential fittings to the trace, and the time constants are indicated at the bottom left. **c**, Note two time constants in the low-CF neuron attributable to a double-exponential fitting. **B–D**, Membrane time constant (**B**), input resistance (**C**), and input capacitance (**D**). Here and in subsequent figures, the numbers in parentheses are the numbers of cells. Statistical significance is evaluated between middle-CF neurons and neurons in the other two regions and is indicated by an asterisk for $p < 0.01$ in this and the following figures. Between high- and low-CF neurons, the difference is significant for the input resistance and the input capacitance ($p < 0.05$) but not significant for the membrane time constant ($p = 0.58$). Here and in the following figures, error bars indicate SE.

The depolarizing limiting slope conductance measured between 3.6 and 4 nA current injection was largest in the middle-CF neurons and decreased in both high-CF and low-CF neurons ($p < 0.01$) (Fig. 4Ca, open bars). The limiting slope conductance with hyperpolarization was similarly measured between -1.6 and -2 nA current injection and increased monotonically from the high-CF to the low-CF neurons ($p < 0.01$) (Fig. 4Cb, open bars). The outward conductance was sensitive to dendrotoxin (DTX)-I (40 nM; Alomone Labs) (Fig. 4Ca, filled bars). The difference between the open and the filled bars is the conductance from I_{LTK} , and difference is greatest in the middle-CF neuron. In the presence of DTX, NL neurons generated a few or several spikes at the onset of current injection but did not fire persistently; therefore, the slope conductance was measured at the pulse end similarly as in the control (data not shown) (Kuba et al., 2002b). The inward conductance and the voltage sag were sensitive to 4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)pyridinium chloride (ZD7288), a blocker of I_{h} (100 μM ; Tocris Cookson, Bristol, UK) (Fig. 4Cb,

filled bars). The conductance of I_h increased monotonically from high- to low-CF neurons.

The input resistance measured in DTX by injecting a small hyperpolarizing current (0.04 nA) was 1.7 times larger than the control in the high-CF, approximately twice as large in the middle-CF, and 1.3 times larger in the low-CF neurons (Table 2). ZD7288 increased the input resistance 1.2 times in the high-CF, 1.5 times in the middle-CF, and ~ 2 times in the low-CF neurons (Table 2). These results indicate that a significant part of the input resistance is set by I_{LTK} in both high-CF and middle-CF neurons, whereas I_h is most responsible in low-CF neurons.

Suprathreshold responses

The threshold current required to generate an action potential was larger in the middle-CF neurons (3.3 ± 0.1 nA; $n = 39$) than in the high-CF (2.3 ± 0.2 nA; $n = 23$) and the low-CF (2.5 ± 0.2 nA; $n = 27$) neurons ($p < 0.01$). In the high- and middle-CF regions, all of the neurons generated a single action potential during depolarization. In the caudolateral end (low CF), however, some neurons generated several spikes after the first action potential (17 of 42 cells; data not shown). In these multiple spiking neurons, the limiting slope conductance at depolarization was significantly smaller (188.8 ± 12.8 nS; $n = 17$) than that of single spiking neurons (257.3 ± 19.2 nS; $n = 25$) in the low-CF region ($p < 0.01$ by unpaired t test). The limiting slope conductance of low-CF neurons presented in Figure 4C*a* was the mean of multiple spiking neurons and single spiking neurons ($n = 42$). The slope conductance at hyperpolarization was not different ($p = 0.93$ by unpaired t test): 177.1 ± 11.8 nS ($n = 17$) for the multiple and 175.7 ± 11.2 nS ($n = 25$) for the single spiking neurons.

The size of action potentials was two to three times smaller in the high-CF and the middle-CF neurons than that in the low-CF neurons (Fig. 4D). In the high- and the middle-CF neurons, the maximum rate of rise of action potential was smaller by three times than that in the low-CF neurons (Fig. 4E). The inward-going Na^+ current may be counterbalanced by I_{LTK} in the high- and the middle-CF neurons, but the maximum rate of rise increased only 1.4 times in the middle-CF neuron after block of I_{LTK} by DTX (200.3 ± 29.7 V/s; $n = 5$; $p < 0.05$ by unpaired t test compared with the control).

EPSC and EPSP

In Figure 5A, the EPSC and EPSP recorded in the same neurons were size-normalized and superimposed to facilitate comparison of the time course. Stimulus intensities were adjusted to evoke EPSPs of similar sizes, as follows: 6.2 ± 0.3 mV ($n = 27$) in the high-CF neuron, 6.5 ± 0.5 mV ($n = 22$) in the middle-CF neuron, and 7.0 ± 0.3 mV ($n = 14$) in the low-CF neuron ($p = 0.36$).

Figure 4. Membrane conductances along the tonotopic axis. **A**, Voltage responses to depolarizing (4 nA; top) and hyperpolarizing (−2 nA; bottom) current injections of 70 ms duration. **a**, High-CF neuron; **b**, middle-CF neuron; **c**, low-CF neuron. **B**, Voltage–current relationships measured at the end of current injection from −2 to 4 nA, with a step of 0.2 nA; 35 cells in the high-CF neuron; (open circles), 58 cells in the middle-CF region (gray triangles), and 42 cells in the low-CF region (filled squares). Error bars were so small and were overlaid by symbols. **C**, Limiting slope conductance. Above (**a**) and below (**b**) the resting potential. Open bars, control; filled bars, in the presence of DTX (**a**) and ZD7288 (**b**). Limiting slope conductances were measured between 3.6 and 4 nA for **a**, and between −1.6 and −2 nA for **b**. After application of DTX or ZD7288 (filled bars), the limiting conductance decreased significantly compared with the control (open bars) ($p < 0.01$ by unpaired t test), except for the low-CF neurons in DTX ($p = 0.1$ by unpaired t test). Controls are from neurons in **B**. For high-, middle-, and low-CF region, records are from 7, 12, and 8 cells in DTX and 7, 7, and 6 cells in ZD7288. **D**, **E**, Amplitude (**D**) and maximum rate of rise (**E**) of the action potentials, induced by a short current injection of 0.5 ms, 5 nA.

Table 2. Effects of DTX and ZD7288 on input resistance

	High CF	Middle CF	Low CF
Control ^a	24.1 ± 2.0 ^b (23)	10.9 ± 0.7 (33)	17.6 ± 1.2 (20)
DTX (40 nM) ^c	39.1 ± 5.5 (7)*	22.2 ± 1.2 (5)*	22.4 ± 2.6 (6)
ZD7288 (100 μM) ^d	30.8 ± 9.9 (7)	15.1 ± 2.9 (9)**	35.7 ± 9.3 (6)*

^aControls are from Figure 3C.

^bValues are given in MΩ.

^cIn DTX, difference was significant between high-CF neurons and neurons in the other two regions ($p < 0.01$) but was not significant between middle- and low-CF neurons ($p = 0.85$).

^dIn ZD7288, difference was not significant among the three CF regions ($p = 0.25$).

*Statistically significant difference ($p < 0.01$ by unpaired t test) compared with the control in each CF.

**Statistically significant difference ($p < 0.05$ by unpaired t test) compared with the control in each CF.

At this stimulus intensity, there was a slight difference in the EPSC amplitude, as follows: 1.3 ± 0.2 nA ($n = 27$) in the high-CF neuron, 2.3 ± 0.2 nA ($n = 22$) in the middle-CF neuron, and 1.8 ± 0.2 nA ($n = 14$) in the low-CF neuron ($p < 0.01$ between high- and middle-CF neurons). However, this difference in EPSC

Figure 5. Synaptic responses along the tonotopic axis. **A**, EPSC (thinner traces, polarity reversed) and EPSP (thicker traces) from the same neurons are size-normalized and superimposed. EPSCs were recorded at -80 mV, and EPSPs were recorded at the resting potential, indicated to the left. **a**, High-CF neuron; **b**, middle-CF neuron; **c**, low-CF neuron. **B**, **C**, Ten to 90% rise time (open bars) and half-amplitude width (filled bars) of EPSC (**B**) and EPSP (**C**). Between the high- and the low-CF neurons, the difference is statistically significant for the half-amplitude width of EPSC ($p < 0.01$) and EPSP ($p < 0.05$). **D**, Ten to 90% rise time histogram of mEPSC from populations of neurons. **a**, High-CF region (1479 mEPSCs from 16 cells); **b**, middle-CF region (2850 mEPSCs from 33 cells); **c**, low-CF region (2332 mEPSCs from 19 cells). Mean values are indicated by arrows. Inset, Ensemble averaged mEPSC. Note a progressively larger distribution of slower components toward the low-CF neurons.

amplitude had little effect on the time course. When stimulus intensities were changed in neurons of the middle-CF region, the half-amplitude width was 0.46 ± 0.02 ms for EPSCs of ~ 1.3 nA but 0.48 ± 0.03 ms at 2.2 nA ($n = 4$; $p = 0.76$ by paired t test).

The EPSC time course changed along the tonotopic axis (Fig. 5A, thinner traces, B); both the 10–90% rise time (open bar) and the half-amplitude width (filled bar) of the EPSC was smallest in the high-CF neuron and became larger in the lower-CF neuron. Because neurons had more extensive dendrites toward the low-CF region (Fig. 2), this systematic prolongation of EPSC time course along the tonotopic axis may indicate the influence of dendritic filtering. In dendrites of lower-CF neurons, voltage clamp might not be adequate, which might have distorted the EPSC time course (Spruston et al., 1994) (see also Discussion). In parallel with the EPSC time course, the mEPSC showed a tendency of prolongation toward the low-CF region (Fig. 5D) ($p < 0.01$ by Kolmogorov–Smirnov test). The median of 10–90% rise time of mEPSCs was 0.08, 0.10, and 0.14 ms in the high-, middle-, and low-CF neurons, respectively (Fig. 5D, arrows), and their ratio (1:1.25:1.75) was similar to that of EPSC rise time: 0.15:0.20:0.28 ms (1:1.33:1.87) (Fig. 5B). The time course of the EPSC and

mEPSC could be affected by other factors, such as the distribution and the kinetics of receptor molecules (Mosbacher et al., 1994). However, we have not tested this possibility.

In contrast, the time course of the EPSP did not vary monotonically along the tonotopic axis (Fig. 5A, thicker traces, C). The 10–90% rise time (Fig. 5A, open bar) of EPSPs was small and similar both in the high- and the middle-CF neuron and large in the low-CF neuron. However, the half-amplitude width of EPSPs (Fig. 5A, filled bar) was smallest in the middle-CF neurons, followed by the high-CF neurons, and then by the low-CF neurons. This distribution was consistent with the distribution of the membrane time constant (Fig. 3B), the input resistance (Fig. 3C), and the depolarizing limiting slope resistance (Fig. 4Ca, reciprocal of the conductance). A small hyperpolarizing afterpotential followed the EPSP in the middle-CF neuron (Fig. 5Ab), which is consistent with the idea that I_{LTK} is activated during depolarization and accelerates the falling phase of EPSP (Kuba et al., 2003).

During posthatch days from P3 to P11, the EPSP time course was slightly accelerated, but the difference was not statistically significant ($p > 0.1$ by unpaired t test in each CF region); the half-amplitude width of EPSP in P3–P6 was 1.05 ± 0.09 ms ($n = 23$) in the high-CF, 0.65 ± 0.04 ms ($n = 16$) in the middle-CF, and 1.84 ± 0.33 ms ($n = 10$) in the low-CF neuron, whereas that in P9–P11 was 0.85 ± 0.10 ms ($n = 3$), 0.59 ± 0.03 ms ($n = 4$), and 1.43 ± 0.23 ms ($n = 3$), respectively.

Expression of Kv1 channels in NL

The sensitivity of I_{LTK} conductance to DTX (Fig. 4Ca) suggests that Kv1 channels are responsible for I_{LTK} (Grismer et al., 1994). *In situ* hybridization studies have shown that NL neurons expressed Kv1.1 and Kv1.2 subunits; in particular, Kv1.2 was predominant in NL (Fukui and Ohmori, 2004). However, the immunopositivity of Kv1.1 in NL was only in the neuropil region (ventral and dorsal sides of cell bodies) and Kv1.2 was negative [sc-11188 (Santa Cruz Biotechnology, Santa Cruz, CA), APC-010 (Alomone Labs)] (Fukui and Ohmori, 2004). In the present study, we modified the fixation procedure (see Materials and Methods) and observed a clear immunoreactivity of Kv1.2 in NL. The immunoreactivity was strong in the soma and weak but present in the neuropil region (Fig. 6A–G). Preadsorption of the antibody with Kv1.2–GST fusion protein blocked the staining. We evaluated the expression level of Kv1.2 quantitatively by measuring the fluorescence intensity of cell soma (see Materials and Methods) (Fig. 6A, E–G). In both transverse (Fig. 6A) and coronal (Fig. 6E–G) sections (see orientation of sections in Fig. 6H), the level of Kv1.2 expression changed systematically along the tonotopic axis ($p < 0.01$ by Mann–Whitney U test in each slice); it was maximum in the middle region (Fig. 6C, middle-CF) and decreased toward both the ros-

tromedial (Fig. 6B, high-CF) and caudolateral (Fig. 6D, low-CF) regions. The graphs plotted in Figure 6, A and E–G, show the intensity of fluorescence relative to the mean of the vestibular nucleus in the same section (see Materials and Methods). The relative fluorescence intensity of each sector was summarized for four chicks in Figure 6I (see Materials and Methods). Within one slice, the higher intensity was observed in the lower CF sector in the rostral slices (sector 3 higher than sector 2), and in the higher CF sectors in the caudal slices (sectors 7 and 8 higher than sector 9; sector 10 higher than sector 11) ($p < 0.05$). These indicate that the expression of Kv1.2 channels is high in the middle-CF region (gray bars).

The immunoreactivity of Kv1.1 in NL was weak and positive especially in the neuropil region even by the present fixation procedure (APC-009; Alomone Labs) (Fukui and Ohmori, 2004). This is in contrast to the observation by Lu et al. (2004); Kv1.1 labeling in NL was predominant in the neuronal soma rather than in the neuropil, and the difference may be attributable to the different polyclonal antibody raised against Kv1.1. The neurons in NM showed a slight gradient of Kv1.2 immunoreactivity (Fig. 6G); the fluorescence intensity was higher in the lateral region ($p < 0.01$ by Mann–Whitney U test).

Tonotopic specialization of coincidence detection

The time course of the EPSP showed a strong positive correlation with the acuity of coincidence detection (Kuba et al., 2003). This might indicate that the coincidence detection of NL is specialized in the middle-CF region (Fig. 5A,B,C). In Figure 7, electrical stimuli were applied to evoke EPSPs bilaterally, and coincidence detection was evaluated by changing the time interval between the two sides (see Materials and Methods). In all three CF regions, Δt of 0 ms was set at the peak firing probability (Fig. 7A). With increase in the stimulus time interval, the firing probability decreased and formed a bell-shaped distribution when plotted against Δt (Fig. 7B). The time window, determined as the time interval to give the half-maximum firing probability, was 0.54 ms for the high-CF, 0.31 ms for the middle-CF, and 1.35 ms for the low-CF neurons. In Figure 8A, the firing probability from populations of neurons was calculated for each CF, and the curve was the sharpest in the middle-CF neuron (gray triangles), still sharp in the high-CF neuron (open circles), and broad in the low-CF neuron (filled squares).

The acuity of coincidence detection was evaluated by two measures; the time window (Fig. 8B) and the percentage maximum slope, defined as percentage maximum change in firing probability over 10 μ s of Δt (Fig. 8C). The time window was

Figure 6. Distribution of Kv1.2 channel protein along the tonotopic axis. **A**, Transverse slice stained with the Kv1.2 antibody. Boxes show the regions of NL magnified in **B–D**. The left panel in **A** and bottom panels in **E–G** show the relative fluorescence intensity of individual NL neurons (see Materials and Methods). Numbers on the abscissa indicate the sector of NL. **B–D**, Immunoreactivity is strong in the middle region (**C**) and decreases in both the rostromedial (**B**) and caudolateral (**D**) region ($p < 0.01$ by Mann–Whitney U test among the three CF regions in a slice). **E–G**, Coronal slices stained with the antibody. R, Rostral; M, medial; D, dorsal. The immunoreactivity increases from sector 2 to sector 3 in slice **E** (high CF) but decreases in slice **F** from sectors 7–8 (middle CF) to sector 9 (low CF) and decreases in slice **G** toward the lower-CF sector ($p < 0.01$ by Mann–Whitney U test among sectors in each slice). **H**, This gradient is developed along the tonotopic axis, which is directed from the rostromedial high CF to the caudolateral low CF. Each slice is illustrated by a dotted line in **H**. **I**, Relative fluorescence intensity in each sector. Data from four chicks were averaged, and comparisons were made among sectors within the same slices (see Materials and Methods). #Statistical significance for $p < 0.05$.

smallest and the percentage maximum slope was largest in the middle-CF neurons, indicating that the acuity is highest in the middle-CF neurons, whereas it is lowest in the low-CF neurons. For two low-CF neurons, we could not determine the time window, because the firing probabilities did not fall below the half-maximum value even at a Δt of ± 1 ms. Although the stimulus intensity was tuned as described in Materials and Methods (Kuba et al., 2002b, 2003), the peak firing probability was slightly lower in the high-CF neuron (Fig. 7B): 0.26 ± 0.02 in the high-CF neuron ($n = 10$), 0.42 ± 0.06 in the middle-CF neuron ($n = 15$),

Figure 7. Coincidence detection along the tonotopic axis. **A**, Four superimposed voltage traces in response to bilateral stimuli with three different time intervals between the two sides (Δt in milliseconds) (see Materials and Methods). In **A** and **B**, high-CF neuron (**a**), middle- (**b**), and low-CF neuron (**c**). **B**, Probability of spike generation as a function of Δt calculated from the neurons in **A**. Ipsi, ipsilateral; contra, contralateral.

and 0.43 ± 0.10 in the low-CF neuron ($n = 6$) ($p < 0.1$). This might be attributable to the different extent of synaptic depression during the stimulus train (Fig. 7A at Δt of -0.4 ms). During the train, the amplitude of EPSP decreased from 6.9 ± 0.5 to 2.3 ± 0.2 mV in the high-CF neuron ($n = 10$), from 6.9 ± 0.6 to 4.7 ± 0.4 mV in the middle-CF neuron ($n = 15$), and from 7.2 ± 1.3 to 3.6 ± 0.4 mV in the low-CF neuron ($n = 6$). The amplitude of the first EPSP during the train was not statistically different among three CF neurons ($p = 0.94$), whereas the fourth EPSP was significantly small in the high-CF neuron ($p < 0.01$). This slight difference in the firing probability would not affect the acuity of coincidence detection significantly. When groups of neurons in the high CF were compared in terms of those with a relatively high peak firing probability (0.40 ± 0.07 , ranging from 0.3 to 0.5; $n = 3$) and those with a relatively small peak firing probability (0.23 ± 0.01 , ranging from 0.2 to 0.3; $n = 7$), the acuity was not different ($p > 0.1$ by unpaired *t* test); the time window was 0.63 ± 0.09 ms ($n = 3$) and 0.57 ± 0.05 ms ($n = 7$), and the percentage maximum slope was 4.3 ± 0.4 ($n = 3$) and 5.3 ± 0.6 ($n = 7$), respectively, for the high and the low firing-probability neurons.

Discussion

Specialization of membrane properties and coincidence detection in middle-CF neurons

The middle-CF neuron is specialized in its membrane properties and in the accuracy of its coincidence detection, as follows: the

membrane time constant was the smallest (Fig. 3), the I_{LTK} conductance was the largest (Fig. 4Ca), the EPSP time course was the shortest (Fig. 5C), and the coincidence detection was the most accurate (Fig. 7). Other features of neurons showed some gradients in tonotopic axis.

Electrophysiological properties of NL neurons were previously examined along the tonotopic axis in the slice preparation of embryonic chicken [embryonic day 19 (E19) to E21] (Reyes et al., 1996). They observed that the EPSC time course increased at the low-CF region (two cells), although it did not change in the rostral three-fourths of NL (23 cells). However, neither the slope resistance measured below nor that measured above resting potential varied systematically along the tonotopic axis. This absence of tonotopic difference in the postsynaptic membrane property might be related to the development of animals, because significant developmental changes were observed around the time of hatching in the dendritic morphology (Smith, 1981) and the membrane excitability (Kuba et al., 2002b).

Tonotopic specialization of low-threshold K^+ currents

The immunoreactivity of Kv1.2 showed a distribution similar to the I_{LTK} conductance along the tonotopic axis, and was the strongest in the middle-CF neuron (Figs. 4Ca and 6I). The expression of Kv1.1 mRNA and protein was weak in NL compared with NM (Fukui and Ohmori, 2004; Lu et al., 2004). Therefore, it is conceivable that Kv1.2-containing channels mediate the I_{LTK} and contribute to the specialization of middle-CF NL neuron as a coincidence detector. Additional studies with subunit-specific toxins will be needed to determine the fraction of contribution of each subunit to the I_{LTK} .

Graded expression of Kv1 channels along the tonotopic axis is also reported in various auditory nuclei. In NM of the chicken, the monotonic gradient of the expression of both mRNA and protein of Kv1.1, as well as the DTX-sensitive current were found, increasing toward the high-CF region (Fukui and Ohmori, 2004). In the lateral superior olive of the rat (Barnes-Davies et al., 2004), neurons in the low-CF region expressed higher levels of Kv1 channels than those in the high-CF region, which allows the low-CF neurons to integrate precisely timed binaural signals.

Action potentials in the high- and middle-CF neurons

Action potentials were small in the high- and middle-CF neurons (Fig. 4A,D). This could be partly attributable to the large I_{LTK} conductance counterbalancing the inward current and also attributable to a small Na^+ conductance, because these cells exhibited a small maximum rate of rise (Fig. 4). Action potentials of <20 mV in amplitude were also reported in neurons specialized for processing auditory temporal signals, such as octopus cells of mammalian ventral cochlear nucleus (Golding et al., 1999) and chicken NM neurons (Reyes et al., 1994; Fukui and Ohmori, 2004). In these neurons, the DTX-sensitive I_{LTK} is robust. More-

Figure 8. Coincidence detection is most accurate in the middle-CF neuron. **A**, Firing probabilities normalized to the maximum value were calculated from 10 cells in the high-CF region (open circles), 15 cells in the middle-CF region (gray triangles), and 6 cells in the low-CF region (filled squares) and were plotted against the time difference (Δt). The firing probabilities from six high-CF cells at Δt of ± 1 ms were connected with dotted lines. The time intervals between ± 1 and ± 0.5 ms were not tested. **B**, **C**, Time window (**B**) and percentage maximum slope (**C**), the maximum change in firing probability per 10 μ s of Δt , were evaluated from each coincidence detection curve and plotted for three CF regions. * $p < 0.01$; # $p < 0.05$.

over, in the octopus cells, a block of I_{LTK} or I_h only moderately increased the spike size, which might indicate a small density of Na^+ channels (Golding et al., 1999).

Higher-CF NL neurons may be structurally specialized, because of myelination around the initial segment, but not in the low-CF neurons (in the chicken) (Carr and Boudreau, 1993b). Such myelination likely makes the spike size small because of the distance of the spike initiation site from the soma, probably at the first node of the axon. Similar observations were made in the barn owl NL but not in NM (Carr and Boudreau, 1993a,b). The roles of small action potentials in processing the auditory temporal information remain to be investigated.

Gradient of dendritic arborization in NL

The morphology of dendrites changes systematically along the tonotopic axis, and their length increases with a decrease in the

CF of neurons (Smith and Rubel, 1979) (Fig. 2). In the high- and middle-CF neuron, dendrites are not long (5–30 μ m) and showed little tapering but did exhibit a swelling near their ends (Smith and Rubel, 1979) (Fig. 2A,B,D,E). Accordingly, the membrane voltage responses in these neurons had a single exponential time course when induced by a small current injection (Fig. 3A); thus, the neurons in these regions seem electrically compact (Rall, 1969). This property should be ideal for processing fast synaptic responses and for improving coincidence detection.

In the low-CF neuron, the membrane voltage responses to a hyperpolarizing current injection showed a double-exponential time course (Fig. 3Ac), where τ_1 is the faster time constant related to dendrites and τ_0 is the slower time constant related to the membrane time constant of the cell soma (Rall, 1969). The electrotonic length (L), calculated from these time constants using the equation [$L = \pi/\sqrt{(\tau_0/\tau_1 + 1)}$] (Rall, 1969), was 1.36 ± 0.13 ($n = 19$), which is larger than those reported in other neurons [0.9 in hippocampal neurons (Brown et al., 1981) and 0.6 in cerebellar Purkinje cells (Rapp et al., 1994)]. This suggests that EPSPs in low-CF neurons are extensively filtered (Spruston et al., 1994). The relatively large electrotonic length of the low-CF NL neuron may partly result from the thinner dendrites (thinner than 2 μ m) (Table 1) compared with the other neurons (9–10 μ m in the hippocampal neurons and 3–4 μ m in Purkinje cells).

Computer simulation studies have demonstrated that the systematic changes in the dendritic structures of NL neurons along the CF are appropriate for optimizing the coincidence detection; the electrical segregation of binaural synaptic inputs as a result of the long dendrites can improve the coincidence detection toward the lower frequencies (Agmon-Snir et al., 1998; Grau-Serrat et al., 2003). At high frequencies (especially >2 kHz), it has been proposed that the input jitter could deteriorate the coincidence detection severely when synaptic inputs are electrically segregated. The simulation is based on the assumption that the conductance of the postsynaptic membrane is constant along the tonotopic axis. The model might be too simplified to explain the specialization of coincidence detection observed in the middle-CF neurons and the marked deterioration of coincidence detection in the low-CF neurons (Fig. 8).

A significant amount of membrane surface area is contributed by the dendrites in NL neurons. The calculated membrane surface area from Lucifer yellow-filled neuronal soma was only 10–12% of the recorded input capacitance (Fig. 3D, Table 1). We calculated the surface area of dendrites and added them to the soma surface (Table 1, simulated capacitance), but it yielded only $\sim 50\%$ of the recorded input capacitance (Fig. 3D). Several factors may contribute to this discrepancy. First, tissue shrinkage attributable to fixation and the dehydration process could cause the surface area to be underestimated by as much as 50% (Nitzan et al., 1990). In the present study, 20–30% shrinkage was observed when somatic axes were compared between the live neuron observed under Nomarski optics before fixation and the reconstructed Lucifer yellow image after fixation. Second, the dendritic surface area might be underestimated by the assumption made about its shape. We assumed the dendritic shaft to be cylindrical; however, dendrites are actually rough-surfaced and have swellings toward their ends (Smith and Rubel, 1979) (Fig. 2). Third, labeling neurons with Lucifer yellow or another hydrophilic dye might have affected the estimation. In Di-I-labeled neurons, dendrites of 40–70% greater width were observed than we reported in Table 1 ($p < 0.01$ by unpaired t test at each region): 2.0 ± 0.1 μ m ($n = 6$) versus 1.4 μ m in the high-CF,

$2.7 \pm 0.2 \mu\text{m}$ ($n = 5$) versus $1.6 \mu\text{m}$ in the middle-CF, and $2.8 \pm 0.2 \mu\text{m}$ ($n = 5$) versus $1.9 \mu\text{m}$ in the low-CF neuron. This might be because Di-I labels the membrane (Fig. 2*D,E*). On the contrary, because Lucifer yellow fills the cytosol, the pass length of cytosol would affect the intensity of fluorescence signals and decrease at the edge; this may affect the dimension of dendrites (Fig. 2). Previous data obtained with Golgi staining also provided a larger dendritic width by 40–70% than those in Table 1 (Smith and Rubel, 1979).

Comparison with *in vivo* studies in the barn owl

We reevaluated the ITD sensitivity of NL neurons of the barn owl on the frequency axis (1–8 kHz) by recalculating the time window and the percentage maximum slope (see definition in Results) from the reported ITD response curve (Carr and Konishi, 1990; Pena et al., 1996; Viete et al., 1997). The ITD sensitivity is critically dependent on the tonal period when the ITD response curve was normalized between the peak and the baseline activity (Fujita and Konishi, 1991). However, the ITD sensitivity showed a slight improvement in the middle-CF region, when the slope and the time window were calculated without normalization. The time window and the percentage maximum slope were 0.66 versus 2 ms (%/10 μs) in the low-CF neurons (~ 1 –2 kHz), 0.13 versus 13 ms in the middle-CF neurons (~ 3 –5 kHz), and 0.13 versus 11 ms in the high-CF neurons (~ 6 –8 kHz). The maximum slopes are approximately twice as steep as we observed in this paper: 1.7 (%/10 μs) in the low-CF neurons, 5.8 in the middle-CF neurons, and 5.0 in the high-CF neurons (Fig. 8*C*). However, both barn owl and chicken NL neurons showed a similar tendency of ITD sensitivity along the tonotopic axis, the higher acuity in the middle- to high-CF neurons than the low-CF neurons (Fig. 8).

The essential differences between our slice experiments and those done *in vivo* would be the absence of effects from inhibitory controls and temporal integration of signals on the coincidence detection. The roles of inhibitory inputs to control the sensitivity of ITD detection were proposed (Fujita and Konishi, 1991; Pena et al., 1996; Bruckner and Hyson, 1998; Funabiki et al., 1998; Yang et al., 1999), and the improvement of coincidence detection by depression of synaptic transmission during stimulus trains was also reported (Kuba et al., 2002a; Cook et al., 2003). Although additional studies must be conducted, both in behavioral and in *in vivo* experiments in the chicken, the finding of specialization of the middle-CF neurons as coincidence detectors in NL may help our understanding of CF dependency of sound-source localization in birds.

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