

An approach to identify the functional transduction and transmission of an activated pathway

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In this study, we investigated the features of latency-amplitude (L - A) functions at different sound frequencies, using extracellular recording from auditory neurons in the central nucleus of the inferior colliculus (ICC) in mice. Isofrequency L - A functions from single neurons could be fit with a newly developed equation based on Pieron's law. The high degree of fitness indicates that the curvatures of all isofrequency L - A functions for a given neuron are similar, and that the difference between L - A functions is due to a shift in their positions in the coordinate system. When we normalized the L - A functions to match the position of the L - A function obtained at the neuronal characteristic frequency (CF), all isofrequency L - A functions from a given ICC neuron were highly superimposed. The similar shapes of the L - A functions at different frequencies may reflect the physical laws of sound being transferred into bioelectric signals. The position of a non-CF L - A function could be measured as the differences of the asymptotic L and A (ΔL and ΔA) compared to the L - A function at a reference frequency such as the CF. The nerve fibers and synapses connecting to a neuron for acoustic information processing can be functionally simplified as a single "wire" (as the total length of nerve fibers) and "joint" (as the summated size/strength of synapses). The wire and joint mediate information transmission and transduction, respectively. Thus, ΔL and ΔA may be measurements of the total length of nerve fibers and the strength of summated synapses in the activated auditory pathway. ΔL and ΔA differed between frequency channels and neurons, suggesting that the differences of acoustic neuronal responses are always caused by activation of different pathways, and that the pathways that process sounds are diverse.

central nucleus of inferior colliculus, acoustic response, transmission efficacy, transduction efficacy

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Sound perception is affected by four basic components: the travel of sound through the air, the frequency-specific response of the basilar membrane, the propagation of action potentials along axons, and signal processing at synapses [1]. Once the sound arrives at the ear, peripheral and central auditory systems are involved in auditory processing. Both serial and parallel processing of acoustic information occur in the central auditory nervous system [2–5]. This involves a large number of nerve fibers and synapses, connecting to an auditory neuron directly or indirectly (Figure 1(a1)). Theoretically, acoustic responses of all these basic components, including each activated nerve fiber and synapse,

should be recorded to study the activated pathway. Although the quantity of nerve fibers and synapses onto a neuron activated by a given acoustic stimulus (AS) are certain, it is impossible to measure anatomically the lengths of the nerve fibers and the strengths of the synapses, because of recording technique limitations.

Although the acoustic pathway comprises several different physical media (air, liquid (e.g., perilymph and endolymph), axons, pre- and post-synaptic membranes, and the synaptic cleft), only two functional processes are involved: (1) the travel of signals through one medium, and (2) the transfer of signals between media. These processes can be considered as "wires" and "joints". Each type of physical medium is considered a wire, whereas each process that

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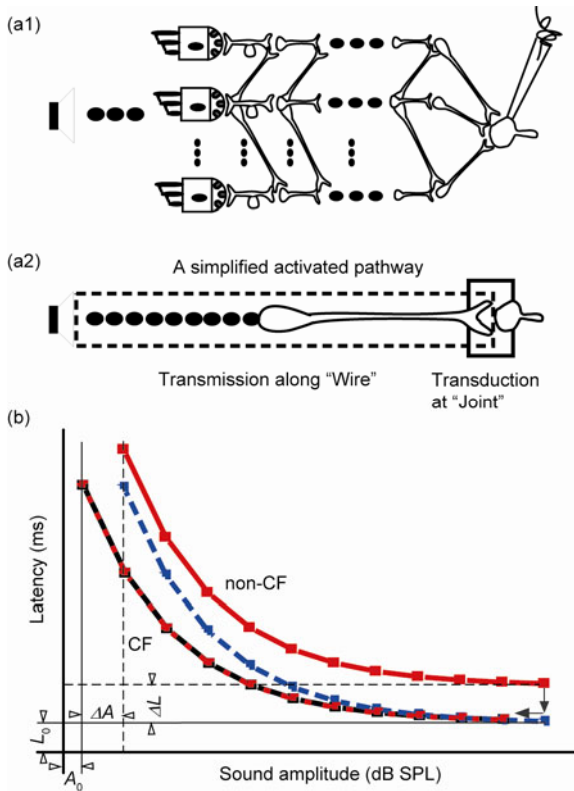


Figure 1 Schematic illustration of the pathway from the sound source to a central auditory neuron (a) and our hypothesis about the superimposition of isofrequency L - A functions (b). (a1) Schematic representation of the nerve fibers and synapses connecting to a neuron that responds to a given AS. (a2) Simplified representation of the activated pathway as a single wire through which transmission occurs (dashed rectangle) and a joint where transduction occurs (rectangle). (b) Our hypothesis about superimposition of L - A functions. The L - A curve for a non-characteristic frequency (CF) AS (red) can be superimposed on the CF L - A curve (black) if it is moved over ΔL along the y axis (blue) then over ΔA along the x axis. L_0 and A_0 indicate the horizontal and vertical asymptotes, respectively.

occurs between two different media, such as pre-synaptic neurotransmitter release or the post-synaptic membrane response, is treated as a joint. In this study, we defined the signal traveling along the wire as “transmission”, and the transfer of the signal at the joint as “transduction”. To separate the effects of transmission and transduction, we simplified the structures involved in the acoustic pathway functionally into one wire and one joint (Figure 1(a2)). The wire and joint may be regarded as the total length of the fibers and the total strength of synapse, respectively. Despite this, no methods exist to analyze the functional transmission and transduction of an activated pathway.

Neuronal spike timing, generally measured as the latency of the first acoustic evoked spike (L), has recently received increasing attention as a potential neural code for sensory representation [1,6,7]. Evidence for this has been found in several systems, including somatosensory [8], olfactory [9], auditory [10,11], visual [12], and in the hippocampus [13]. L is more precise and reliable than average spike rates or counts alone in the representation of sound stimulus information

[11,14–17], although this might not be the case for all stimuli and neurons [18]. Therefore, L might provide information on the functional quantity of activated nerve fibers and synapses for a given neuron responding to a certain AS.

Generally, neurons in the central auditory nervous system respond at the shortest delay when the AS is at or near their characteristic frequency (CF; the AS frequency to which a neuron is most sensitive. That is, the lowest sound amplitude is needed to evoke the neuronal response). And L is positively related to the difference between the AS frequency and the CF of the neuron [11,16,19,20]. L decreases as the amplitude (A) of the AS increases, and can be plotted as a function of the AS amplitude (L - A function) [15–17,21,22]. The L - A functions for acoustic stimuli of different frequencies, such as CF and non-CF stimuli, are similar in shape but shifted in location on the coordinate system (Figure 1(b)) [12,17]. The L - A function can be well described by Pieron’s law [23], as well as by other models derived from Pieron’s law [11,17,24–28].

The basic form of Pieron’s law [23] is:

$$L - L_0 = bP^{-a}, \quad (1)$$

where L_0 is the minimum response latency and P is sound pressure in Pascal. L_0 , a , and b are fit constants.

In these models, the first-spike latency is separated into two components (eq. (1)): the stimulus-dependent latency (bP^{-a}) and the constant stimulus-independent minimum latency (L_0) which is the horizontal asymptote of the L - A function [11] (Figure 1(b)). L_0 was referred to as the “transmission delay” in our previous study [17], and reflects the transmission efficacy from the sound source to the recorded neuron. It comprises sound travel time in the air, frequency-specific delays in the response of the basilar membrane, the fixed delay of synapses, and axonal action potential propagation time. In contrast, the stimulus-dependent latency (bP^{-a}) is the L corrected for the theoretical minimum L ($L - L_0$), and is a function of the AS sound pressure.

Considering the L - A curve, there seems to be another constant that describes the response properties along the x axis. We carefully analyzed our published data on the neuronal response to an AS at the CF [17], and found that the response can be fit by a modified model derived from Pieron’s law with four fit constants (Appendix D):

$$L - L_0 = \lambda e^{-(A-A_0)/\tau}, \quad (2)$$

where A is the stimulus sound pressure and A_0 is the minimum sound pressure level (dB SPL). L_0 , A_0 , τ , and λ are fit constants. At the CF, L_0 is the horizontal asymptote, and A_0 is the vertical asymptote (Figure 1(b)).

In this modified model, the stimulus-dependent latency ($\lambda e^{-(A-A_0)/\tau}$) is a value relative to a constant that is dependent on A_0 ($\lambda e^{A_0/\tau}$). The vertical asymptote A_0 represents the sensitivity threshold of the pathway to the AS, and therefore reflects the reliability of transduction between

different media [17]. If the pathway is functionally simplified into one wire and one joint (Figure 1(a2)), A_0 can be seen as a parameter that describes the transduction efficacy, i.e., the summed efficacy of pre-synaptic neurotransmitter release and the post-synaptic membrane responses for all activated synapses. A_0 and L_0 determine the position of the L - A curve, while τ and λ determine the curvature.

When neurons responded to two different AS frequencies (e.g., CF and nearby non-CF) varying in amplitude, two isofrequency L - A functions were obtained (Figure 1(b), [16]). We hypothesized that these two curves could be superimposed after normalization by moving one of them horizontally and vertically (Figure 1(b)). If this is the case, a neuron will respond in a similar way to different AS frequencies, with unchanged values of τ and λ (i.e., similar curvatures), and with the differences originating solely from changes in A_0 and L_0 . The differences of L_0 and A_0 between the two L - A functions may be calculated by fitting the data with eq. (2) using the same values of τ and λ or with eq. (3) with the same values of L_0 , A_0 , τ and λ .

$$L - (L_0 + \Delta L) = \lambda e^{-[A - (A_0 + \Delta A)]/\tau}, \quad (3)$$

where L_0 , A_0 , λ , and τ are the constants obtained when the CF L - A data were fit with eq. (2). ΔA and ΔL are the constants from this fit, and indicate the differences between the asymptotes L_0 and A_0 of the two functions (Figure 1(b), the dashed lines).

According to the quality and meaning of L_0 and A_0 , ΔL and ΔA may be interpreted as the differences in transmission and transduction efficacy of the activated nerve fibers and synapses in the two frequency-specific pathways converging onto the neuron (Figure 1(a)).

The inferior colliculus is an important nucleus with no analogs in other sensory neuronal systems. It receives convergent inputs from the parallel ascending streams of auditory information [29]. We have previously studied neuronal processing of sound in the central nucleus of inferior colliculus (ICC) using L as the measurement of the acoustic response [16,17,20,30]. The features of the ICC neuronal acoustic response include relatively low levels of spontaneous firing, an onset firing pattern, and reproducibility of the first spike latency across different frequencies and amplitudes of the AS. Therefore, we used the L - A curve obtained from the response of ICC neurons fit by our newly developed eqs. (2) and (3) to test our "superimposition of L - A functions" hypothesis. We tried to develop a method to study the relative efficacy of transmission and transduction, the functional differences between frequency channels, and the properties of frequency channels of ICC neurons.

1 Materials and methods

1.1 General

Forty-five healthy BALB/c mice of either sex (12–18 g; 4–6 weeks old) without hearing defects were provided by the

Experimental Animal Center of the Southern Medical University, Guangzhou, China. The methods for animal preparation, acoustic stimulation, data acquisition, and processing were similar to our previous study [20]. The study was approved by the Animal Care and Use Committee of the Southern Medical University.

1.2 Surgical preparation

Atropine sulfate (0.25 mg/kg, s.c., Sigma-Aldrich, St Louis, MO, USA) was administered at the start of the experiment to reduce bronchial mucous secretion. Anesthesia was induced with sodium pentobarbital (60–70 mg/kg, i.p., Sigma) and maintained by supplemental doses (13 mg/kg) of the same anesthetic being administered every hour or on indication by the pedal withdrawal reflex.

The mouse was held in a polyethylene foam body-mold box placed in a double-walled soundproof room (temperature maintained at 24–26°C). The head was fixed to a stereotaxic apparatus with a 1.5-cm nail attached to the dorsal surface of the skull vertically with dental cement. A 2 mm × 2 mm area was opened on the cranium, and the dura was removed under a surgical microscope (WPI, Sarasota, FL, USA) to expose the IC of one hemisphere for extracellular recording. Vaseline was used to cover the exposed brain and the pinnae were maintained as in normal awake animals during recordings.

1.3 Acoustic stimulation

A Tucker-Davis Technologies System 3 (TDT 3, Tucker-Davis Technologies, Alachua, FL, USA) was used to generate pure tone bursts as the AS. They were applied to the animal by a free-field loudspeaker (ES1, frequency range 2–110 kHz) located 30 cm from the front of the head. The loudspeaker was calibrated using 1/8 and 1/4 inch microphones (Brüel and Kjaer 4138, 4135, Naerum, Denmark) and an amplifier (Brüel and Kjaer 2610) at the start of the experiment. The duration of the acoustic pure tone bursts was 50 ms and the rise-fall time was 5 ms. Pure tone bursts were played using a computer running BrainWare software, and the frequency and amplitude were controlled manually or automatically.

1.4 Data acquisition

Glass micropipettes were filled with 2 mol/L sodium acetate and 3% pontamine sky blue (~1 μm tip diameter; impedance: 10–20 MΩ), and used for extracellular recording of ICC neuronal activity. The signal was amplified (×2000–10000), band-pass filtered (0.3–3 kHz), and processed using the TDT 3.

The shapes and feature spaces (1st peak to 2nd peak) of action potentials were stored and monitored during data acquisition to isolate single units by their similar action potential shapes.

Frequency-amplitude scans were performed using frequencies of $CF \pm 5$ kHz varied in 0.5 or 1 kHz steps, and amplitudes of 10–90 dB above the minimum threshold (MT) varied in 5–20 dB steps. The MT was defined as the AS amplitude that elicited a spike with a probability of 0.1 at the CF [31]. Each AS was applied randomly 15 times at 1/s. After recording, pontamine sky blue was applied to the recording site by microiontophoresis ($-20 \mu\text{A}$, 15 min; Neurophore BH-2, Holliston, MA, USA) to confirm the recording site. Data from neurons outside the ICC were discarded.

1.5 Data processing

The frequency-amplitude scan provided a frequency-amplitude field of an ICC neuron, from which the basic features of the neurons, CF and MT, were identified.

In a frequency-amplitude field of a neuron, the points containing one or more spikes in response to the 15 presentations of a given AS were used to generate a post-stimulus time histogram, in which an appropriate analysis window including only onset responses was selected to calculate the mean L (\pm SD) and spike count (SC) [17]. The L - A and SC- A curves for each AS frequency were plotted, as well as the L -frequency and SC-frequency curves for each AS amplitude.

Since the CF is an important parameter describing the features of a recorded auditory neuron, we used the re-

sponses to the AS at the CF as the reference for processing the datasets in this study.

First, the L - A curve for CF acoustic stimuli was fit using eq. (2) to obtain the constants L_0 , A_0 , λ , and τ . Then, the L - A curves for the non-CF acoustic stimuli in the same neuron were fit with eq. (3) using the four constants L_0 , A_0 , λ , and τ from the first fit, to obtain ΔA and ΔL . Finally, we plotted ΔA and ΔL against the AS frequency normalized to the CF (AS frequency – CF).

Microsoft Excel 2003 and OriginPro 7.5 were used for calculating the values of the relevant parameters and for data fitting and plotting. One-way ANOVA in SPSS 11 (SPSS Inc., Chicago, IL, USA) was used to test the difference.

2 Results

2.1 General

Extracellular recordings were obtained at recording depths of 320–1794 μm from 52 ICC neurons with single-peak frequency-amplitude responses. The CFs of these neurons ranged from 6 to 40 kHz (20 ± 8.3 kHz) and their MTs were between 3 and 64 dB SPL (35 ± 14.9 dB SPL). Most recorded ICC neurons ($n = 51/52$ neurons) fired at the onset, and only one neuron fired over the entire duration of the AS. There was no correlation between the CF and MT of the recorded ICC neurons (Figure 2(a)). Acoustic responses

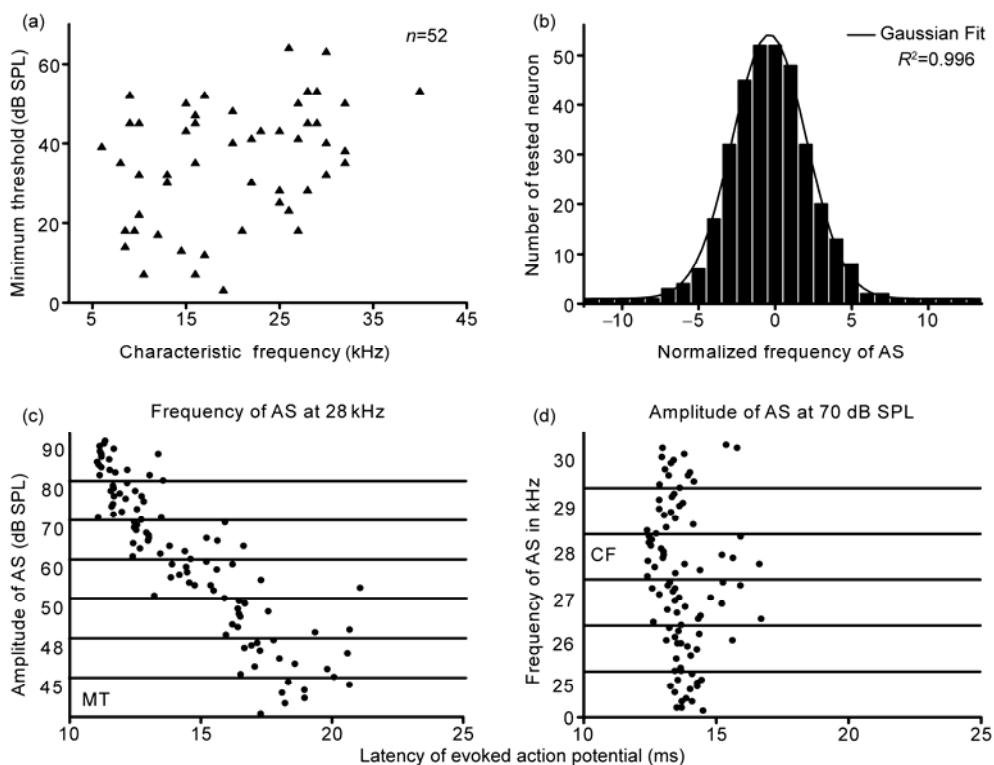


Figure 2 Basic features of ICC neuronal responses to pure tone bursts. (a) There was no correlation between the CF and the MT. (b) The AS frequencies used to test the neuronal response were normally distributed around the CFs (Gaussian Fit, $R^2 = 0.996$). (c) Raster plot of response latencies of an ICC neuron at its CF (28 kHz) at different amplitudes of the AS (MT: 45 dB SPL). (d) Raster plot of the responses of the same neuron at different AS frequency at 70 dB SPL.

were tested at frequencies that were normally distributed around the CF of the ICC neurons (Gaussian Fit, $R^2 = 0.996$; Figure 2(b)). The responses occurred at a shorter L for CF stimuli compared to non-CF stimuli (Figure 2(d)), and L increased when the AS amplitude decreased (Figure 2(c)).

2.2 First spike latency versus spike count

The L and SC were plotted as isoamplitude SC-frequency and L -frequency diagrams (Figure 3(a),(b)) and as isofrequency SC-amplitude and L -amplitude plots (Figure 3(c) and (d)). The SCs of the neuronal responses showed large variance and did not well reflect the amplitude and frequency of the AS (Figure 3(a) and (c)). However, both the isoamplitude L -frequency (Figure 3(b)) and the isofrequency L -A plots (Figure 3(d)) showed accurate, stable, and reliable relationships between stimuli and responses.

According to criteria based on the monotonicity ratio (SC at the highest amplitude/SC at the best amplitude of the AS [32,33]), neuronal SC-A responses to the pure tone bursts in the present study could be divided into monotonic (monotonicity ratio ≥ 0.8 ; $n = 24/52$ neurons; 46.2%) and non-monotonic (monotonicity ratio < 0.8 ; 28/52 neurons; 53.8%). The isoamplitude SC-frequency and L -frequency functions used the same raw data as the isofrequency SC-A and L -A functions. Thus, we could only compare the isofrequency SC-A/ L -A functions of the monotonic amplitude-response neurons to the non-monotonic ones. In both cases, the L -A functions showed more accurate, stable, and reliable results (Figure 4(b) and (d)) than SC-A functions

(Figure 4(a) and (c)). The L -A function showed a similar appearance regardless of whether the response was non-monotonic or monotonic.

These results were consistent with previous reports [16,34,35]. In all isofrequency L -A functions, L decreased with increasing AS amplitudes. The position of the L -A functions differed for different AS frequencies (Figures 3(d), 4(b) and (d)), but the functions were similar in shape.

2.3 Superimposition of L -A functions

The CF and non-CF L -A curves ($n = 346$) for all recorded neurons were fit well with eqs. (2) and (3), respectively ($R^2 > 0.90$ for 46 recorded ICC neurons). The fits yielded the two constants ΔA and ΔL , which describe the differences between the asymptotes L_0 and A_0 for the non-CF and CF L -A curves. Using the obtained values of ΔA and ΔL , all the non-CF L -A curves were normalized by subtraction ($L - \Delta L$ and $A - \Delta A$). Then, the CF and non-CF L -A curves of each neuron were re-plotted together, and the normalized non-CF data were fit to eq. (2). The normalization process amounted to moving the non-CF L -A curve horizontally and vertically over a distance of ΔA and ΔL , preserving the AS-dependent component of L , i.e., the shape of the curve. Figure 5(a)–(c) illustrates that the non-CF and CF L -A curves were highly superimposed after this procedure (R^2 values: 0.99, 1.00 and 0.99).

The data from most of the recorded ICC neurons ($n = 50/52$ neurons) showed good fits, with $R^2 > 0.85$ (mean $R^2 = 0.95 \pm 0.07$; Figure 6). The L -A curves for a relative small

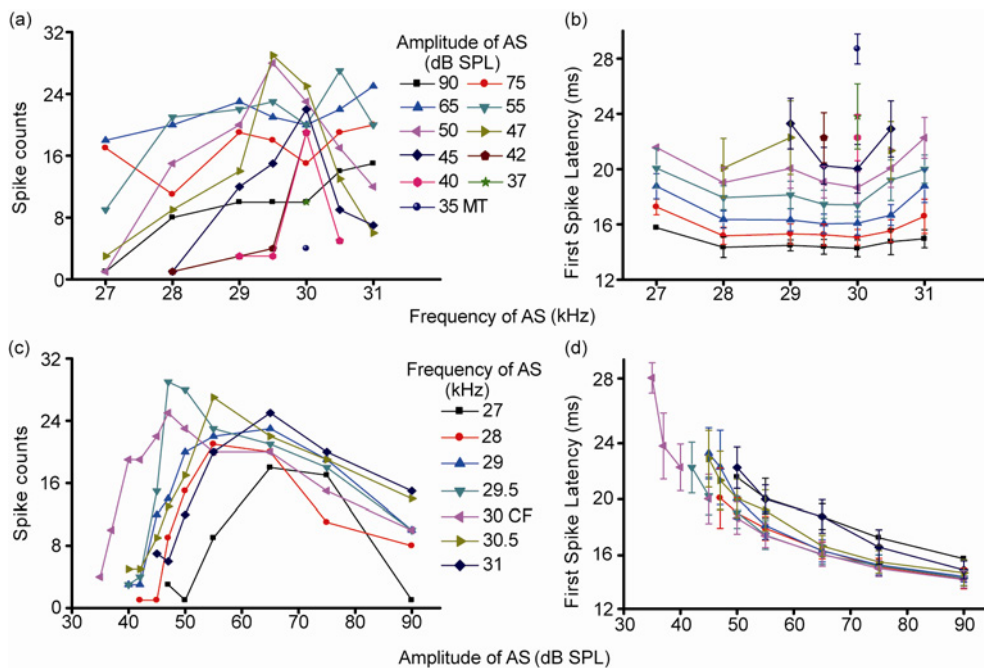


Figure 3 Comparison of the SC and L responses of an ICC neuron (No. 032401; CF: 30 kHz. MT: 35 dB SPL) to pure tones varying in frequency and amplitude. (a) and (b) The isoamplitude SC-frequency (a) and L -frequency (b) functions. (c) and (d) The isofrequency SC-A (c) and L -A (d) functions.

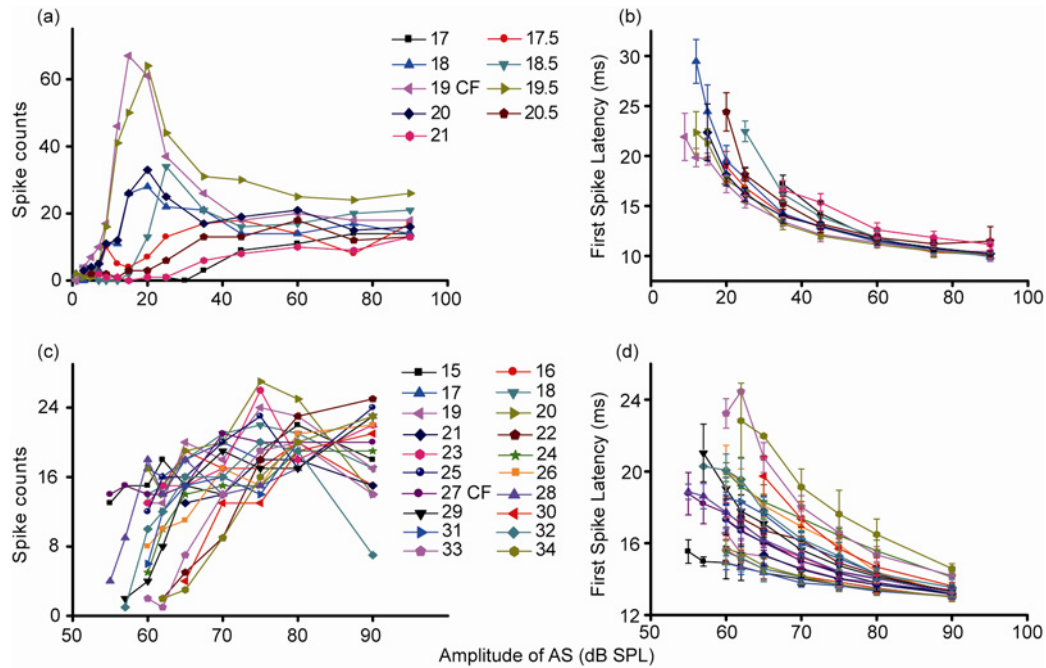


Figure 4 Comparison of isofrequency SC-A ((a) and (c)) and L-A ((b) and (d)) plots in one non-monotonic neuron (No. 070702; CF: 19 kHz; MT: 3 dB SPL) ((a) and (b)) versus one monotonic neuron (No. 033001; CF: 27 kHz; MT: 50 dB SPL) ((c) and (d)).

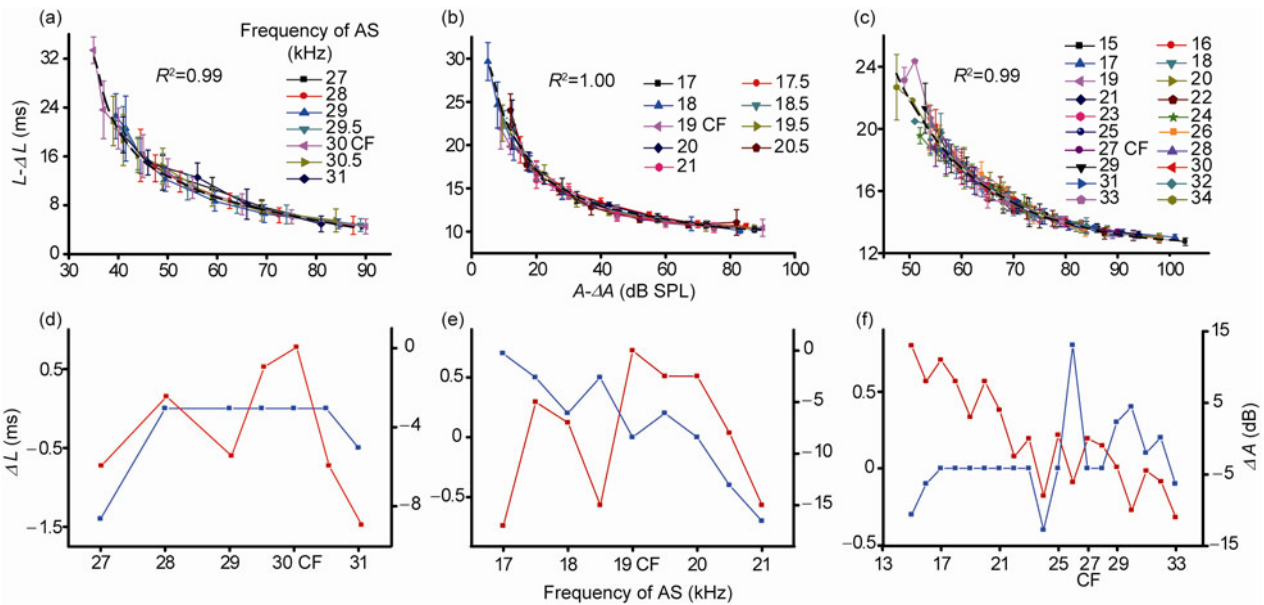


Figure 5 Three examples of superimposition of L-A functions (data from the same neurons as in Figures 3 and 4). Superimposed L-A functions for neurons No. 032401 (a), 070702 (b), and 033001 (c) are shown. (d)–(f) Plots show ΔA (red line, secondary y axis) and ΔL (blue line, primary y axis) obtained from data fitting using eq. (3) as a function of AS frequency.

range of frequencies including CF and non-CF had similar shapes while only their positions in the coordinate system were different. This indicates that an ICC neuron responds to pure tone stimuli of different frequencies and amplitudes in an identical way.

According to our hypothesis, ΔL and ΔA can be interpreted as the difference in transmission and transduction efficacy of two different frequency channels. Therefore, we

plotted the values of ΔA (red lines) and ΔL (blue lines) obtained from a given neuron against the stimulus frequency (Figure 5(d)–(f)), to check for any regular pattern in the processing of frequency and amplitude. However, the ΔA (Figure 5(d)–(f); red lines) and ΔL (Figure 5(d)–(f); blue lines) were different at different frequencies. This may mean that stimuli at different frequencies activate different sets of synapses and fibers.

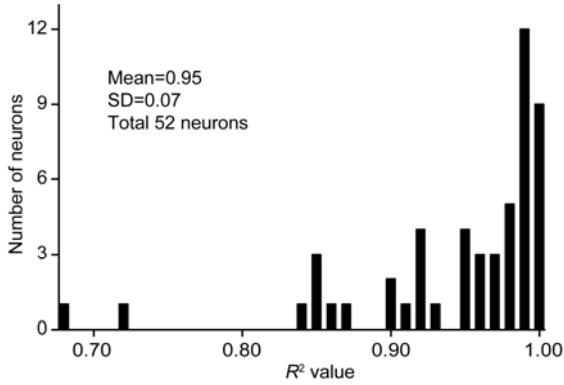


Figure 6 Histogram of the distribution of R^2 values for the 52 recorded ICC neurons.

2.4 Properties of ICC neurons expressed by ΔA and ΔL

The fibers and synapses connecting to a neuron activated by a given AS frequency can be treated as a frequency channel. To find some regularity of frequency channels in the ICC neuron population, we plotted ΔA and ΔL against the AS frequencies normalized to the CFs for all recorded neurons, and categorized the changes in ΔA and ΔL (Figure 7). ICC neurons that had not been tested at more than two non-CF frequencies were excluded ($n = 15/52$). In the remaining 37 neurons, we identified four categories of ΔA (Figure 7, upper panels) or ΔL (Figure 7, lower panels) when plotted against AS frequency. The ΔA or ΔL increased monotonically with increasing frequency for type I (Figure 7(a) and

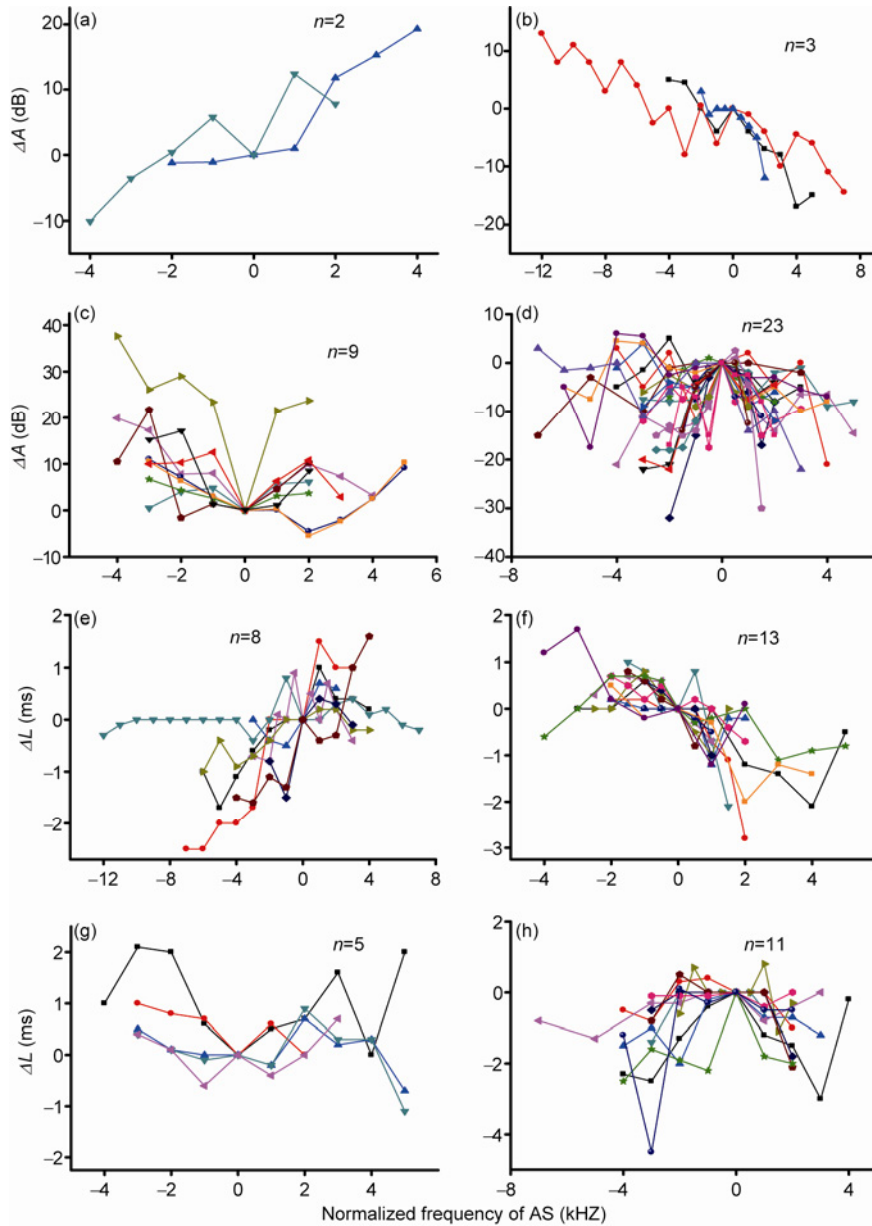


Figure 7 Four categories of ΔA and ΔL as a function of normalized frequencies ($n = 37$). The ΔA and ΔL increased monotonically with increasing frequency (type I; (a) and (e)), decreasing monotonically with increasing frequency (type II; (b) and (f)), changed non-monotonically with frequency in a “V” shape (type III; (c) and (g)) or changed non-monotonically in an inverse “V” shape (type IV; (d) and (h)).

(e) and increased with decreasing frequency for type II (Figure 7(b) and (f)). For type III, ΔA or ΔL changed non-monotonically with an increase in frequency, forming a “V” shape (Figure 7(c) and (g)), and type IV showed an inverse “V” shape (Figure 7(d) and (h)). For ΔA , 86.5% of neurons showed non-monotonic change (Figure 7(c) and (d)), with 62.2% of neurons showing an inverse “V” shape (type IV; Figure 7(d)). The remaining 13.5% of neurons showed monotonic change (Figure 7(a) and (b)). For ΔL , 43.2% of neurons showed non-monotonic change (Figure 7(g) and (h)), while 56.8% neurons showed monotonic change (Figure 7(e) and (f)). Although these four categories of ΔA and ΔL changes could be identified, the change of ΔA and ΔL with the AS frequency varied between neurons.

Each parameter, ΔA or ΔL , could not fully describe neuronal properties in terms of wire transmission and joint transduction. Therefore, we categorized neuronal properties using changes with AS frequency for both ΔA and ΔL . Eleven types were identified in total (Figure 8(a)). In 23 out of 37 neurons, ΔA first increased then decreased. ΔL monotonically decreased with increasing AS frequency in 29.7% of neurons, non-monotonically increased then decreased in 18.9%, and monotonically increased in 10.8%. Of 9 neurons in which ΔA first decreased then increased, ΔL monotonically increased in 8.1%, first increased then decreased in 8.1%, and first decreased then increased in 8.1%. All other types occurred with an incidence of < 5%. The above categories may be related to some functions of sound processing. Then, the functional structures of wire transmission and joint transduction onto most sampled neurons, implied by the changes of ΔL and ΔA referenced to the CF response, were exhibited (Figure 8(b)). The most common type of ΔL and ΔA changes may be explained by the functional structure shown in Figure 8(b5). For this type, the ΔL changes monotonically while the ΔA changes non-monotonically as an inverse V-shape. This means that transmission efficacy increased as the frequency of the AS increased, while the transduction efficacy increased when the frequency of the AS shifted away from the CF.

3 Discussion

In the present study, we investigated the ICC neuronal responses to acoustic pure tones of varying frequencies and amplitudes, using the L - A curves obtained for different AS frequencies. There were three main findings. (1) The first spike latency is much more accurate, stable, and reliable than SC, and is a function of the AS amplitude. (2) The L - A functions for different AS frequencies can be superimposed irrespective of their positions, confirming our hypothesis. (3) The AS-independent asymptotes L_0 and A_0 obtained from the fit of the L - A plot, as well as the differences in the asymptotes (ΔL , ΔA) between the CF and non-CF L - A functions, may be suitable parameters to describe the efficacy of wire transmission and joint transduction of an ICC neuron.

The first finding supports previous results [11,14–17]. The latter two findings may provide a method to analyze the relative functional transduction and transmission by using the response at the CF as a reference.

3.1 The implications of superimposition of L - A functions

The latency of the first acoustically evoked spike is determined by the integration of the sound envelope, which contains both amplitude and time course [11,25,26]. Both excitation and inhibition occur in the central auditory nervous system during the processing of acoustic information. However, we are only concerned with the acoustic information that is able to reach the recorded ICC neuron, that is, the excitatory integrated neuronal activities passed from one relay station to another in the ascending auditory pathway, to evoke acoustic perception. Since the excitatory integrated neuronal activities can be recorded extracellularly, the L obtained by extracellular recording can be used as a parameter that reflects the response properties of the auditory neuron.

Although the isofrequency L - A functions of a neuron

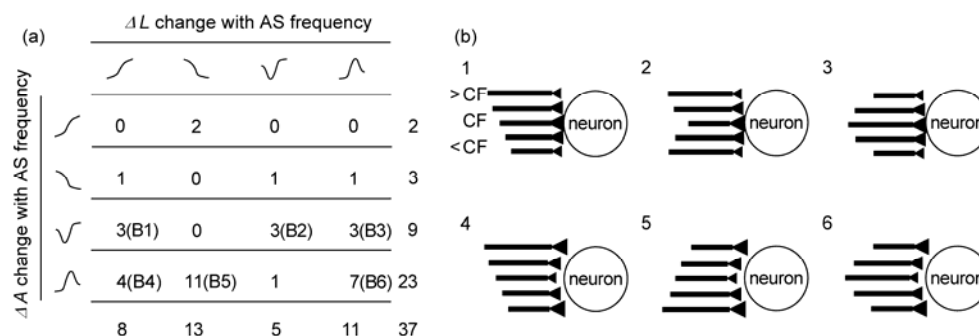


Figure 8 Summary of ICC neurons ($n = 37$) categorized by ΔA and ΔL . (a) The number of neurons in each category (see text for details). (b) The functional wire and joint structures onto neurons implied by the changes of ΔL and ΔA relatively to the CF response. The length of the wire represents the transmission efficacy and the size of the joint represents the transduction efficacy. The central wire and joint represent the pathway activated by the AS at the CF of the recorded neuron. The upper two sets represent the pathways activated by the AS at $> CF$, and the bottom two set represent those activated by the AS at $< CF$.

were different from each other at different AS frequencies (Figures 3(d), 4(b) and 4(d)), all L - A functions were similar in shape and were superimposed when their x - y position was adjusted (Figure 5(a)–(c)). This behavior of auditory neurons implies that sound amplitude processing obeys the same law (the curvature of the L - A function as described by λ and τ) irrespective of AS frequency. Different AS frequencies can be processed referenced to one frequency, and this can be treated as filtering. Thus, the frequency processing of an ICC neuron might work as a filter. However, previous studies have also demonstrated that the L - A functions in different auditory neurons can be superimposed by normalization of their positions to that of a reference neuron, indicating that the filtering is not achieved by the auditory neuron itself [11,25,26]. It is well known that the basilar membrane works as a filter. Thus, the behavior of ICC auditory neurons faithfully represents the physical laws of sound being transferred into bioelectric signals, and this may originate in the basilar membrane.

Regardless of the asymptotes L_0 and A_0 that determine the position of the L - A curves, the superimposition of the L - A functions suggests that auditory neurons are not concerned with the frequency input quality or the frequency input channel when processing the acoustic information. Only the input magnitude must be processed in the recorded neuron. The curvature of the superimposed L - A functions, determined by λ and τ (see Appendix I; $\lambda = bP_0^{-a}$ and $\tau = 20/a \ln 10$, developed from Pieron's law [23]), might reflect the sensitivity of the recorded neuron, which may be determined by the properties of the neuronal membrane. This possibility should be tested by further experiments, such as whole-cell recording *in vivo* and *in vitro*.

All ICC neurons sampled in this study showed single-peak V-shape receptive fields. Therefore, we only tested the neuronal responses at frequencies ranging from CF – 5 to CF + 5 kHz, which is limited to the central range of the receptive fields. The AS at other frequencies either evoked fewer neuronal responses requiring much higher sound levels, or induced no neuronal response at all. This might slightly decrease the success of data fitting. However, based on the consistent results obtained from ICC neurons with a large range of CFs (6–40 kHz), the superimposition of L - A functions for CF and non-CF stimuli is likely to be valid for a much larger range of frequencies within the neuron's receptive field.

3.2 The meanings of ΔA and ΔL

As discussed above, the superimposition of the L - A functions could be seen regardless of neurons [11,25,26] and sound frequencies (Figure 5(a)–(c)). The important feature of the L - A function is described by its curvature, i.e., the constants λ and τ . Thus, λ and τ are determined by the response properties of the basilar membrane, which works as a filter. In contrast, the position of the L - A function is de-

termined by the horizontal and vertical asymptotes, that is, the constant L_0 and A_0 , or the differences relative to a reference, expressed by ΔA in dB SPL and ΔL in ms. As discussed in the Introduction, L_0 and A_0 may represent the efficacy of wire transmission and joint transduction, respectively. Therefore, ΔL and ΔA for a given non-CF channel reflect the differences in transmission and transduction efficacy compared to the CF channel.

When ΔL is zero, the non-CF channel carries the acoustic information with a similar transmission efficacy to the CF channel, but they do not necessarily represent the same channel. However, the channels for CF and non-CF acoustic information processing are likely to be different when $\Delta L \neq 0$ or $\Delta A \neq 0$. When ΔA and ΔL of a neuron are plotted against AS frequency, the functions (Figures 5(d)–(f) and 7) may represent a neuron's relative functional structure of the wire and joint. The wire transmission reflected by ΔL includes transmission of the acoustic signal in the air, the cochlear liquid, the synaptic gap, and the axon [17]. The latter two can vary with different frequencies, whereas the first two are relative independent of sound frequencies. In contrast to ΔL , ΔA represents the sensitivity of the joint transduction, which includes the summation of pre-synaptic neurotransmitter release and postsynaptic membrane responses for all activated synapses. In summary, ΔL can be a functional measurement of the difference in the length of the wire, i.e., the sum of the involved synapses, compared to a reference such as the CF channel (Figure 8(b), the lines), while ΔA can be a functional measurement of the strength of the joint, i.e., the sum of the sensitivity of the involved synapses (Figure 8(b), the dark triangles).

3.3 The implications of the functional structure of inputs to ICC neurons as estimated by ΔL and ΔA

By taking ΔL and ΔA as the measurements of the functional structure of a frequency channel, we plotted ΔA and ΔL of all studied ICC neurons against the AS frequency. The results showed the ΔL and ΔA were different for different frequency channels and neurons (Figures 7 and 8(a)). This suggests that differences in neuronal responses are always caused by activation of different pathways, and that the auditory pathways that process different sound frequencies are diverse. The functional structure of the wire and joint of a simplified frequency channel could be categorized into 11 types (Figure 8(a)). Six of these were relatively frequently observed in the studied neurons. The functional structures of these neurons were schematically demonstrated in Figure 8(b). Because ΔA indicates the strength of the summated synapses, and ΔL indicates the total length of the fibers (including synaptic gaps), the arrangement of the frequency channels to a neuron is clearly outlined. We can easily observe the regularities of the neuron's functional structure. However, the exact function of these neurons in sound information processing should be tested in further studies.

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Appendix I

Pieron's empirical law [23]:

$$RT - RT_{\min} = bP^{-a},$$

where RT and RT_{\min} represent the response time and minimum response time, respectively (they are expressed as L and L_0 in the present study). a and b are constants, while P is the sound pressure in Pascal. The sound pressure is converted into sound amplitude in dB SPL as follows:

$$A = 20 \log(P / P_{\min}),$$

$$A_0 = 20 \log(P_0 / P_{\min}),$$

$$A - A_0 = 20 \log(P / P_0),$$

where A is the amplitude of an acoustic stimulus in dB SPL, P_0 is the minimum sound pressure required to activate the recorded ICC neuron, while the P_{\min} is the minimum sound pressure. Then,

$$P = P_0 e^{(A-A_0)\ln 10/20}.$$

When P in Pieron's law is replaced, the function changes into

$$L - L_0 = b(P_0 e^{(A-A_0)\ln 10/20})^{-a},$$

$$L - L_0 = bP_0^{-a} e^{-a(A-A_0)/20/(a \ln 10)}.$$

If $\lambda = bP_0^{-a}$ and $\tau = 20/a \ln 10$, then Equation (2) used in this study can be developed from Pieron's law:

$$L - L_0 = \lambda e^{-(A-A_0)/\tau}.$$