Chinese Journal of Physiology 50(5): 258-268, 2007

Neurons in the Inferior Colliculus of the Big Brown Bat Show Maximal Amplitude Sensitivity at the Best Duration

Chung-Hsin Wu*, and Philip H. -S. Jen

Division of Biological Sciences and Interdisciplinary Neuroscience Program
University of Missouri-Columbia
Missouri 65211, USA

Abstract

The big brown bats, *Eptesicus fuscus*, emit ultrasonic signals and analyze the returning echoes in multi-parametric domains to extract target features. The variation of different pulse parameters during hunting predicts that analysis of an echo parameter by bats is inevitably affected by other co-varying echo parameters. In this study, we presented data to show that the bat inferior collicular (IC) neurons have maximal amplitude sensitivity at the best duration (BD). A family of rate-amplitude function (RAF) of each IC neuron is plotted with the BD and non-BD sound pulses. The RAF plotted with BD pulses has sharper slope (SL) and smaller dynamic range (DR) than the RAF plotted with non-BD pulses has. All RAFs can be described as monotonic, saturated or non-monotonic. IC neurons with monotonic RAF are mostly recorded at deeper IC and they have the largest average BD, best amplitude (BA) and DR. Conversely, IC neurons with non-monotonic RAF are mostly recorded at upper IC and they have the smallest average BD, BA and DR. Low best frequency (BF) neurons at upper IC have shorter BD, smaller BA and DR than high BF neurons at deeper IC have. These data suggest that IC neurons that tune to an echo duration also have the greatest sensitivity to echo amplitude. These data also suggest that sensitivity in frequency, duration and amplitude appears to be orderly represented along the dorso-ventral axis of the IC.

Key Words: bat, amplitude and duration sensitivity, echolocation, inferior colliculus, rate-amplitude function

Introduction

During hunting, the big brown bats, *Eptesicus fuscus*, systematically shorten duration, lower frequency, decrease amplitude and increase repetition rate of emitted pulses as they search, approach, and finally intercept insects or negotiate obstacles (10, 16, 28, 31). Presumably, this variation in pulse parameters allows bats to obtain as much information as possible about the localized target through analysis of returning echoes for successful hunting. However, this variation in pulse parameters also predicts that analysis of an echo parameter by bats is inevitably affected by other co-varying echo parameters. For example, previous studies have shown that many bat's IC neurons discharge maximally to a specific pulse duration (the best duration, BD) such that they behave like band-, short- and long-pass filters to pulse duration (2-4, 7-9, 15, 17, 18, 20, 26, 32, 33). The duration selectivity of IC neurons, which plays an essential role in echo recognition, improves with increasing pulse repetition rate (20, 32, 37). Furthermore, duration selectivity of many IC neurons becomes poor at very strong sound intensity (35). When determined with a pair of sound pulses,

*Present address: Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, R.O.C.
Corresponding author: Dr. Chung-Hsin Wu, Department of Life Science, National Taiwan Normal University, Taipei, Taiwan, R.O.C. Tel: +886-2-29326234, Fax: +886-2-29312904, E-mail: megawu@ntnu.edu.tw
Received: April 27, 2007; Revised: June 2, 2007; Accepted: June 5, 2007.
 ISSN : 0304-4920. http://www.cps.org.tw

duration selectivity of IC neurons to the second sound pulse becomes sharper with shortening of gap between the two pulses (33). Also, the response of bat IC neurons to the BD sound is suppressed when the BD sound is preceded, overlapped or followed by a nonexcitatory sound within a specific temporal window (5).

A previous study in our laboratory examined the effect of sound duration on amplitude sensitivity of bat IC neurons by plotting a family of rate-amplitude function (RAF) of IC neurons with different pulse durations (36). This study showed that "RAFs of one group (n = 47, 57%) of IC neurons changed form one type to another with sound duration and one third of these neurons were tuned to sound duration. As a result, the best amplitude, dynamic range, and slope of most neurons also varied with decreasing sound duration." However, this study did not specifically study if the amplitude sensitivity of IC neurons is sharper when determined with BD sound than with non-BD sounds.

The main objective of the present study is to show that duration selectivity plays an important role in amplitude sensitivity of IC neurons. To achieve this objective, we compared the RAF of duration tuned IC neurons obtained with BD and non-BD sounds. We report here that IC neurons have the sharpest RAF when determined with BD pulses. Furthermore, low BF neurons at upper IC have shorter BD, smaller best amplitude (BA) and sharper amplitude sensitivity than high BF neurons at deeper IC have.

Materials and Methods

Animals and Surgery

Eleven *Eptesicus fuscus*, (7 males, 4 females, 11-38 g, body weight, b.w.) were used for this study. As described in previous studies (19), the flat head of a 1.8 cm nail was glued onto the exposed skull of each Nembutal anesthetized bat (45-59 mg/kg b.w.) with acrylic glue and dental cement one or two days before the recording session. Exposed tissue was treated with an antibiotic (Neosporin, Johnson & Johnson, Inc., New Brunswick, NJ, USA) to prevent inflammation. Ortho-McNeil, Inc., South Paritan, NJ, USA. During recording, the bat was administered the neuroleptanalgesic, Innovar-Vet (Fentanyl 0.08 mg/kg b.w. Droperidol 4 mg/kg b.w., FortéBio, Inc., Menlo Park, CA, USA), and placed inside a bat holder (made of wire mesh) that was suspended in an elastic sling inside a double-wall sound-proof room (temperature 28°-30°C). The ceiling and inside walls of the room were covered with 3-inch convoluted polyurethane foam to reduce echoes. After fixing the bat's head with a set screw, small holes (about 25-30 μm in diameter) were made in the skull above the IC for insertion of 3 M KCl glass pipette electrodes (impedance: 5-10 MΩ). Additional doses of Innovar-Vet were administered during later phases of recording when bats showed signs of discomfort. A local anesthetic (Lidocaine, Astra Pharmaceutical Ins., Södertälje, Sweden) was applied to the open wound area. The recording depth was read from the scale of a microdrive (David Kopf, Tujunga, CA, USA). A common indifferent electrode (silver wire) was placed at the nearby temporal muscles. Each bat was used in one to five recording sessions on separate days and each recording session typically last for 2-6 h. The experiments were conducted according to NIH publication No. 85-23, "Principles of Laboratory Animal Care" and with the approval of the Institutional Animal Care and Use Committee of the University of Missouri- Columbia.

Acoustic Stimulation

Acoustic stimuli (4 ms with 0.5 ms rise-decay times delivered at 2 pps) were generated with an oscillator (KH model 1200) and a homemade electronic switch driven by a stimulator (Grass S88). These stimuli were then amplified after passing through a decade attenuator (HP 350D, Palo Alto, CA, USA) before they were fed to a small condenser loudspeaker (AKG model CK 50, 1.5 cm diameter, 1.2 g) that was placed 23 cm away from the bat and 30° contralateral to the recording site. Calibration of the loudspeaker was performed with a 1/4 inch microphone (B & K 4135, Norcross, GA, USA) placed at the position of the bat's head during recording using a measuring amplifier (B & K 2607, Norcross, GA, USA). The output of the loudspeaker was expressed in dB SPL in reference to 20 μPa root mean square. The maximum output was flat between 50 and 80 kHz (100 ± 5 dB SPL). The output then fell at low and high frequency range at about 15 dB /octave.

Experimental Protocol

Upon isolation of an IC neuron with 4 ms sound pulses, its BF was determined by changing the frequency and amplitude of sound stimuli. The minimum threshold (MT) at the BF was defined as the sound level that elicited 50% response probability from the neuron. The neuron's duration tuning curve was then plotted using the number of impulses in response to BF sound pulses that were varied in 8 durations (1, 1.5, 2, 4, 6, 8, 10 and 20 ms) and delivered at 10 dB above the MT (see Fig. 1). Rise-decay times for these different durations were typically 0.5 ms, but they were 0.25 ms for 1 ms pulse duration. The BD to which a duration-tuned IC neuron discharged the maximal number of impulses was identified.

Recorded action potentials were amplified, band-pass filtered (Krohn-Hite 3500), and then fed through a window discriminator (WPI 121) before being sent to an oscilloscope (Tektronix 5111, Beaverton, OR, USA)

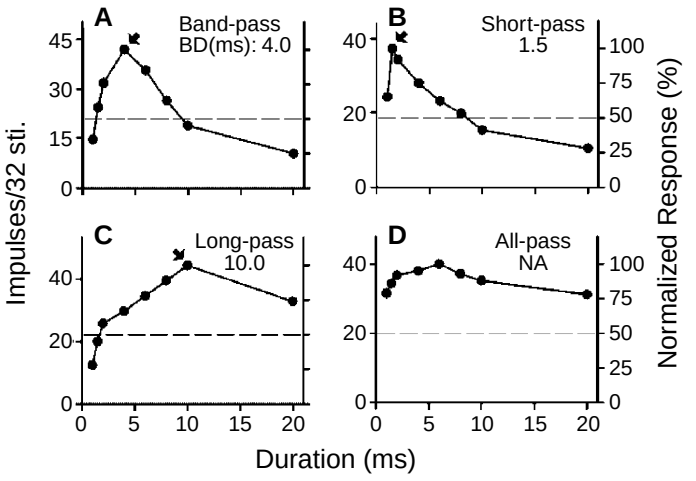

Fig. 1. A-D: Representative band-, short-, long-, and all-pass duration tuning curves of inferior colliculoar (IC) neurons of the big brown bat, *Eptesicus fuscus*, showing variation in the number of impulses with pulse duration. The horizontal dashed lines indicate the 50 % maximal response. The best duration (BD) of the band-, short- and long-pass duration tuning curves is indicated by a filled arrowhead. The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of these neurons were 32.5, 11.0, 37, 415 (A); 30.2, 12.0, 35, 335 (B); 38.5, 13.0, 43, 836 (C); and 42.8, 13.5, 45, 985 (D).

and an audio monitor (Grass AM6, Tilburg, North Brabant, Netherland). They were then sent to a computer (Gateway 2000, 486) for acquisition of peri-stimulus-time (PST) histograms (bin width: 500 μs, sampling period: 300 ms) to 32 pulse presentations. The number of impulses of each investigated IC neuron in response to presented sound pulses at selected amplitudes was then used to plot the RAF as described above.

Data Analysis

A family of RAF was then plotted for the recorded neuron with the BD and two non-BD sound pulses that were randomly presented. As in previous study (21), each RAF was plotted using the number of impulses in response to presented sound pluses delivered at the MT and 10 dB increments above the MT. The amplitude sensitivity of the neuron was then expressed with the best amplitude (BA) and a dynamic range (DR, See Fig. 2). The DR of the RAF of IC neurons obtained with BD and non-BD sound pulses was then quantitatively examined and statistically compared using repeated measures one-way ANOVA followed by a Student-Newman-Keuls multiple comparisons post-test with significance established at the $P < 0.05$ level.

Results

In this study, 122 IC neurons which discharged 3-7 impulses to each presented sound pulse were recorded at depths between 145 and 1780 μm (average: 618 ± 256 μm) with BFs of 18.0-66.4 kHz (average: 34.6 ± 8.2 kHz) and MTs of 19-45 dB SPL (average: 36 ± 6.7 dB SPL). The first-spike latency of these neurons in response to BF sounds at 10 dB above the MT was between 9.0 and 18.5 ms (average: 12.5 ± 1.9 ms). These basic response properties are similar to those reported in our previous studies (17, 26, 27, 34).

Duration Tuning Curves of IC Neurons

Duration tuning curves plotted for these 122 IC neurons can be described as the following four types. [1] IC neurons with *the band-pass duration tuning curve* discharged a maximal number of impulses to a specific duration and the maximal number of impulses decreases at least 50% at both limbs (Fig. 1A, n = 32, 26%). [2] IC neurons with *the short-pass duration tuning curve* discharged a maximum number of impulses to a short duration. The maximum number of impulses of these neurons decreased more than 50% at a longer duration but less than 50% at a shorter duration (Fig. 1B, n = 25, 20%). [3] IC neurons with the *long-pass duration tuning curve* discharge maximally to a long duration. The maximum number of impulses of these neurons decreased more than 50% at a shorter duration but less than 50% at a longer duration (Fig. 1C, n = 18, 15%). These three types of IC neurons are called duration tuned or selective neurons. The duration that elicited the maximum number of impulses from these neurons is called the BD (filled arrowhead in Fig. 1A,B,C). [4] The number of impulses of IC neurons with *the all-pass duration tuning curve* often varied more than 25% but never more than 50% at all durations tested (Fig. 1D, n = 47, 39%). These 47 IC neurons are called duration non-selective neurons. They do not have a BD.

The BD of those 75 IC neurons with band-, short- and long-pass duration tuning curves ranged

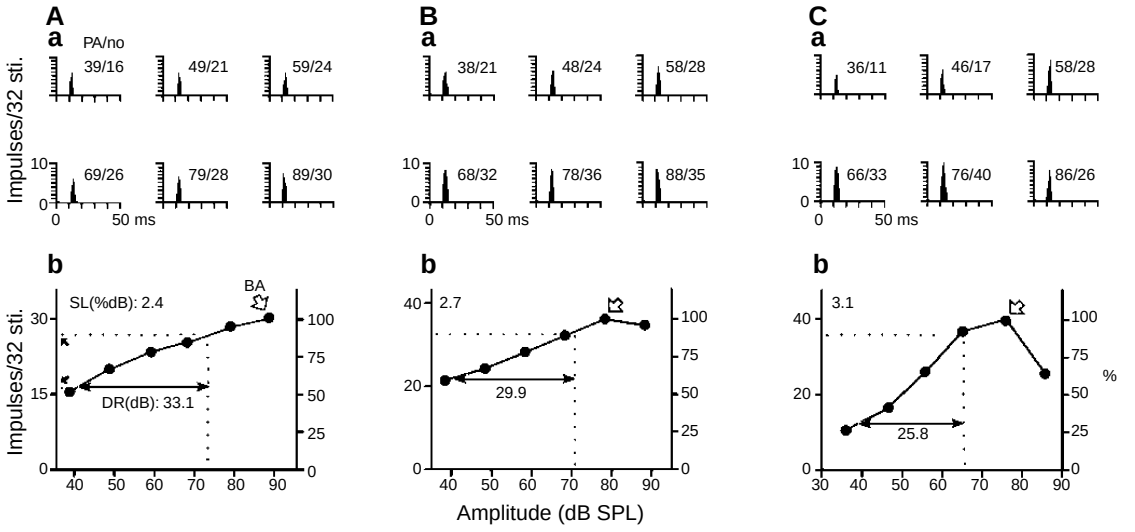

Fig. 2. Aa,Ba,Ca: The peristimulus (PST) histograms showing the discharge pattern of three representative IC neurons obtained with 4 ms best frequency (BF) pulses delivered at the minimum threshold (MT) and at 10 dB increments above the MT. The pulse amplitude (PA) used to obtain the discharge pattern and the discharged number of impulses (no) are shown in each histogram. Ab,Bb,Cb: The rate-amplitude function (RAF) of each neuron plotted with the number of impulses obtained at selected pulse amplitudes. The left ordinate indicates the number of impulses per 32 presented pulses and the right ordinate indicates the normalized percent response (%). The abscissa represents the pulse amplitude in dB SPL. The RAF of these three neurons is monotonic (Ab), saturated (Bb) and non-monotonic (Cb)(see text for definition). The best amplitude (BA) of each RAF is indicated by an unfilled arrowhead. The sharpness of the RAF is expressed with a dynamic range (DR) which is defined as the amplitude range corresponding to the number of impulses that is 10% below the maximum and 10% above the minimum. The slope of the linear portion of the ascending limb of the RAF is shown by SL (%/dB). The BF (kHz), latency (ms), MT (dB SPL) and recording depth (μm) of these neurons were 38.5, 13.0, 46, 785 (A); 35.8, 12.5, 40, 656 (B); and 28.5, 11.0, 34, 355 (C).

between 1.5 and 10 ms. For simplicity of comparison, we conveniently divided these neurons into three groups based on their BD. The first group of (n = 27, 36%) neurons with a BD of 1.5 (n = 7) and 2.0 (n = 20) ms; the 2nd group of (n = 30, 40%) neurons with a BD of 4.0 (n = 21) and 6.0 (n = 9) ms, and the 3rd group of (n = 18, 24%) neurons with a BD of 8.0 (n = 9) and 10 (n = 9) ms. The BD of these three groups of neurons approximately corresponded to the duration of pulses emitted by the big brown bat during the search, approach and terminal phases of hunting (10, 28, 31).

RAF of IC Neurons

The discharge pattern and RAF of three representative IC neurons obtained with 4 ms BF sound pulses are shown in Fig. 2. Clearly, the number of impulses of these IC neurons varies with pulse amplitude (Fig. 2, Aa,Ba,Ca). The RAF of the first neuron shows that the number of impulses progressively increased to the strongest pulse amplitude available by the stimulus system. This type of RAF is called the monotonic RAF (Fig. 2Ab). The RAF of the second neuron shows that the number of impulses increased with pulse amplitude from the minimum to the maximum and then decreased less than 25% at stronger pulse amplitude. This type of RAF is called the saturated RAF (Fig. 2Bb). The RAF of the third neuron shows that the number of impulses increased with pulse amplitude from the minimum to the maximum and then decreased more than 25% thereafter at stronger pulse amplitude. This type of RAF is called the non-monotonic RAF (Fig. 2Cb).

We used two parameters of a RAF to express a neuron's amplitude sensitivity. The first parameter is the DR which is defined as the amplitude range corresponding to the number of impulses that is 10% below the maximum and 10% above the minimum (DR in Fig. 2, Ab, Bb, Cb, double arrowhead). Within this DR, the neuron's number of impulses linearly increased with pulse amplitude. As such, the DR is a measure of the breadth of the RAF and a RAF with a small DR has a sharper slope (SL) in its ascending limb than a RAF with a large DR. The second parameter is the BA. For IC neurons with saturated and non-monotonic RAFs, the BA is the pulse amplitude that elicited the maximal number of impulses (Fig. 2, Bb, Cb, unfilled arrowhead). Because the number of impulses of IC neurons with monotonic RAF progressively increased with pulse amplitude, the BA is approximated as the strongest pulse amplitude available by the stimulus system (Fig. 2Ab, unfilled arrowhead).

Table 1. Percent distribution of three types of RAF curves of IC neurons determined with BD and non-BD sound pulses

	Monotonic	Saturated	Non-monotonic
BD (N = 75)	30 (40%)	25 (33%)	20 (27%)
Non-BD (N = 150)	79 (53%)	56 (37%)	15 (10%)

N: the number of RAF curves of IC neurons

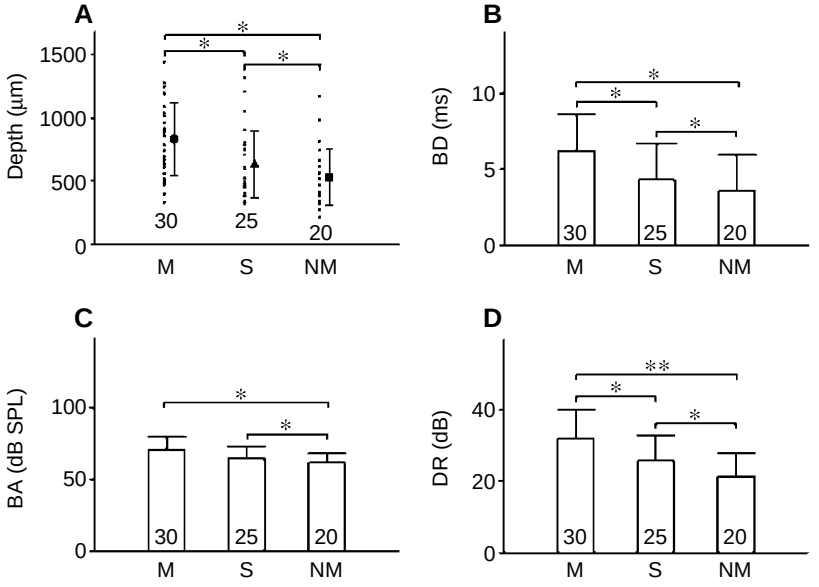

Fig. 3. A: The distribution of the recording depth of IC neurons with monotonic (M), saturated (S) or non-monotonic (NM) RAF. The mean recoding depth and standard deviation are shown by the distribution. The three means are significantly different (repeated measure one-way ANOVA, $P < 0.01$). B,C,D: Bar histograms showing the average BD (B), BA (C) and DR (D) of 75 IC neurons based on the three types of RAFs. The average BD, BA and DR obtained from the three types of RAFs are significantly different (repeated measures one-way ANOVA, $P < 0.05$).

Amplitude Sensitivity of IC Neurons with Different Type of RAF

In this study, 225 RAFs were plotted with BD and non-BD sound pulses from 75 duration selective neurons. The type of RAF and recording depth of these neurons are as follow: [1] monotonic (90 RAFs from 30 neurons, 40%), recorded between 336-1780 μm (average: 838 ±293 μm), [2] saturated (74 RAFs from 25 neurons; 33%), recorded between 305-1325 μm (average: 633 ±267 μm), and [3] non-monotonic (61 RAFs from 20 neurons; 27%), recorded between 145-1175 μm (average: 531 ±255 μm). These three types of IC neurons differ significantly in recording depth, BD, BA and DR (Fig. 3, repeated measures one-way ANOVA, $P < 0.05$). A Student-Newman-Keuls multiple comparisons post test showed significant differences between each set of data ($P < 0.05$).

These comparisons show that monotonic IC neurons are mostly recorded at deeper IC and they have the largest average BD, BA and DR (Fig. 3, A, C,D, M). Conversely, non-monotonic IC neurons are mostly recorded at upper IC and they have the smallest average BD, BA and DR. IC neurons with saturated RAF have intermediate recording depth, BD, BA and DR (Fig. 3, A,C,D,S).

Table 1 shows the percent distribution of three types of RAF of IC neurons determined with BD and non-BD sound pulses. Clearly, there is always a large percent monotonic RAF than either saturated or non-monotonic RAF when plotted with both BD and non-BD pulses. However, the percent of both monotonic and saturated RAFs decreased while the percent of non-monotonic RAF greatly increased (from 10 to 27%) when the RAF was plotted with BD pulses.

RAF of IC Neurons Plotted with BD and Non-BD Pulses

The envelope of three pulse durations (1.5, 4.0

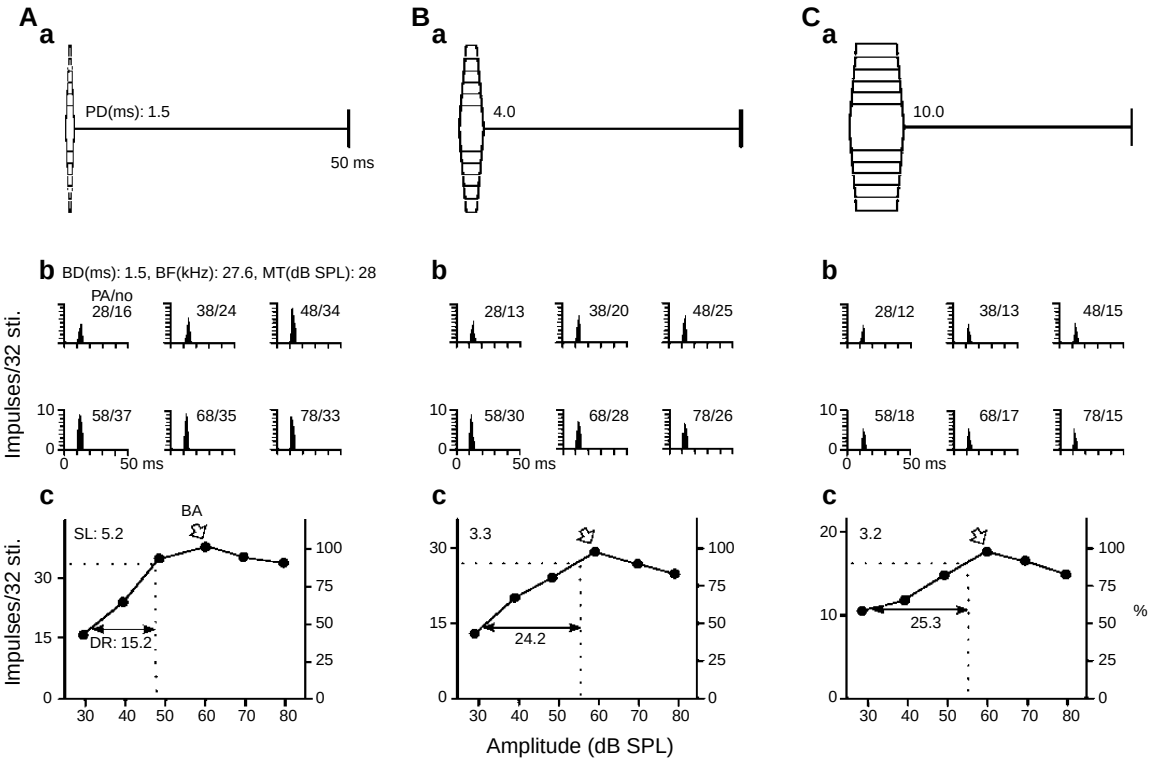

Fig. 4. Aa,Ba,Ca: Sketches showing the envelopes of three BF pulses (pulse duration, PD = 1.5, 4.0 and 10 ms) at several selected amplitudes. Ab,Bb,Cb: PST histograms showing the discharge pattern and the number of impulses (no) of an IC neuron in response to BF sounds delivered at selected PA. This neuron had a 1.5 ms best duration (BD). Ac,Bc,Cc: The neuron's RAF plotted with the number of impulses shown in the Ab, Bb, Cb. Note that this neuron always had a saturated RAF when determined with three PDs. However, it had the smallest DR when the RAF was plotted with the BD pulses (Ac)(See Fig. 2 for legends).

and 10 ms) at several selected amplitudes, the discharge patterns and RAFs of three representative IC neurons are shown in Figs. 4-6. The first neuron had a BD of 1.5 ms, and it discharged the largest number of impulses to the BD pulse than to other two non-BD pulses at 58 dB SPL (Fig. 4, Ab *vs.* Bb *vs.* Cb, impulses number, 37 *vs.* 30 *vs.* 18). When stimulated with all three pulse durations, the neuron always had a saturated RAF with varied DR and SL. However, the RAF had the smallest DR with the largest SL when plotted with 1.5 ms BD pulses (Fig. 4, Ac *vs.* Bc,Cc).

The second neuron had a BD of 4.0 ms. It discharged maximally to 1.5 and 4.0 ms pulses at 71 dB SPL but to 10 ms pulses at 81 dB SPL (Fig. 5, 71 dB SPL at Ab,Bb, 81 dB SPL at Cb). However, the neuron discharged the largest number of impulses to the 4.0 ms BD pulses (Fig. 5Bb *vs.* Ab *vs.* Cb, impulses number, 34 *vs.* 27 *vs.* 20). The neuron's RAF changed from saturated to non-monotonic and then to monotonic when the sound pulse lengthened from 1.5 to 4.0 and then to 10 ms (Fig. 5Ac,Bc,Cc). The neuron's RAF had the smallest DR with the largest SL when determined with 4.0 ms BD pulses (Fig. 5, Bc *vs.* Ac,Cc).

The number of impulses of the third neuron with a BD of 10 ms also varied with pulse amplitude and duration (Fig. 6, Ab,Bb,Cb). This neuron discharged maximally to 1.5 and 4.0 ms at 89 dB SPL but to 10 ms pulses at 79 dB SPL (Fig. 6, Ac *vs.* Bc *vs.* Cc). The neuron's largest number of impulses was obtained with the 10 ms BD pulses (Fig. 6, Cb *vs.* Bb *vs.* Ab, impulses number, 29 *vs.* 23 *vs.* 17). The neurons had a monotonic RAF when determined with 1.5 and 4.0 ms pulses. However, the neuron had a saturated RAF with the smallest DR and largest SL when determined with the 10 ms BD pulse (Fig. 6, Ac, BC *vs.* Cc).

To show the variation of DR of IC neurons with BD and non-BD pulses, we compared the average DR of IC neurons obtained with 1.5, 4.0 and 10 ms pulses in relation to the BD (Fig. 7). It is clear that the DR differed significantly among IC neurons with different BDs (Fig. 7A,B,C, repeated measures one-way ANOVA, $P < 0.01$). However, IC neurons always had the smallest DR when determined with the pulse duration that was equal to the BD (Fig. 7, A,B,C, filled arrowhead).

To more clearly show the difference in DR of IC neurons obtained with BD and non-BD pulses, we plotted the average DR of IC neurons in relation to the

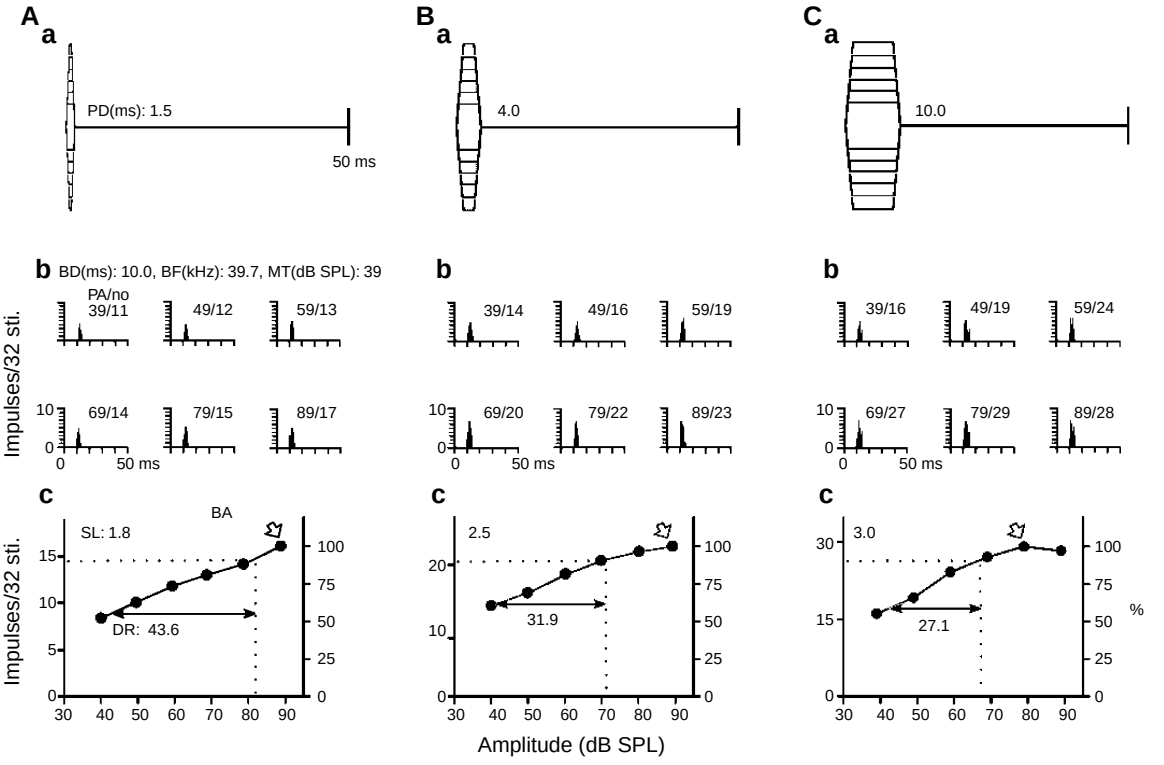

Fig. 5. Aa,Ba,Ca: The pulse envelope, discharge pattern and RAF of an IC neuron that had a BD of 4.0 ms. Note that the neuron's smallest DR was obtained when the RAF was plotted with the BD pulses (Bc). Note also that the neuron's RAF changed from saturated to non-monotonic and then monotonic when determined with 1.5, 4.0 and 10 ms BF pulses (See Fig. 4 for legends).

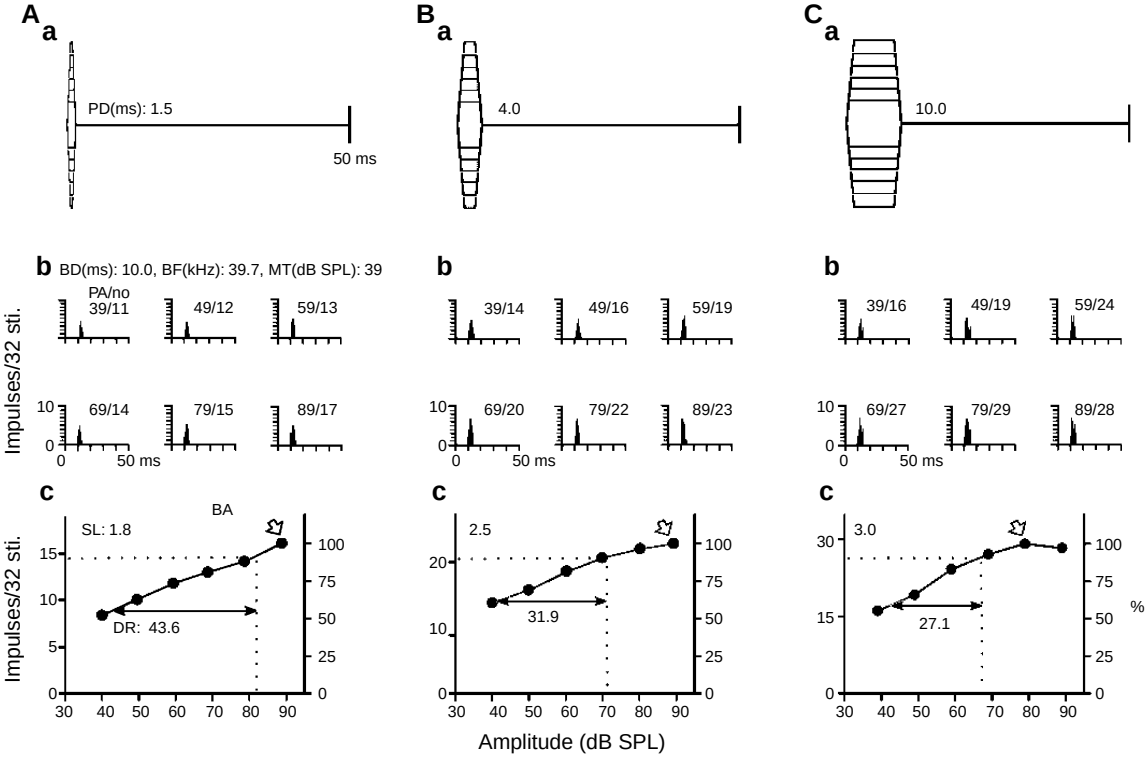

Fig. 6. Aa,Ba,Ca: The pulse envelope, discharge pattern and RAF of an IC neuron that had a BD of 10 ms. Note the neuron's smallest DR was obtained when the RAF was plotted with the BD pulses (Cc). Note also that the neuron had a monotonic RAF when determined with 1.5 and 4.0 ms pulses but had a saturated RAF when determined with 10 ms BF pulses (See Fig. 4 for details).

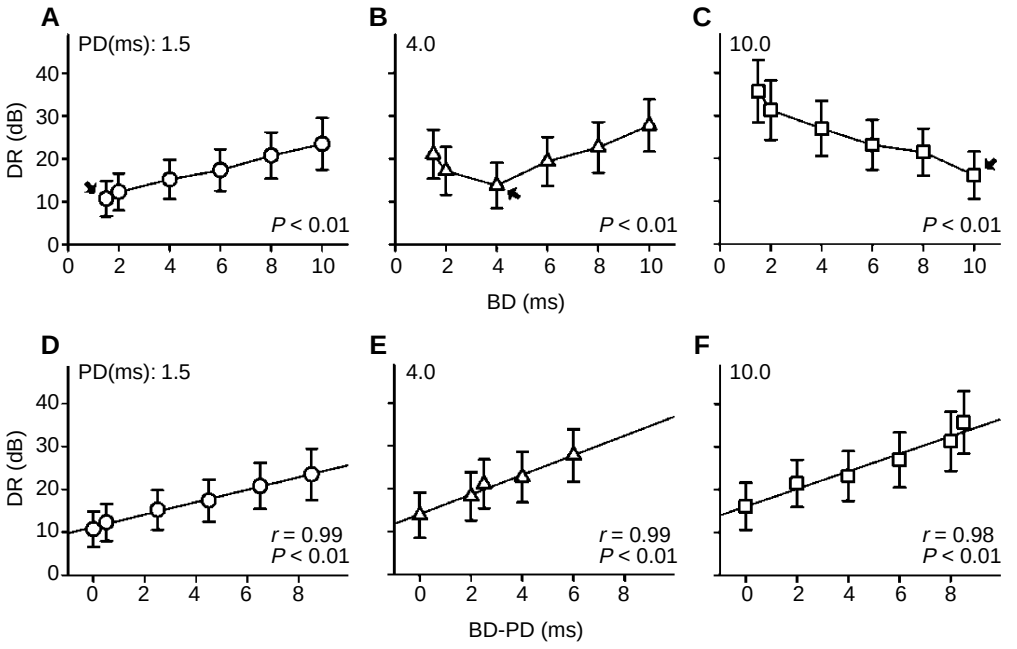

Fig. 7. A-C: The average DR of IC neurons determined with BF pulses at three PDs (A, 1.5 ms; B, 4.0 ms; C, 10 ms) in relation to the BD. The number and vertical bar at each data point represent the standard deviation and the number of neurons tested. P: significance level. While the DR is significantly different among IC neurons with different BDs, IC neurons have the smallest DR when PD is equal to the BD (indicated with a filled arrowhead). D,E,F: The average DR of IC neurons determined with BF pulses at three PDs in relation to the difference between BD and PD (BD-PD). The solid line, r and P represent the linear regression line, correlation coefficient and significance level. Note that the DR progressively increases with increasing the BD-PD.

differences between BD and pulse duration. The average DR of IC neurons increased significantly with increasing differences between the BD and PD (Fig. 7, D,E,F, repeated measures one-way ANOVA, $P < 0.01$). The smallest average DR of IC neurons was obtained when the pulse duration was equal to BD. Furthermore, IC neurons with 1.5 ms BD had significantly smaller average DR (10.7 ± 4.1, Fig. 7D) than IC neurons with 4.0 ms and 10 ms BD (13.8 ± 5.3 and 16.1 ± 5.5, respectively, Fig. 7, E,F, repeated measures one-way ANOVA, $P < 0.01$).

Correlation of BA with BF, BD, DR and Recording Depth

Because the RAF of IC neurons recorded at different depths varied in BF, BA, BD and DR, we studied the correlation among these different measurements by performing linear regression analysis of different scatter plots of these measurements. However, we did not include the data obtained from those 30 monotonic IC neurons because we could not predict if the RAF of these neurons would remain monotonic or change into saturated or non-monotonic when still stronger pulse amplitude was available. As such, the BA for those monotonic IC neurons was only an approximate value and was not as accurate as the BA determined for saturated and non-monotonic IC neurons.

Our linear regression analyses reveal that the BA of those 45 saturated and non-monotonic IC neurons is significantly correlated with the recording depth, BF, BD and DR (Fig. 8, $P < 0.05$). As such, neurons with large BA are mostly recorded at deeper IC with high BF than neurons with small BA at upper IC with low BF (Fig. 8, A,B). Also most neurons with large BA have longer BD and larger DR than neurons with small BA have (Fig. 8, C,D).

Discussion

Pulse Parameters Affect the SL and DR of RAF

Previous studies show that shorter pulse rise times, higher pulse repetition rates or shortening of pulse duration increases the SL but decreases the DR of the RAF of auditory neurons (6, 9, 21, 25, 36). In parallel with these studies, we observed that the SL and DR of the RAF of IC neurons is dependent on the difference between the BD and stimulus pulse duration. This is supported by the following findings. [1]. The RAF of IC neurons has the smallest DR and largest SL when obtained with BD pulses (Figs. 4,5, 6). [2]. The smaller the differences between the BD and stimulus pulse duration, the smaller the DR would be (Fig. 7). [3]. When non-BD pulses were replaced

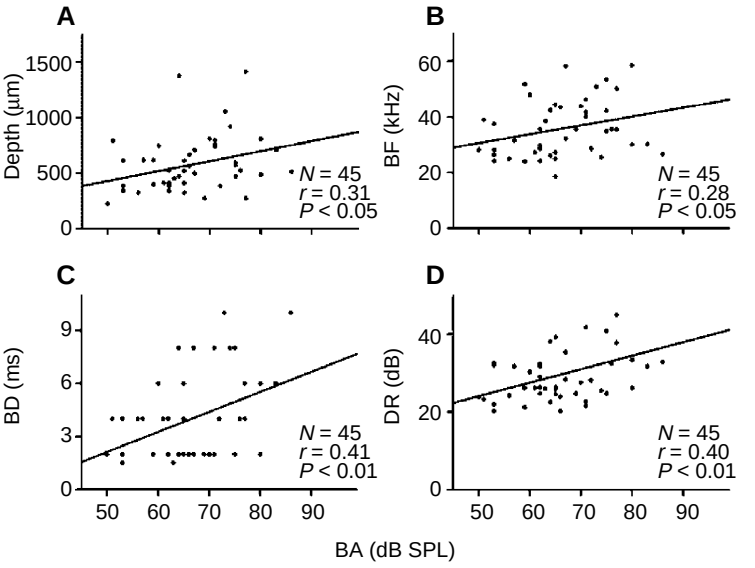

Fig. 8. A-D: Scatter plots showing the distribution of the recording depth, BF, BD and DR in relation to the BA of 45 saturated and non-monotonic IC neurons. The linear regression line and correlation coefficient for each plot are shown with a solid line and r. *P*: significance level (See text for details).

with BD pulses as stimuli, there is a great increase in the percent of non-monotonic RAF and a great decrease in the percent of monotonic RAF (Table 1). All these findings suggested that IC neurons had the greatest amplitude sensitivity when the stimulus pulse duration matches a neuron's BD. What might be the possible mechanism underlying these findings?

Previous studies show that GABA-mediated inhibition shapes the duration selectivity of IC neurons (2-4, 7, 15, 32, 33). In addition, our earlier study shows that GABA-mediated inhibition sharpens duration selectivity of IC neurons by producing a greater decrease in the response magnitude for non-BD pulses than for BD pulses (32). Conceivably, this duration-dependent GABA-mediated inhibition is also the underlying mechanism for shaping the amplitude sensitivity of IC neurons. If this is true, then our data suggest that sharpening of RAF by GABA-mediated inhibition would be stronger for BD than for non-BD pulses as evidenced by the greater SL of RAF plotted with the BD pulses (Figs. 4, 5, 6). Similarly, GABA-mediated inhibition would be stronger for IC neurons with shorter BD than for longer BD pulses as shown by the fact that IC neurons with 1.5-ms BD had significantly smaller average DR than IC neurons with 4.0-ms and 10-ms BD (Fig. 7, D,E,F). Future works are necessary to study the neural mechanism underlying these observations.

Amplitude Sensitivity in the IC

In agreement with previous studies (1, 13, 21, 24, 36), we observed that IC neurons with non-monotonic RAF were mostly recorded at upper IC, and they typically had the smallest BD and BA and DR (Fig. 3). Because the BA of IC neurons is significantly correlated with the recording depth, the BF, BD and DR, it follows that the DR is also significantly correlated with the BD and recording depth (Fig. 8). We have shown previously that the BF of IC neurons progressively increases with recording depth (17, 26, 27, 34). We also showed that low BF neurons at upper IC have shorter BD and sharper duration selectivity than high BF neurons in the deeper IC have (18, 32, 33). All these studies plus the present data suggest that low BF neurons at upper IC have smaller BA, shorter BD and smaller DR and sharper amplitude sensitivity than high BF neurons at the deeper IC have. In other words, auditory sensitivity of IC neurons appears to be systematically organized along the dorso-ventral axis of the IC in time, frequency and amplitude domains.

Behavioral Relevance

In amplitude domain, the ear of insectivorous bats such as *Eptesicus fuscus* is constantly bombarded with intense self-emitted pulses, pulses emitted by other bats and various echoes during hunting. Previous studies have shown that bats systematically decrease pulse amplitude during hunting to compensate for progressively increasing echo amplitude so as to ensure the echoes reaching the ear at an optimal level (11, 12, 16, 23). Bats also contract their middle ear

muscles during pulse emission to attenuate stimulation by the intense self-emitted pulses (14, 30) and increase the threshold for target detection and discrimination (11, 12, 22, 29). All these studies indicate that the bat auditory system is well-prepared for analysis of amplitude of echoes whose duration systematically shortens throughout the entire course of hunting. This notion is supported by our present study.

For example, the range of BD of IC neurons (Figs. 7A-C, 8C) obtained from the present study covers the duration of pulses emitted by the *Eptesicus fuscus* during the search, approach and terminal phases of hunting (10, 28, 31). Furthermore, IC neurons have greater amplitude sensitivity to BD pulses than to non-BD pulses (Figs. 4-7). Also, low BF IC neurons have shorter BD and sharper amplitude sensitivity than high BF IC neurons with longer BD (Fig. 8). All these data suggest that echo duration at different phases of hunting can be successfully encoded by IC neurons with different BD. Our data also suggested that IC neurons that tune to an echo duration also have the greatest sensitivity to echo amplitude, in particular during the terminal phase of hunting when the echo duration becomes very short. Since the BF, BD and DR appeared to be orderly organized along the dorso-ventral axis of the IC (Fig. 8), we tempted to suggest that multi-parametric echo analysis might be systematically processed by different populations of IC neurons throughout the entire course of hunting.

Acknowledgments

We thank the two anonymous reviewers for comments on an earlier version of this manuscript. This work was supported by a grant and fellowship from the Graduate School, the Division of Biological Sciences and College and Arts and Sciences of University of Missouri-Columbia.

References

1. Aitkin, L. Rate-level functions of neurons in the inferior colliculus of cats measured with the use of free-field sound stimuli. *J. Neurophysiol*. 65: 383-392, 1991.
2. Casseday, J.H., Ehrlich, D. and Covey, E. Neural tuning for sound duration: role of inhibitory mechanisms in the inferior colliculus. *Science* 264: 847-850, 1994.
3. Casseday, J. H., Ehrlich, D. and Covey, E. Neural measurement of sound duration: control by excitatory-inhibitory interaction in the inferior colliculus. *J. Neurophysiol*. 84: 1475-1487, 2000.
4. Ehrlich, D., Casseday, J. H. and Covey, E. Neural tuning to sound duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *J. Neurophysiol*. 77: 2360-2372, 1997.
5. Faure, P. A., Fremouw, T., Casseday, J. H. and Covey, E. Temporal masking reveals properties of sound-evoked inhibition in duration-tuned neurons of the inferior colliculus. *J. Neurosci.* 23: 3052-3065, 2003.
6. Fusessery, Z. M. Response selectivity for multiple dimensions of frequency sweeps in the pallid bat inferior colliculus. *J. Neurophysiol.* 72: 1061-1079, 1994.
7. Fuzessery, Z. M. and Hall, J. C. Sound duration selectivity in the pallid bat inferior colliculus. *Hear. Res.* 137: 137-154, 1999.
8. Galazyuk, A. V. and Feng, A. S. Encoding of sound duration by neurons in the auditory cortex of the little brown bat, Myotis lucifugus. *J. Comp. Physiol.* A 180: 301-311, 1997.
9. Galazyuk, A. V., Liano, D. and Feng, A. S. Temporal dynamics of acoustic stimuli enhance amplitude tuning of inferior colliculus neurons. *J. Neurophysiol*. 83: 128-138, 2000.
10. Griffin, D.R. Listening in the Dark. New Haven, CT: Yale Univ. Press, 1958 (Reprinted by Comstock Pub. Asso. Cornell Univ. Press 1986).
11. Hartley, D. J. Stabilization of perceived echo amplitudes in echolocating bats. I. Echo detection and automatic gain control in the big brown bat, *Eptesicus fuscus*, and the fishing bat. Noctilio leporinus. *J . Acoust. Soc. Am*. 91: 1120-1132, 1992a.
12. Hartley, D. J. Stabilization of perceived echo amplitude in echolocating bats. II. The acoustic behavior of the big brown bat, *Eptesicus fuscus*, while tracking moving prey. *J. Acoust. Soc. Am.* 91: 1133-1149, 1992b.
13. Heil, P., Rajan, R. and Irvine, D. R. F. Sensitivity of neurons in cat primary auditory cortex to tones and frequency-modulated stimuli. I: Effects of variation of stimulus parameters. *Hear. Res*. 63: 108-134: 1992.
14. Henson, O. W. Jr. The activity and function of the middle ear muscle in echolocation bats. *J. Physiol. (Lond)* 180: 871-887, 1965.
15. Jen, P. H. S. and Feng, R. B. Bicuculline application affects discharge pattern and pulse-duration tuning characteristics of bat inferior collicuar neurons. *J. Comp. Physiol*. A 184: 185-194, 1999.
16. Jen, P. H. S. and Kamada, T. Analysis of orientation signals emitted by the CF-FM bat, Pteronotus parnellii and the FM bat, *Eptesicus fuscus* during avoidance of moving and stationary obstacles. *J. Comp. Physiol*. 148: 389-398, 1982.
17. Jen, P. H. S. and Schlegel, P. Auditory physiological properties of the neurons in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *J. Comp. Physiol*. A 147: 351-363, 1982.
18. Jen, P. H. S. and Wu, C. H. The role of GABAergic inhibition in shaping the response size and duration selectivity of bat inferior collicular neurons to sound pulses in rapid sequences. *Hear. Res.* 202: 222-234, 2005.
19. Jen, P. H. S., Sun, X. D., Chen, D. and Teng, H. B. Auditory space representation in the inferior colliculus of the FM bat, *Eptesicus fuscus*. *Brain Res*. 419: 7-18, 1987.
20. Jen, P. H. S. and Zhou, X. M. Temporally patterned sound trains affect duration tuning characteristics of bat inferior collicular neurons. *J. Comp. Physiol*. A 185: 471-478, 1999.
21. Jen, P. H. S., Zhou, X. M. and Wu, C. H. Temporally patterned sound trains affect intensity and frequency sensitivity of inferior collicular neurons of the big brown bat, *Eptesicus fuscus. J. Comp. Physiol*. A 187: 605-616, 2001.
22. Kick, S. A. and Simmons, J. A. Automatic gain control in the bat's sonar receiver and the neuroethology of echolocation. *J. Neurophysiol*. 4: 2725-2737, 1984.
23. Kobler, J. B., Wilson, B. S., Henson, O. W. and Bishop, A. L. Echo intensity compensation by echolocating bats. *Hear. Res*. 20: 99-108, 1985.
24. Phillips, D. P. and Hall, S. E. Spike-rate intensity function of cat cortical neurons studied with combined tone-noise stimuli. *J. Acoust. Soc. Am*. 80: 177-187, 1986.
25. Phillips, D. P. Effect of tone-pulse rise time on rate-level functions of cat auditory cortex neurons: excitatory and inhibitory processes shaping responses to tone onset. *J. Neurophysiol*. 59: 1524-1539, 1988.
26. Pinheiro, A. D., Wu, M. and Jen, P. H. S. Encoding repetition rate and duration in the inferior colliculus of the big brown bat, *Eptesicus fuscus*. *J. Comp. Physiol. A* 169: 69-85, 1991.
27. Poon, P. W., Sun, X. D., Kamada, T. and Jen, P. H. S. Frequency

and space representation in the inferior colliculus of the FM bat, *Eptesicus fuscus*. *Exp. Brain Res*. 79: 83-91, 1990.
28. Simmons, J. A., Fenton, M. B. and O'Farrell, M. J. Echolocation and pursuit of prey by bats. *Science* 203: 16-21, 1979.
29. Simmons, J. A., Moffat, A. J. and Masters, W. M. Sonar gain control and echo detection thresholds in the echolocating bat, *Eptesicus fuscus*. *J. Acoust. Soc. Am*. 91: 1150-1163, 1992.
30. Suga, N. and Jen, P. H. S. Peripheral control of acoustic signals in the auditory system of echolocating bats. *J. Exp. Biol.* 62: 277-311, 1975.
31. Surlykke, A. and Moss, C. F. Echolocation behavior of big brown bat, *Eptesicus fuscus*, in the field and the laboratory. *J. Acoustic. Soc. Am.* 108: 2419-2429, 2000.
32. Wu, C. H. and Jen, P. H. S. The role of GABAergic inhibition in shaping duration selectivity of bat inferior collicular neurons determined with temporally patterned sound trains. *Hear. Res.* 215: 55-65, 2006a.
33. Wu, C. H. and Jen, P. H. S. GABA-mediated echo duration selectivity of inferior collicular neurons of *Eptesicus fuscus*, determined with single pulses and pulse-echo pairs. *J. Comp. Physiol*. A 192: 985-1002, 2006b.
34. Wu, M. and Jen, P. H. S. Encoding of acoustic stimulus intensity by inferior collicular neurons of the big brown bat, *Eptesicus fuscus*. *Chinese J. Physiol.* 34: 145-155, 1991.
35. Zhou, X. M. and Jen, P. H. S. The effect of sound intensity on duration-tuning characteristics on bat inferior collicular neurons. *J. Comp. Physiol*. A 187: 63-73, 2001.
36. Zhou, X. M., Jen, P. H. S. The effect of sound duration on rate-amplitude functions of inferior collicular neurons in the big brown bat, *Eptesicus fuscus*. *Hear. Res.* 166: 124-135, 2002.
37. Zhou, X. M. and Jen, P. H. S. Duration selectivity of bat inferior collicular neurons improves with increasing pulse repetition rate. *Chinese J. Physiol*. 49: 46-55, 2006.