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Recovery Cycle of Neurons in the Inferior Colliculus of the FM Bat Determined with Varied Pulse-Echo Duration and Amplitude

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Abstract

The recovery cycle of auditory neurons is an important neuronal property which underlies a bat's ability in analyzing returning echoes and to determine target distance (*i.e.* echo ranging). In the same token, duration selectivity of auditory neurons plays an important role in pulse recognition in bat echolocation. Because insectivorous bats progressively vary the pulse parameters (repetition rate, duration, and amplitude) during hunting, the recovery cycle of auditory neurons is inevitably affected by their selectivity to other co-varying echo parameters. This study examines the effect of pulse duration and amplitude on recovery cycle of neurons in the central nucleus of the inferior colliculus (IC) of the FM bat, *Pipistrellus abramus*, using biologically relevant pulse-echo (P-E) pairs with varied duration and amplitude difference. We specifically examine how duration selectivity may affect a neuron's recovery cycle. IC neurons have wide range of recovery cycle and best duration (BD) covering P-E intervals and duration occurring different phases of hunting. The recovery cycle of most IC neurons increases with P-E duration and amplitude difference. Most duration-selective IC neurons recover rapidly when stimulated with biologically relevant P-E pairs. As such, neurons with short BD recover rapidly when stimulated with P-E pairs of short duration and small P-E amplitude difference. Conversely, neurons with long BD recover rapidly when stimulated with P-E pairs of long duration and large P-E amplitude difference. These data suggest that bats may potentially utilize the response of IC neurons with different BD and recovery cycle to effectively perform echo detection, recognition of echo duration and echo ranging throughout a target approaching sequence.

Key Words: bat, amplitude, duration, recovery cycle, inferior colliculus, pulse-echo pairs

Introduction

During hunting, insectivorous bats progressively shorten the duration, decrease the amplitude and increase the repetition rate of emitted pulses (P) as they search, approach and finally intercept insects or negotiate obstacles (4, 7, 15, 17). This dynamic variation of multiple parameters of emitted pulses predicts that analysis of an echo parameter by bats would be inevitably affected by other co-varying echo parameters. Indeed, we have previously shown that duration selectivity of most neurons in the central

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nucleus of the inferior colliculus (IC) improves with increasing pulse repetition rate and frequency but becomes poor at very strong pulse intensity (9-11, 21, 26, 29). When stimulated with pulse-echo (P-E) pairs, the echo duration selectivity of IC neurons varies with P-E amplitude difference and improves with shortening pulse duration and P-E interval (8, 22). Conversely, pulse duration profoundly affects the amplitude and frequency selectivity of IC neurons (23-25, 27).

Sound duration is an important acoustic parameter that contributes to the distinct spectral and temporal attributes of individual biological sounds. As such, duration selectivity of auditory neurons plays an important role for sound recognition in animal communication and in bat echolocation. In the same token, the recovery cycle of auditory neurons is an important neuronal property that determines a neuron's ability in responding to closely spaced sound pulses. As such, it underlies a bat's ability to analyze the returning echoes and to determine the target distance, *i.e.* echo ranging. For this reason, many studies have devoted to examining the recovery cycle of bat IC neurons using P-E pairs (3, 5, 12-14, 16, 18-20, 28). These studies showed that IC neurons have wide range of recovery cycles, and GABAergic inhibition contributes significantly to shaping the recovery cycle of these neurons. Although two studies showed that the recovery cycle of bat IC neurons tended to increase with P-E duration, they did not examine variation of recovery cycle of IC neurons with changing P-E duration and amplitude (5, 20). To further explore the dynamic aspects of auditory temporal processing, we examined the variation of the recovery cycle of IC neurons using P-E pairs with varied interval, duration and amplitude mimicking those occurring during search, approach and terminal phases of hunting (4, 15, 17). In particular, we examined how a neuron's duration selectivity might affect its recovery cycle.

We report here that the recovery cycle of most IC neurons increases with the duration and amplitude difference of P-E pairs. Most duration-selective neurons recover rapidly when tested with P-E pairs delivered at the best duration (BD) and at biologically relevant P-E amplitude difference. As such, IC neurons with short BD recover rapidly when stimulated with P-E pairs with short duration and small P-E amplitude difference. Conversely, IC neurons with long BD recover rapidly when stimulated with P-E pairs with long duration and large P-E amplitude difference. A preliminary report of this study has been presented recently (19).

Materials and Methods

Twelve adult *Pipistrellus abramus* (4-6.5 g, b.w.) were used for this study. As described in the previous study (19), the flat head of 1.8-cm nail was glued onto the exposed skull of each Nembutal-anesthetized (45-59 mg/kg b.w.) bat with acrylic glue and dental cement. After securing the bat to an aluminum plate inside a sound-proof room (temperature 28-30°C), its head was immobilized by fixing the shank of the nail into a brass rod with a set of screws. The bat's head was oriented with the eye-nostril line pointed to 0° in azimuth and 0° in elevation with respect to the frontal auditory space. A small hole (diameter: 200-500 μm) was drilled in the skull above the IC for orthogonal insertion of 2 M NaCl glass pipette electrodes (impedance: 5-10Ω) to record sound-evoked response. A silver-wire indifferent electrode was placed on the nearby temporal muscles. A neuron's recording depth was read from the scale of a hydraulic drive (Model 640, David-Kopf Instruments, Tujunga, CA, USA). Each bat was used in one to three recording sessions on separate days and each recording session typically lasted for 2-6 h. The experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Central China Normal University, Wuhan, Hubei, PRC.

Two sound generation systems were used for this study. To generate sound stimuli, continuous sine waves from a function generator (GFG-8016G, Good Will Inst Co., Ltd, Bayan Lepas, Penang, Malaysia) were formed into tone pulses (40 ms with 2 ms rise-decay times), delivered at 2 pulses per second by a custom-made tone burst generator (electronic switch) driven by a stimulator (Model SEN-7203, Nihon Kohden Co, Shinjuku, Tokyo, Japan). The tone pulses were then amplified (custom-made amplifier) after passing a decade attenuator (LAT-45, Leader, Kohokuku, Yokohama, Japan) before they were fed into a loudspeaker (AKG model CK 50, 1.5 cm in diameter, 1.2 g, frequency response 1 ~ 100 kHz). The loudspeaker was placed 30 cm away from the bat and at 30° contralateral to the recording site. Calibration of the loudspeaker was conducted with a 1/4 inch microphone (4939, B & K, Narum Denmark) placed at the bat's ear using a measuring amplifier (2610, B & K, Nærum, Denmark). The output of the loudspeaker was expressed in dB SPL in reference to 20 μPa root mean square.

Upon isolation of an IC neuron with 4 ms pulses (0.5 ms rise-decay times), its threshold at each responsive frequency was then audio-visually determined by changing the amplitude of sound stimuli such that the sound amplitude on average elicited 50% response probability from the neuron. The frequency that elicited the neuron's response with the lowest amplitude was defined as the best frequency (BF). The threshold at the BF was defined as the minimum

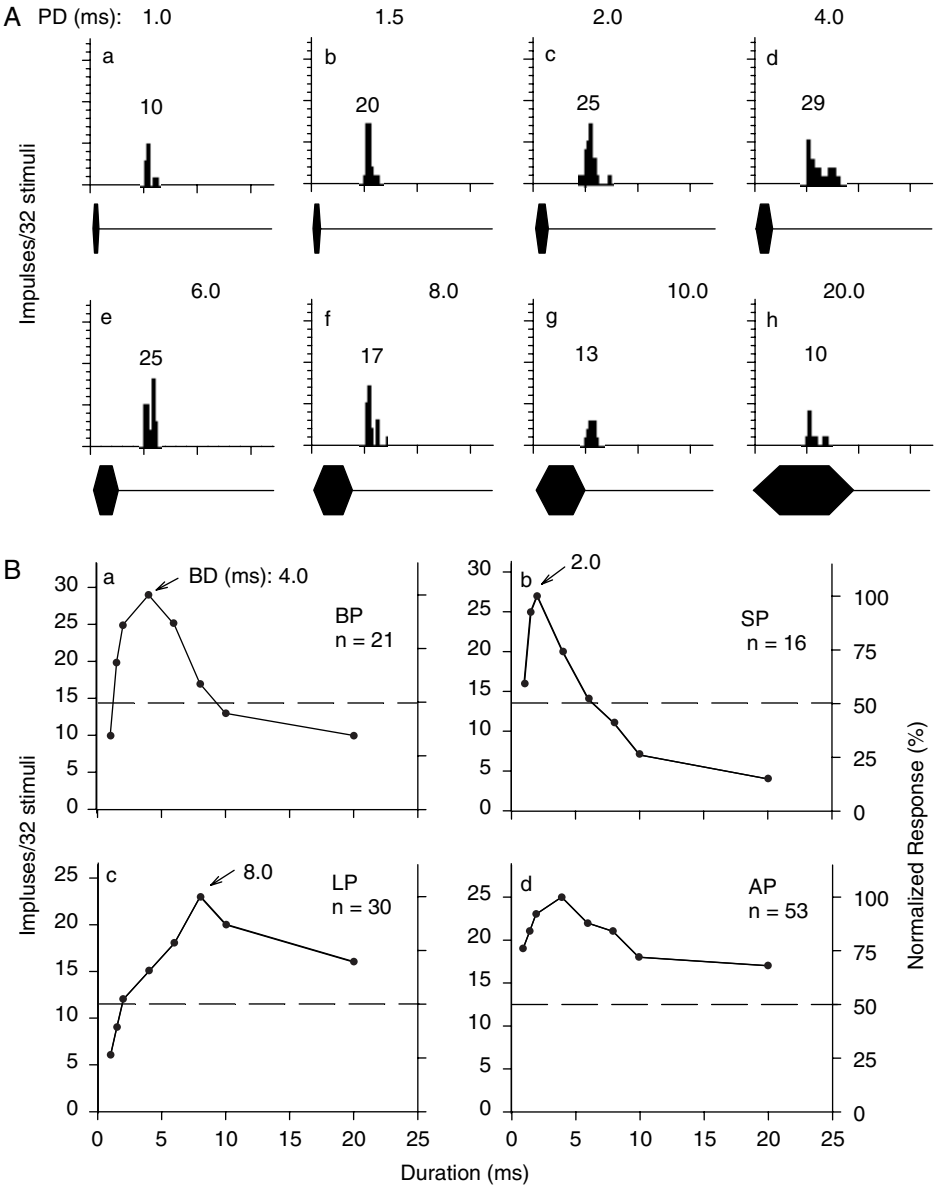

Fig. 1. A: Peri-stimulus-time histogram (PST, bin width: 500 µm and sampling period: 300 ms) showing the discharge pattern of a neuron in the central nucleus of the inferior colliculus (IC) determined with varied pulse duration (PD, shown in ms above each histogram). The envelope of each pulse is shown below each PST histogram. The number of impulses is used to plot the neuron's duration tuning curve (Ba). B: Four representative duration tuning curves of IC neurons. These duration tuning curves are described as band-pass (Ba, BP), short-pass (Bb, SP), long-pass (Bc, LP), and all-pass (Bd, AP). The best duration (BD) is shown with an arrow. The BF (kHz), MT (dB SPL) and recording depth (µm) of these neurons were 59, 88.7,1795 (Ba); 36.4, 67.3, 436 (Bb); 57.2, 61.1, 957 (Bc); 62.8, 66.9, 1355 (Bd) (see text for details).

threshold (MT). The number of impulses in response to BF pulses of 8 durations (1, 1.5, 2, 4, 6, 8, 10 and 20 ms) delivered at 10 dB above the MT was recorded (Fig. 1A). Rise-decay times for these different pulse durations were typically 0.5 ms but they were 0.25 ms for 1-ms pulse duration. The neuron's duration tuning curve was then obtained by plotting the number of impulses against the duration. The neuron's BD, which elicited maximal number of impulses from the neuron, was identified from its duration tuning curve (Fig. 1Ba, b, c, arrow).

The neuron's recovery cycle was studied using P-E pairs that are biologically relevant to those occurring during three phases of hunting (4, 15, 17). These P-E pairs were delivered at 2 pairs/s with P-E intervals of 1.5, 4, 10, 20, 30, 40, 50, 75, 100, 150 and 200 ms. The amplitude of P and E was set at 10-10 dB, 30-20 dB and 30-10 dB above the neuron's MT such that the P-E amplitude difference was 0, 10 and 20 dB, respectively (Fig. 2Aa, Ba, Ca). The duration of

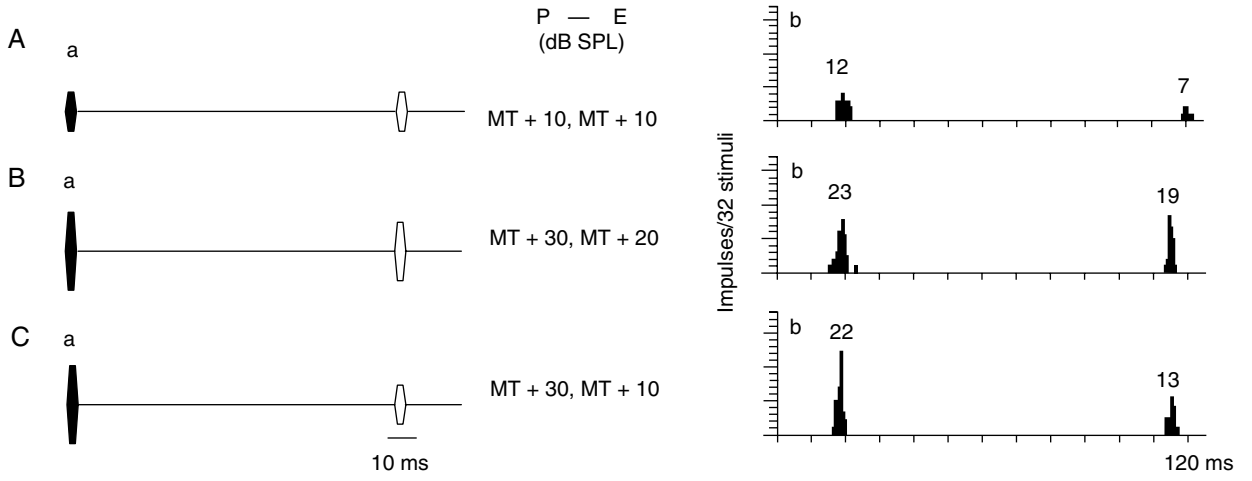

Fig. 2. Aa, Ba, Ca: The envelope of three representative pulse-echo (P-E) pairs of varied P-E amplitude difference used to study the response of IC neurons. The duration of P (filled) and E (unfilled) was 4 ms. The amplitude of P and E was set between 10 and 30 dB above the neuron's minimum threshold (MT). Ab, Bb, Cb: PST histograms obtained from a representative IC neuron with 32 presentations of these three P-E pairs. The neuron's number of impulses in response to each pulse (P) and echo (E) is shown above each histogram.

P and E (abbreviated as P-E duration) was set at 1.5, 4 and 10 ms. The neuron's number of impulses discharged to P and E alone as well as at each P-E interval was systematically recorded (Fig. 2Ab, Bb, Cb; Fig. 3A). The neuron's recovery cycle was then plotted with the percent recovery against the P-E interval. The percent recovery was obtained by dividing the number of impulses discharged to E pulse of each P-E by the number of impulse discharged to the E pulse alone. As in previous studies (12, 19, 28), we used the 50% recovery time to characterize a neuron's recovery property. Whenever possible, we obtained a family of 9 recovery cycles for each neuron with the P-E pair delivered at three P-E amplitude differences and durations.

Recorded action potentials were amplified and band-pass filtered (ISO-DAM, WPI, Sarasota, Florida, USA) before being sent to an oscilloscope (PM3084, Fluke, Avenel, NJ, USA) and an audio monitor (Grass AM9, Warwick, RI, USA). They were then sent to a computer for acquisition of peri-stimulus-time (PST) histograms (bin width: 0.5 ms; sampling period: 100 ms) of the neuron's response to 32 stimulus presentations. The PST histograms show the neuron's temporal discharge pattern to sound stimulus (Figs. 1, 2, 3). The total number of impulses in each histogram was used to measure a neuron's duration tuning curve and recovery cycle as described above.

Results

The Duration Tuning Curve of IC Neurons

In this study, 120 IC neurons were isolated at depths between 161 and 2,533 μm (1045.5 ± 576.5 μm). These neurons discharged phasically (3-7 impulses) to present 4 ms sound pulses. The BF and MT of these neurons ranged from 18.6 to 74.8 kHz (average: 45.1 ± 12.3 kHz) and from 48.6 to 101.1 dB SPL (average: 74.7 ± 8.6 dB SPL).

These neurons discharged different numbers of impulses to varied pulse duration and they often discharged maximally to a specific duration (*e. g.* Fig. 1Ad). The duration tuning curves measured for these 120 neurons can be described as band-, short-, long- and all-pass using the same criterion adopted in previous studies (6, 8, 9, 11, 21-25). A band-pass duration tuning curve showed a maximal number of impulses at a duration and the maximum decreased at least 50% at both limbs (Fig. 1Ba, n = 21, 17.5%). A short-pass duration tuning curve showed a maximal number of impulses at a short duration and the maximum decreased at least 25 % at a short duration and more than 50% at a long duration (Fig. 1Bb, n = 16, 13.3%). Conversely, a long-pass duration tuning curves showed a maximal number of impulses at a long duration and the maximum decreased at least 25% at a long duration and more than 50% at a short duration (Fig. 2Bc, n = 30, 25%). The number of impulses of an all-pass duration tuning curve often differed by more than 25% but never more than 50% at all durations tested (Fig. 1Bd, n = 53, 44.2%). As such, neurons with all-pass duration tuning curves were not selective to any pulse duration.

The 67 neurons with band-, short- and long-pass duration tuning curves are called duration-selective neurons. Their BDs ranged between 1.5 and 20 ms covering the duration of pulses emitted by the bat

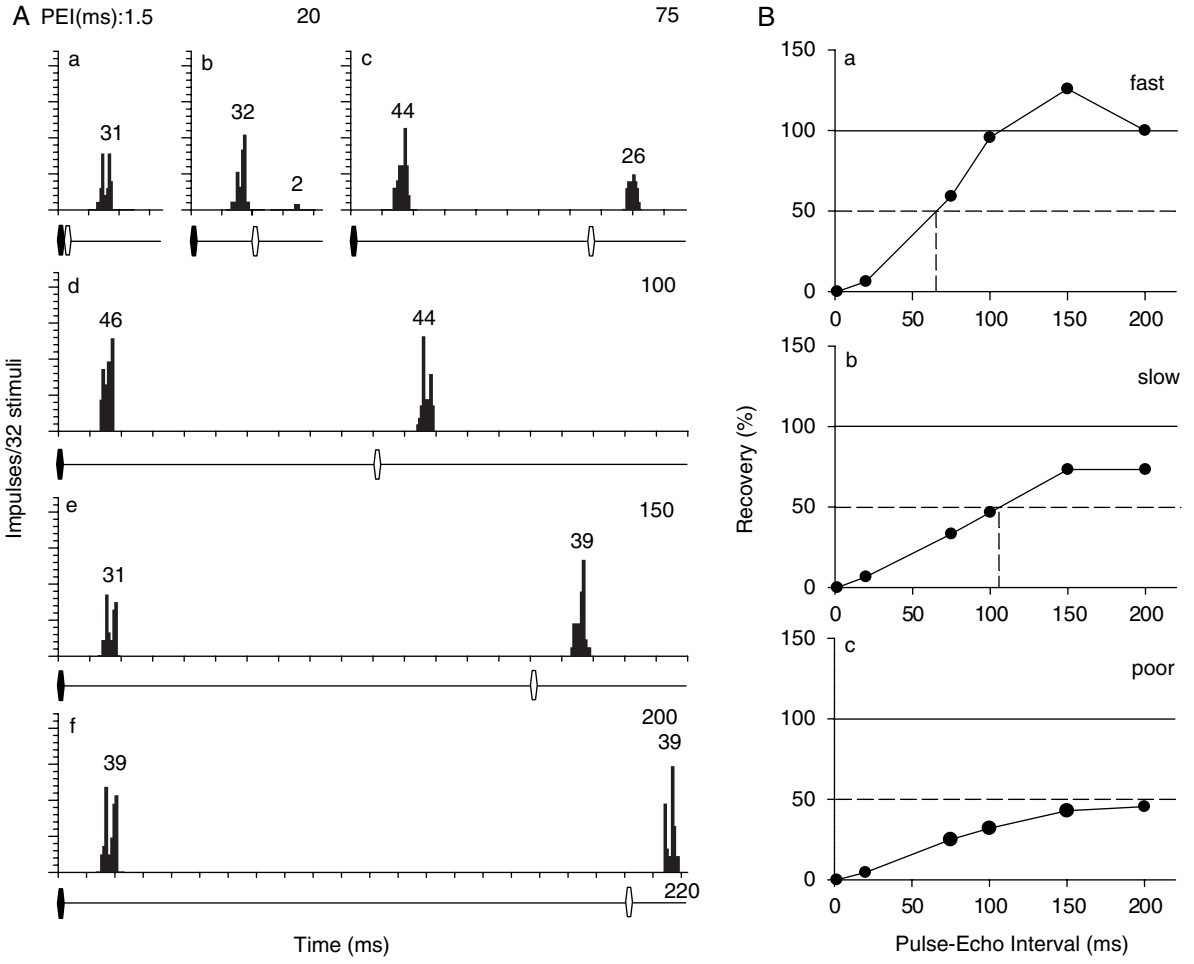

Fig. 3. A: PST histograms obtained from a representative IC neuron with a P-E pair (1.5 ms duration, envelope shown below each histogram) at varied P-E intervals (PEI, ms, shown at upper right of each panel). The neuron's number of impulses in response to each P and E is shown above each histogram and is used to plot its recovery cycle (Ba). B: The fast, slow and poor recovery cycles of three representative IC neurons. Ordinate and abscissa represent percent recovery (%) and P-E interval (ms). The solid and dashed horizontal lines represent 100% and 50% recovery in response. The vertical dashed line represents 50% recovery time (ms). The BF (kHz), MT (dB SPL) and recording depth (μm) of these neurons were 56.6, 80.7, 883 (Ba); 67, 59.2, 2031 (Bb); 45.5, 74.5, 516 (Bc) (see text for details).

during search (8.0-20 ms, 29 neurons, 43.3%), approach (4.0-6.0 ms, 24 neurons, 35.8%) and terminal (1.0-2.0 ms, 14 neurons, 20.9%) phases of hunting (4, 15, 17).

Recovery Cycle of IC Neurons

Because of time constrain and loss of neurons throughout the course of recording, we obtained the recovery cycle of 83 neurons with 3 P-E pairs that varied at 3 durations at equal P-E amplitude. In addition, we obtained the recovery cycle of 62 neurons with all 9 P-E pairs that varied at 3 durations and P-E amplitude differences.

Fig. 3A shows the discharge pattern of a representative IC neuron obtained with a 1.5 ms P-E pair at varied P-E interval. Clearly, the neuron's number of impulses in response to the E pulse progressively increased with P-E interval. The number of impulses in response to P and E pulses was used to measure the neuron's recovery cycle (Fig. 3Ba). Based on the 50% recovery time, the recovery cycle of IC neurons studied can be described as fast, slow and poor. A fast-recovery cycle often recovered more than 100% within the 200 ms P-E interval (Fig. 3Ba, number of curves, n: 242, range: 1.5-185 ms, average: 63.37 ± 38.00 ms). A slow-recovery cycle recovered more than 50% but never reached 100% within the 200ms P-E interval (Fig. 3Bb, n: 320, range: 1.5-200 ms, average: 109.47 ± 53.6 ms). A poor-recovery cycle never reached 50% within the 200 ms P-E interval (Fig. 3Bc, n: 30).

The recovery cycle of a representative IC neuron obtained with 9 different P-E pairs is shown in

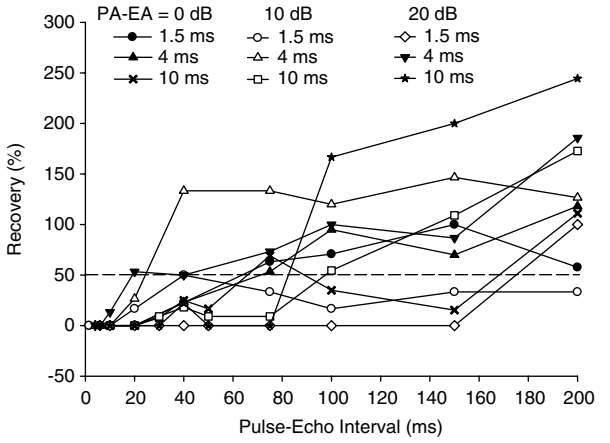

Fig. 4. A family of 9 recovery cycles of a representative IC neuron obtained with P-E pairs at varied duration and P-E amplitude difference (shown in different symbols). The BF (kHz), MT (dB SPL) and recording depth (μm) of the neuron was 27.3, 77.7, 278 (see text in details).

Fig. 4. Clearly, this neuron's recovery cycle and 50% recovery time varied from one type to another with different P-E pairs. Table 1 shows the 50% recovery time of fast and slow recovery neurons determined under different stimulation conditions. When plotted with all three P-E durations, the number of fast recovery neurons progressively decreased while the number of slow recovery neurons increased with P-E amplitude difference. Nevertheless, the total number of fast and slow recovery neurons decreased with P-E amplitude difference because some neurons became poor recovery ones without 50% recovery time.

The 50% recovery time was always shorter for fast than for slow recovery neurons and the difference in 50% recovery time greatly increased with P-E amplitude difference. As such, the difference in 50% recovery time became significant when determined under 10 and 20 dB P-E amplitude difference (*t*-test, $P < 0.01$).

When determined with P-E pairs of equal amplitude, the number of fast and slow recovery neurons progressively decreased and increased with P-E duration, respectively. In addition, the 50% recovery time of both types of neurons significantly increased with P-E duration (One-way ANOVA, $P < 0.05$). The 50% recovery time of both type of neurons also increased with P-E duration when determined with P-E pairs of unequal amplitude. However, significant increase in 50% recovery time with P-E duration was only observed for slow recovery neurons at 10 dB P-E amplitude difference (One way ANOVA, $P < 0.01$).

Recovery Cycle of All-Pass and Duration-Selective Neurons

The recovery cycle of all-pass and duration selective neurons varied differently with P-E duration and amplitude difference. Among 49 all-pass neurons studied, the shortest 50% recovery time of 8 (16%) neurons was obtained when stimulated with 4 ms P-E pair at equal amplitude, with 1.5 ms P-E pair at 10 dB P-E amplitude difference and with 10 ms P-E pair at 20 dB P-E amplitude difference (Fig. 5A-1, A-2, A-3, filled arrow). Another 24 (49%) neurons recovered rapidly when stimulated with 1.5 ms P-E pair at all three P-E amplitude differences (Fig. 5B-1, B-2, B-3, filled arrow). The remaining 17 (35%) neurons did not recover predictably with P-E duration and amplitude difference. As such, each neuron recovered rapidly at different P-E duration and amplitude difference. For example, one neuron had the shortest 50% recovery time when stimulated with 1.5 ms P-E pair at equal amplitude, with 4 ms P-E pair at 10 and 20 dB P-E amplitude differences (Fig. 5C-1, C-2, C-3, filled arrow). Each of the remaining 17 neurons recovered rapidly at different combinations of P-E duration and amplitude difference.

Among the 30 duration-selective neurons studied, the recovery cycle of 10 (33.3%) neurons, regardless of BD, varied unsystematically with P-E duration and amplitude difference. Conversely, the recovery cycle of 20 (66.6%) neurons appeared to vary systematically with biologically relevant P-E pairs in relation to BD. As such, 7 (23.3%) neurons with 1-2 ms BD recovered rapidly when stimulated with 1.5 ms P-E duration at 10 dB amplitude difference (*i.e.* P-E pairs in the terminal phase, unfilled arrow in Fig. 6A-2). However, these neurons recovered rapidly at equal amplitude or 20 dB amplitude difference when stimulated 4 and 10 ms P-E duration (Fig. 6A-1, A-3, filled arrow). Another 7 (23.3%) neurons with 4-6 ms BD recovered rapidly when stimulated with 4 ms P-E pairs at both 10 and 20 dB amplitude difference (*i.e.* P-E pairs at the approach phase, unfilled arrow, Fig. 6B-2, B-3). They recovered more slowly when stimulated with 1.5 or 4 ms P-E pair at equal amplitude (Fig. 6B-1, filled arrow). The remaining 6 (20%) neurons with 8-20 ms BD recovered rapidly when stimulated with 10 ms P-E duration at 20 dB amplitude difference (*i.e.* P-E pairs at the search phase) but not at equal or 10 dB amplitude difference (Fig. 6C-3, unfilled arrow *vs.* Fig. 6C-1, C-2, filled arrow).

The 50% recovery time of duration-selective and all-pass neurons obtained under different stimulation conditions is shown in Table 2. Although not significant, the 50% recovery time of IC neurons was typically shorter for duration-selective than for all-pass neurons when obtained with 7 out of 9 P-E pairs (*t*-test, $P > 0.05$). When stimulated at equal P-E amplitude, the 50% recovery time of these two

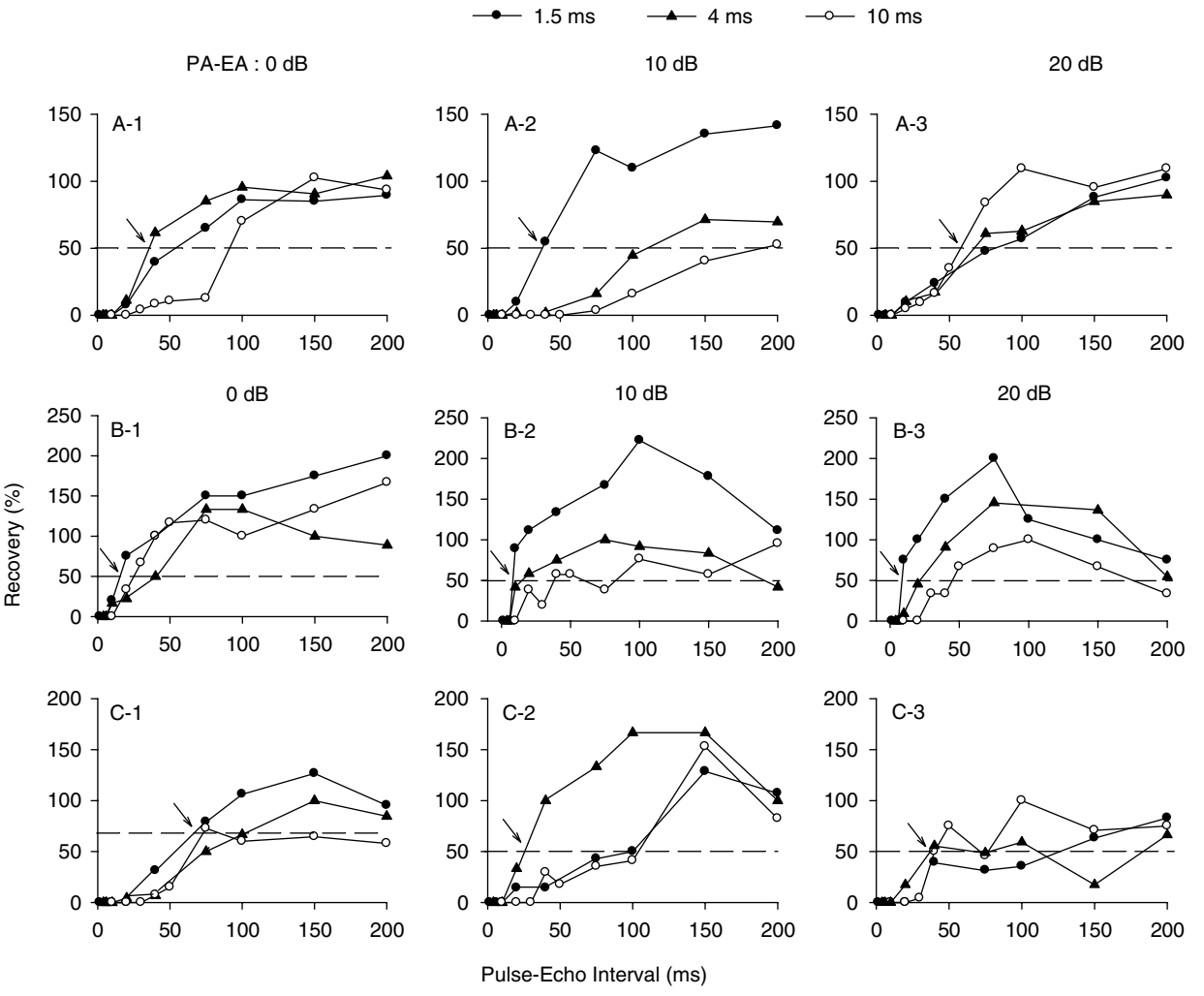

Fig. 5. The recovery cycle of 3 all-pass IC neurons determined with 9 P-E pairs that varied in P-E interval, duration and amplitude difference. In each panel, the filled arrow indicates the shortest 50% recovery time. The BF (kHz), MT (dB SPL) and recording depth (μm) of these neurons were 42.5, 83.3, 561 (A); 26.8, 67.7, 2332 (B); 41.5, 82.4, 612 (C) (see text for details).

types of neurons increased significantly with P-E duration (One way ANOVA, $P < 0.05$). The 50% recovery time also increased with P-E duration when stimulated with P-E pairs at 10 and 20 dB amplitude difference but the increase was only significant for duration all-pass neurons at 10 dB amplitude difference (One way ANOVA, $P < 0.01$).

Discussion

The Recovery Cycle of IC Neurons Is Affected by Other Co-Varying Echo Parameters

In this study, we examined the effect of pulse duration and amplitude on the recovery cycle of IC neurons using biologically relevant P-E pairs. We found the following: 1) IC neurons had a wide range of recovery cycle and BD; 2) the recovery cycle of IC neurons typically increased with P-E duration; 3) the recovery cycle of IC neurons increased with amplitude difference; 4) the recovery cycle of all-pass neurons and one-third of duration selective neurons varied unsystematically with P-E duration and amplitude difference, and 5) the recovery cycle of two-third of duration selective neurons varied systematically with biologically relevant P-E pairs in relation to BD. These findings are in agreement with many previous studies that show the analysis of an echo parameter by neurons affected by other co-varying echo parameters (8-11, 21-27, 29).

Previous studies have shown that temporal interaction of excitation and GABAergic inhibition shape the duration selectivity and recovery cycle of collicular neurons (1, 2, 6, 10, 12, 21, 22, 29). Based on these studies, we have recently proposed that when stimulated with P-E pairs a neuron's E-elicited response is likely suppressed by P through GABAergic inhibition (19). As such, a neuron's recovery cycle is

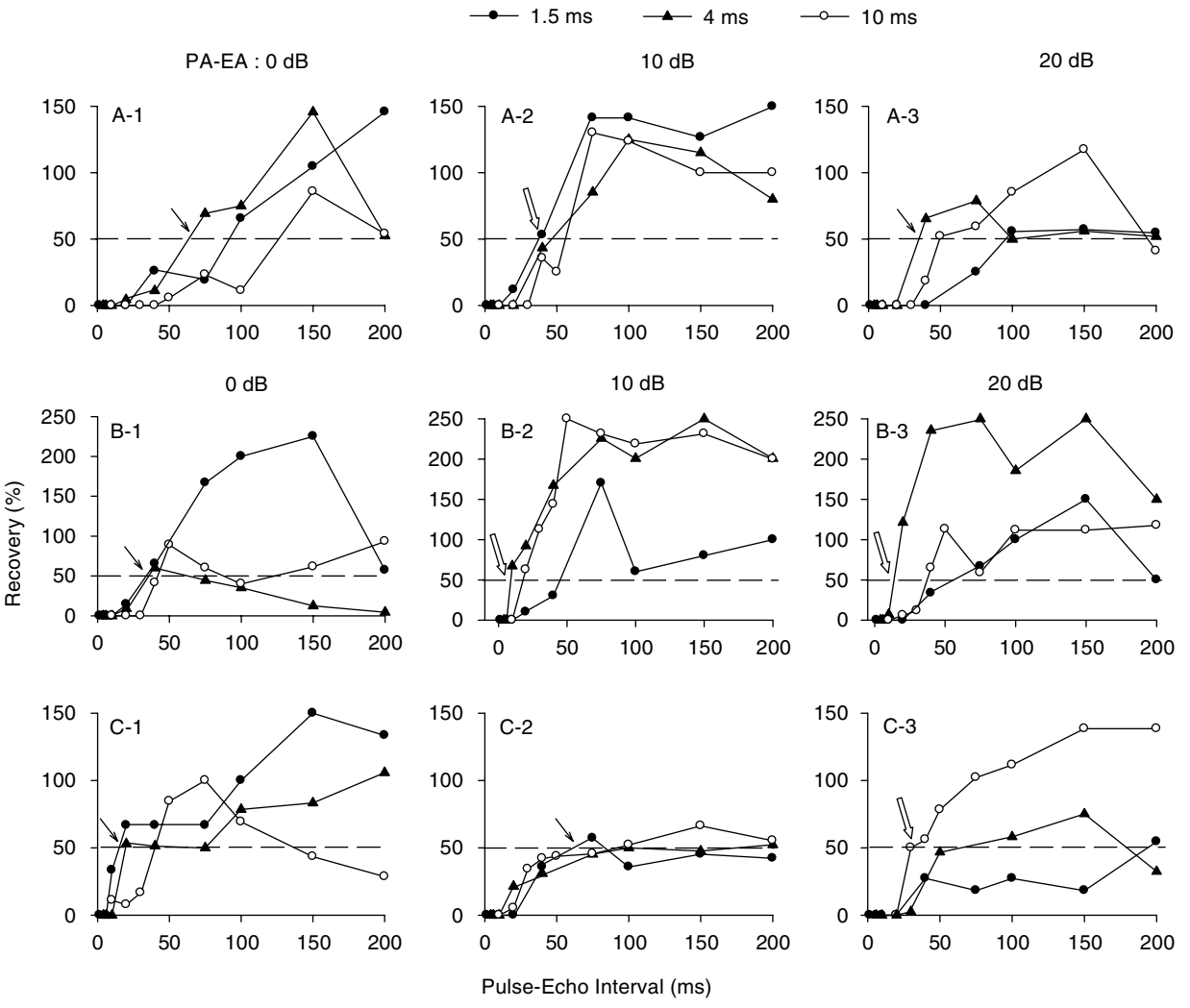

Fig. 6. The recovery cycle of 3 duration-selective IC neurons determined with 9 P-E pairs that varied in P-E interval, duration and amplitude difference. The BD of these 3 neurons was 1.5 ms (A), 4 ms (B) and 10 ms (C). In each panel, the filled and unfilled arrows indicate the shortest 50% recovery time. The BF (kHz), MT (dB SPL) and recording depth (μm) of these neurons were 35.7, 76.9, 905 (A); 27.3, 77.7, 278 (B); 18.6, 71.8, 743 (C) (see text for details).

determined by the strength of P-elicited inhibition relative to the strength of E-elicited excitation. If this is true, the strength of P-elicited inhibition must be conceivably stronger for slow and poor recovery neurons than for fast recovery neurons such that the former had a longer recovery cycle than the latter (Table 1). The strength of P-elicited inhibition might also be stronger for long than for short duration P such that recovery cycle of IC neurons increased with P-E duration. In the same token, the strength of P-elicited inhibition must become stronger with increasing P-E amplitude difference resulting in decreasing fast recovery neurons and increasing slow recovery neurons. We noted that the recovery cycle of IC neurons in most cases did not increase significantly with P-E duration at 10 and 20 dB P-E amplitude differences. This observation suggests that the strength of P-elicited inhibition in shaping the recovery cycle of IC neurons increased more effectively with P-E amplitude difference than with P-E duration. Conceivably, the strength of P-elicited inhibition at increasing P-E amplitude difference was already so strong that further increase in the strength of P-elicited inhibition with P-E duration was minimal. Alternatively, this observation might simply be due to the small sampling size of IC neurons.

The duration-selective neurons discharged maximally to the BD pulse (Fig. 1). For this reason, the strength of P-elicited inhibition in shaping the recovery cycle of IC neurons must be the greatest when the duration of P matched the BD. Conceivably, when duration-selective neurons were stimulated with a P-E pair at BD, integration of excitation and inhibition must result in an optimal time window within

Table 1. The 50% recovery time of fast and slow recovery IC neurons determined under different stimulation conditions

P-E amplitude difference			P-E duration			One-way ANOVA
			1.5 ms	4.0 ms	10 ms	*P*
0 dB	fast	n	58	48	37	
		range	1.5-156	4-150	10-185	
		mean ± sd	52.92 ± 39.44 (1)	53.29 ± 33.19 (2)	72.08 ± 6.62 (3)	< 0.05
	slow	n	25	34	45	
		range	1.5-158	4-200	8-200	
		mean ± sd	69.46 ± 41.61 (4)	101.76 ± 58.29 (5)	101.45 ± 55.04 (6)	< 0.05
		t-test *P*	> 0.05	< 0.01	< 0.05	
10 dB	fast	n	19	17	19	
		range	7-111	8-138	15-183	
		mean ± sd	55.21 ± 30.49 (7)	57.12 ± 37.55 (8)	75.21 ± 44.68 (9)	> 0.05
	slow	n	37	39	34	
		range	10-200	10-200	18-200	
		mean ± sd	93.89 ± 55.48 (10)	123.69 ± 54.92 (11)	137.21 ± 54.98 (12)	< 0.01
		t-test *P*	< 0.01	< 0.001	< 0.001	
20 dB	fast	n	15	15	14	
		range	15-175	14-129	36-142	
		mean ± sd	62.47 ± 41.52 (13)	62.13 ± 37.40 (14)	79.93 ± 30.10 (15)	> 0.05
	slow	n	37	35	34	
		range	15-200	20-200	30-200	
		mean ± sd	110.03 ± 57.84 (16)	121.83 ± 48.37 (17)	125.9 ± 55.87 (18)	> 0.05
		t-test *P*	< 0.01	< 0.001	< 0.01	

n: number of IC neurons, *P*: significance level, A Student-Newman-Keuls multiple comparisons post test showed significance difference between (1) and (3), (2) and (3), (4) and (5), (4) and (6), (10) and (11), (10) and (12) at $P < 0.05$ (see text for details).

which a neuron responded maximally and recovered rapidly at a specific P-E amplitude difference (Fig. 6). As such, the recovery cycle of duration-selective neurons typically had shorter recovery cycle than all-pass neurons had (Table 2).

Behavioral Relevance of the Present Study

Because insectivorous bats progressively decrease the P-E duration, interval and amplitude difference (due to increasing echo amplitude at short bat-to target distance) as they search, approach and finally intercept the prey, they must have neurons that respond effectively to these changing P-E parameters for successful hunting. For example, to perform effective echo detection, they must have neurons responding to echo of varied duration returning throughout the target approaching sequence. To perform effective recognition of echo duration, a bat must have neurons with BD closely matching the echo duration occurred during different phases of hunting. In the same token, a bat must have neurons that recover rapidly when P-E interval progressively shortens throughout a target approaching sequence.

In the present study, the P-E pairs delivered at 10, 4 and 1.5 ms and at 20 and 10 dB amplitude differences approximately correspond to the P-E pairs occurring during search, approach and terminal phases of hunting (4, 15, 17). We observed that all-pass IC neurons responded to pulses presented at varied duration. We also observed that IC neurons had a wide range of recovery cycles covering the P-E interval occurring throughout different phases of hunting. In addition, duration-selective neurons had a wide range of BD and most of them recovered rapidly when stimulated with P-E pairs delivered at the BD and at biologically relevant P-E amplitude difference (Fig. 6). As such, neurons with 1-2 ms BD recovered rapidly with 1.5 ms P-E duration delivered at 10 dB amplitude difference occurring during the terminal phase of hunting (Fig. 6A-2). Those neurons with 8-20 ms BD recovered rapidly with 10 ms P-E duration at 20 dB amplitude difference occurring during the search phase of hunting (Fig. 6C-3). Finally, neurons with 4-6 ms BD recovered rapidly when stimulated with 4 ms P-E duration at both 10 and 20 dB amplitude

Table 2. The 50% recovery time of duration-selective and all-pass IC neurons determined under different stimulation conditions

P-E amplitude difference			P-E duration			One-way ANOVA
			1.5 ms	4.0 ms	10 ms	*P*
0 dB	duration-selective neurons	n	49	49	49	
		range	1.5-156	4-200	8-200	
		mean ± sd	55.08 ± 42.36 (1)	65.86 ± 47.85 (2)	82.53 ± 51.44 (3)	< 0.05
	all-pass neurons	n	31	31	30	
		range	8-158	4-184	15-200	
		mean ± sd	62.13 ± 38.25 (4)	77.42 ± 49.03 (5)	93.3 ± 55.62 (6)	< 0.05
		t-test *P*	> 0.05	> 0.05	> 0.05	
10 dB	duration-selective neurons	n	30	30	30	
		range	7-200	8-200	18-200	
		mean ± sd	85.03 ± 60.34 (7)	97.76 ± 60.66 (8)	103.5 ± 65.10 (9)	> 0.05
	all-pass neurons	n	25	26	24	
		range	8 -200	10-200	15-200	
		mean ± sd	77.36 ± 39.84 (10)	107 ± 56.05 (11)	124.83 ± 48.84 (12)	< 0.01
		t-test *P*	> 0.05	> 0.05	> 0.05	
20 dB	duration-selective neurons	n	28	28	28	
		range	15-200	14-200	30-200	
		mean ± sd	100.25 ± 58.70 (13)	95.08 ± 54.26 (14)	103.59 ± 46.65 (15)	> 0.05
	all-pass neurons	n	23	25	20	
		range	15-200	19-200	45-200	
		mean ± sd	93.17 ± 57.81 (16)	110.56 ± 51.46 (17)	121.3 ± 61.22 (18)	> 0.05
		t-test *P*	> 0.05	> 0.05	> 0.05	

n: number of IC neurons, *P*: significance level, A Student-Newman-Keuls multiple comparisons post test showed significance difference between (1) and (3), (2) and (3), (4) and (6), (10) and (11), (10) and (12) at $P < 0.05$ (see text for details).

differences which occur during the approaching phase of hunting (Fig. 6B-2, B-3). These data suggest that bats may potentially utilize the response of all-pass neurons for effective echo detection. They may also utilize the response of IC neurons with different BD and recovery cycles to effectively perform recognition of echo duration and echo ranging during different phases of hunting.

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