Duration-Sensitive Neurons in the Inferior Colliculus of Horseshoe Bats: Adaptations for Using CF-FM Echolocation Pulses

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Submitted 20 August 2007; accepted in final form 25 October 2007

Luo F, Metzner W, Wu FJ, Zhang SY, Chen QC. Duration-sensitive neurons in the inferior colliculus of horseshoe bats: adaptations for using CF-FM echolocation pulses. J Neurophysiol 99: 284–296, 2008. First published November 14, 2007; doi:10.1152/jn.00935.2007. The present study examines duration-sensitive neurons in the inferior colliculus (IC) of the least horseshoe bat, Rhinolophus pusillus, from China. In contrast to other bat species tested for duration selectivity so far, echolocation pulses emitted by horseshoe bats are generally longer and composed of a long constant-frequency (CF) component followed by a short downward frequency-modulated (FM) sweep (CF-FM pulse). We used combined CF-FM pulses to analyze the differential effects that these two pulse components had on the duration tuning in neurons of the horseshoe bat’s IC. Consistent with results from other mammals, duration-sensitive neurons found in the least horseshoe bat fell into three main classes: short-pass, band-pass, and long-pass. Using a CF stimulus alone, 54% (51/95) of all IC neurons showed at least one form of duration selectivity at one or more stimulus intensities. In 65 of the 95 IC neurons tested with CF pulses, we were also able to test their duration selectivity for a combined CF-FM pulse, which increased the ratio of duration-sensitive neurons to 66% (43/65). Seven to 15 neurons that failed to show duration tuning for CF bursts became duration sensitive for CF-FM pulses, with most of them exhibiting short-pass (depending on stimulus intensity, between 4 and 8 neurons) or band-pass tuning (1–3 neurons). Increasing stimulus intensities did not affect the duration tuning in 53% (23/43) of duration-sensitive neurons for CF bursts and in about 26% (7/27) for CF-FM stimuli. In the remaining neurons, increasing sound levels generally reduced the ratio of duration-sensitive neurons to 33% for CF and 37% for CF-FM stimulation. In those that remained duration sensitive, louder CF bursts shortened best durations in band-pass neurons and cutoff durations in short- and long-pass neurons, whereas louder CF-FM stimuli reduced the cutoff durations only in short-pass neurons. Bandwidths of band-pass neurons were not significantly affected by any stimulus configuration, with only a slight trend for increasing bandwidths for louder CF bursts (but not CF-FM stimuli). Best durations and cutoff durations reached higher values than those in the other bat species examined so far and roughly match the longer durations of echolocation pulses emitted by horseshoe bats. Therefore presentation of a CF-FM stimulus improved the duration sensitivity in IC neurons by increasing the ratio of duration-tuned neurons and making them less susceptible to changes in signal intensity.

INTRODUCTION

Sound duration is a fundamental parameter in animal acoustic communication and orientation, including bat echolocation (Covey and Casseday 1999; Covey et al. 1995). In the central auditory system of a number of species, many neurons are found to be sensitive to the duration of sound stimuli. Based on different behavioral adaptations, duration-tuned neurons show different tuning properties across species. In the midbrain of bats, for example, about 30% of all auditory neurons exhibit duration sensitivity and most of them are tuned to short sound durations <10 ms (e.g., Casseday et al. 1994; Ehrlich et al. 1997). In mammals other than bats, an even greater percentage of auditory neurons is duration selective and most of them are tuned to longer durations (rats: 76%, Pérez-González et al. 2006; mice: 69%, Brand et al. 2000; cats: 59%, He et al. 1997). The difference in the tuning between bat and nonbat species to different ranges of sound durations is likely related to the different durations of vocalizations most commonly produced by these species: echolocation pulses in most bats usually have relatively short durations of ≤10 ms, whereas calls produced by other species are normally longer (Pérez-González et al. 2006).

Each species of bat has a distinct set of echolocation calls, differing in the calls’ frequency composition, duration, and intensity. This species-specific signal design is determined mostly by ecological constraints on each species’ echolocation tasks, and the temporal and spectral characteristics of echoes provide a wealth of vital information about targets (e.g., Griffin 1958; Neuweller 2003; Schnitzler and Kalko 2001). Echolocation pulses can be grouped into different categories, depending on their spectrotemporal composition. One commonly used distinction in pulse design categorizes pulses into J) relatively short, broadband frequency-modulated (FM) pulses; 2) longer, narrowband constant-frequency (CF) pulses that are combined with a terminal (and sometimes also initial) FM (long CF/FM); and J) extremely short, broadband and thus “click-like” signals (Neuweller 2003).

All echolocating bats systematically increase pulse repetition rate and shorten call duration as they switch from the search to the approach phase of echolocation and eventually intercept insects (e.g., Griffin 1958; Simmons et al. 1979), or when facing a perceptually difficult task such as dodging thin wires or when detecting a target in clutter (e.g., Griffin et al. 1960; Moss and Surykke 2001; Moss et al. 2006). These changes in call temporal parameters during target pursuit primarily serve to minimize overlap between the emitted calls and the returning echoes, thereby allowing bats to determine the target range more accurately (Schnitzler and Kalko 2001). Thus for bats, the analysis of echoes with changing duration is essential for successful prey capture and orientation (Neuweller 1990).
Within the ascending auditory pathways, the central nucleus of the inferior colliculus (IC) receives and integrates excitatory and inhibitory inputs from a large number of lower auditory nuclei (Covey 2005; Covey and Casseday 1995; Pollak and Casseday 1986). Neurons sensitive to sound durations have been found abundantly in the IC of several species of bats emitting FM-type echolocation pulses (Eptesicus fuscus, Myotis lucifugus, Antrozous pallidus, and Molossus molossus; Casseday et al. 1994; Condon et al. 1996; Covey and Casseday 1999; Covey et al. 1995; Ehrlich et al. 1997; Faure et al. 2003; Fremouw et al. 2005; Füzessery and Hall 1999; Galazyuk and Feng 1997; Mora and Kössl 2004; Pinheiro et al. 1991), as well as in the IC of mouse (Brand et al. 2000), rat (Pérez-González et al. 2006), chinchilla (Chen 1998), frog (Feng et al. 1990; Gooler and Feng 1997; Mora and Kössl 2004; Pinheiro et al. 1991), as well as in the auditory cortex (He et al. 1997).

Like other bats, horseshoe bats precisely adjust the duration and repetition rate of their sonar pulses depending on the echolocation tasks at hand. In addition, they also precisely control the CF-frequency of their CF-FM echolocation calls (Konstantinov et al. 1978; Schnitzler 1968, 1987; Smotherman and Metzner 2005; Tian and Schnitzler 1997). The CF value varies not only between different species of horseshoe bats but also between individuals of one species (Grinnell 1995; Heller and Helversen 1989; Jones and Rayner 1989; Kingston et al. 2006), chinchilla (Chen 1998), frog (Feng et al. 1990; Gooler and Feng 1997; Mora and Kössl 2004; Pinheiro et al. 1991), as well as in the IC of mouse (Brand et al. 2000), rat (Pérez-González et al. 2006), chinchilla (Chen 1998), frog (Feng et al. 1990; Gooler and Feng 1997; Mora and Kössl 2004; Pinheiro et al. 1991), as well as in the auditory cortex (He et al. 1997).

In addition to echolocation pulses, bats also possess a large repertoire of communication calls, which exhibit more complex spectrotemporal characteristics. In horseshoe bats, the dominant frequency range of communication signals is usually 10–40% below the range covered by the second harmonic of their echolocation pulses. In addition, they are generally several times longer in duration but less frequently uttered than echolocation calls, with each type of communication signal having its characteristic spectral composition and duration (Ma et al. 2006). Communication signals can serve several purposes, such as intra- and even interspecific information transfer (Pfalzer and Kusch 2003), courtship and mating behavior (Behr and Helversen 2004), or mother–infant interaction (Matsumura 1979). Similar to its function in mammals other than bats, duration sensitivity in the bat auditory system may therefore also play an important role in social communication.

We investigated the encoding of sound duration in the IC of R. pusillus by presenting long pure tones as well as combined CF-FM signals with variable CF durations. Compared with all other bat species in which duration tuning was tested with pure tones (Casseday et al. 1994; Ehrlich et al. 1997; Jen and Zhou 1999; Zhou and Jen 2001), pure-tone bursts and especially the CF-FM signal represent a more natural stimulus paradigm in horseshoe bats. The durations and repetition rates used here were comparable to those generated by these bats during their search, approach, and terminal phases of hunting (Luo et al. 2006). Consistent with results from other mammals, we found several types of duration tuning in the horseshoe bat IC. Interestingly, however, combined CF-FM stimuli improved the duration selectivity and made the neurons less susceptible to changes in signal intensities.

**METHODS**

**Animal preparation**

Twelve least horseshoe bats, *R. pusillus* (5.5–6.5 g body weight) of both genders were collected locally in Hubei province, China, and used for this study. The surgical procedures followed those described elsewhere (Chen and Jen 2000). Briefly, 1 or 2 days before the first recording session, the bat was anesthetized with pentobarbital sodium (45–50 mg/kg) and a 1.5-cm-long metal post was attached to the exposed dorsal skull with acrylic glue and dental cement. A small hole 200–500 μm in diameter was made in the semitransparent skull above the IC. For the recording sessions, the bat was placed in a soft body mold, and the head was fixed with the eye–snout axis directing at 0° in azimuth and elevation in the frontal auditory space. A local anesthetic (procaine hydrochloride) was applied to the open wound area. For the neurophysiological recordings, a glass pipette electrode filled with 2 M NaCl (impedance: 5–10 MΩ) was inserted into the brain. An indifferent electrode (Ag–AgCl silver wire) was placed under the adjacent temporals muscle. Recordings were conducted inside a double-walled soundproof room (temperature 28–30°C) with its ceiling and inside walls covered with 3-in. convoluted polyurethane foam to minimize echoes. Each recording electrode was visually aimed at the IC and advanced with a hydraulic microdrive (Model 640, Kopf Instruments, Tujunga, CA). In an attempt to distribute the recording sites evenly over most of the IC, the penetration points on the brain surfaces covered >75% of the dorsally exposed surface of the IC central nucleus for all bats used for this study. Procedures were in accordance with National Institutes of Health guidelines for experiments involving vertebrate animals.

**Acoustic stimulation and recording of neuronal responses**

To generate the acoustic stimuli, continuous sine waves from a function generator (GFG-8016G, Good Will Instruments, Penang, Malaysia) were gated with a homemade tone-burst generator triggered by a stimulator (Sen-7203, Nihon Kohden, Tokyo, Japan) to form pure-tone (CF) pulses. FM signals were generated from another function generator (33220A, Agilent Technologies, Penang, Malaysia) and transformed into FM tone pulses with another homemade tone-burst generator driven by the same simulator. The CF and FM pulses were amplified after passing through decade attenuators (LAT-45, Leader Electronics, Yokohama, Japan) and fed to a small condenser loudspeaker (AKG model, CK 50). The loudspeaker was placed 30 cm away from the bat and 30° contralateral to the recording site. Calibration of the loudspeaker was conducted with a 0.25-in. microphone (4939, Brüel & Kjær, Nærum, Denmark) placed at the position of the bat’s ear using a measuring amplifier (2610, Bruel & Kjær). Search stimuli were CF pulses with a duration of 40 ms (rise-decay times: 0.5 ms) delivered at a rate of 2 Hz.

On isolation of a neuron, its action potentials were amplified (ISO-DAM, WPI, Sarasota, FL) and sent synchronously to an oscilloscope (TDS210, Tektronix, Beaverton, OR), an audio monitor (Grass AM9, Grass Instruments, West Warwick, RI), and a computer for acquisition of poststimulus time histograms (PSTHs) (bin width: 0.5 ms; sampling period: 280 ms). PSTHs were constructed with data.
from 32 sound presentations, using a custom-written program. By systematically changing the frequency and intensity of the CF, we determined each neuron’s best frequency (BF) and minimum threshold (MT) in response to the search stimuli (40-ms-long CF tone bursts). BF was defined as the frequency with the lowest stimulation threshold. At MT, the neuron responded to sounds presented at its BF with a 50% probability. The neuron’s response latency (“first spike latency”) was determined in a PSTH display in response to a stimulus given at its BF at 10 dB above threshold.

The duration sensitivity of IC neurons was investigated by presenting CF bursts at the unit’s BF with durations of 5, 10, 20, 40, 60, 80, or 100 ms and delivered at 10, 20, and 30 dB above MT. Stimulus durations were pseudorandomly chosen and presented at 2 Hz with rise-decay times of 0.5 ms. PSTH and dot-raster displays of the neuron’s response revealed the type of duration sensitivity. For band-pass neurons, we defined the best duration (BD) as the duration that evoked the maximal number of spikes. For short-pass and long-pass neurons, the cutoff duration was determined as the duration at which the number of spikes dropped to <50% of the maximum spike count.

In onset responses, the first spike latency was always shorter than the stimulus duration, even for the shortest durations tested (see the dot-raster pattern in Fig. 1F1). If for short-duration stimuli the first spike latency was longer than the signal, but spikes occurred before the stimulus end for longer durations, with latencies remaining more or less constant for any stimulus duration, the response was also...

![Figure 1](http://jn.physiology.org/)

**Fig. 1.** Representative examples for the 5 types of duration sensitivity and all-pass neurons obtained with pure-tone [constant frequency (CF)] bursts at the units’ best frequencies (BFs) and delivered 10 dB above their minimum thresholds (MTs). For each type (A–F), the units’ discharge patterns (displayed as spike-count functions) are shown in a dot-raster display (top, A1–F1) and the units’ duration-tuning curves are given underneath (A2–F2). Left and right ordinates of the duration-tuning plots represent the spikes per 32 stimuli and normalized responses (%), respectively. The abscissas indicate time (top plots) or sound duration (ms, bottom plots). The horizontal dotted lines in each bottom plot indicate the 50% maximal response. The serial number, BF (kHz), MT (dB SPL), and depth (µm) of each unit are given in each top panel.
classified as onset (see, e.g., Fig. 1A). Conversely, if spikes occurred after the end of the stimulus for all durations tested, the firing pattern was classified as an offset response. Offset responses were also those in which the response occurred after the end of the signal for short durations but then remained at a constant value for longer durations (see Fig. 1C as an example).

To study whether adding a terminal FM component to the CF burst would affect the duration tuning of IC neurons, we presented CF-FM stimuli in which the CF was immediately followed by an FM sweep with a 15-kHz bandwidth, 3-ms duration, and 0.5-ms rise-fall times. The bandwidth used here was similar to the bandwidth of the first- and second-harmonic downward FM sweeps in the bat’s biosonar pulse (Luo et al. 2006). The FM component started at the neuron’s BF and swept 15 kHz downward, and the amplitude of the FM sweep was 10 or 20 dB above MT, measured with a CF burst given at the unit’s BF. The intensities of the CF tone bursts were 10, 20, or 30 dB above MT.

We varied only the duration of the CF component, leaving the duration of the FM component constant. Therefore stimulus durations of CF-FM pulses were 3 ms longer than the durations of the corresponding CF bursts, i.e., 8, 13, 23, 43, 63, 83, and 103 ms. However, we limited the spike counts used to characterize basic response patterns and duration sensitivity to CF-FM signals to the responses to the CF components alone. We therefore discounted any spikes that occurred after a time span longer than the first spike latency plus the duration of the CF burst. Although this approach does not take into account any responses that might have occurred specifically to the FM component, it ensured full compatibility of the data sets for CF and CF-FM stimulation and allowed us to compare our data sets with those published for other systems.

**RESULTS**

**Basic response properties and duration tuning for CF stimulation**

We recorded from a total of 95 single units in the IC of *R. pusillus*. Their BFs ranged from 7.5 to 75.1 kHz depending on the recording site, which ranged from 180 to 1,981 μm in depth beneath the dorsal surface of IC. BFs were topographically organized within the IC in a mammalian-like fashion with higher BFs situated more ventrally (Luo et al. 2006). The MTs of IC neurons ranged from 3 to 70 dB SPL, and the first spike latencies (in response to a 40-ms CF tone burst given at the unit’s BF and 10 dB above MT) ranged from 3.5 to 26.0 ms.

Using CF tone bursts delivered at BF and 10 dB above MT, we found that 45% (43/95) of neurons in our sample showed some form of duration sensitivity. We distinguished five different types of duration selectivity (short-pass, band-pass, long-pass, band-reject, and multi-peak) and one type of non-duration selectivity (all-pass), with a representative example for each type given in Fig. 1A–F. The six types were classified following criteria used elsewhere (Fuzessery and Hall 1999). Thus a short-pass (SP) response (Fig. 1A) consisted of an activity pattern in which the spike count obtained for short durations was high, but with increasing durations eventually dropped to <50% of the peak value at the cutoff duration. SP responses were found in 37% of neurons (16 of 43) that exhibited duration sensitivity (Fig. 2A). In a band-pass (BP) response, one duration or a small range of values yielded...
maximal spike counts [best duration (BD)], and responses dropped to <50% of the peak value for durations above and below BD (Fig. 1B). BP neurons constituted 30% (13 of 43) of the units (Fig. 2A). Long-pass (LP) responses were those for which the spike counts for longer durations were high but fell to <50% of peak value for durations shorter than the cutoff duration (Fig. 1C). Five of 43 units (12%) showed LP responses (Fig. 2A). Two additional neurons showed LP behavior only for low stimulus intensities and lost their duration tuning at higher levels (see following text). They are therefore not considered LP neurons in a strict sense. In a band-reject (BR) response, the neuron responded to both short and long durations but poorly (<50% peak value) to sounds of intermediate durations (Fig. 1D). Six of 43 units (14%) exhibited BR-type duration tuning (Fig. 2A). Multipeaked (MP) responses were those that peaked at multiple durations separated by more than one minimum with spike counts <50% peak value (Fig. 1E). Three of 43 units (7%) were MP neurons. Finally, 55%, or 52 of the 95 units tested, failed to exhibit any significant duration sensitivity for CF bursts (Fig. 2A). In these all-pass (AP) responses, the spike counts for all durations never varied by >50% of the peak value (Fig. 1F). In some of these AP neurons, however, presentation of a CF-FM stimulus, instead of a pure-tone burst, did yield duration selectivity (see following text). It should be noted that AP neurons represent a mixture of neurophysiological response types with inhomogeneous discharge properties; of course, they are not considered to be part of the population of duration-tuned neurons.

The BFs, MTs, recording depths, and latencies for the five types of duration sensitivity tested at 10 dB above MT are given in Table 1. Neurons with different BFs or MTs did not show any significant differences in their duration tuning (one-way ANOVA, P > 0.05).

The discharge patterns of duration-sensitive IC neurons could be classified into phasic, phasic–burster, and phasic–tonic types following criteria outlined elsewhere (Chen and Jen 2000; Gooler and Feng 1992; Jen and Feng 1999). Representative examples of these discharge types are given in Fig. 3; Table 2 lists the discharge patterns observed for the five types of duration sensitivity and AP neurons. Overall, most of the neurons were phasic–burster types (87%, 82/95; Fig. 3B, Table 2) and only 4 AP neurons showed phasic–tonic discharge patterns (Fig. 3C, Table 2). All BP (14%, 13/95) and LP neurons (5%, 5/95) and 8 of the 9 combined BR and MP neurons (which were grouped together due to the relatively low sample size) were phasic–burster types (Table 2). Three SP and one MP neuron showed phasic spike patterns. All discharge patterns were onset responses, except for LP neurons at durations of ≤20 ms, which were offset responses.

**Table 1.** BF, MT, recording depth, and first spike latency of IC neurons with different duration sensitivity tested at 10 dB above MT

<table>
<thead>
<tr>
<th>Tuning Characteristic</th>
<th>n</th>
<th>BF, kHz</th>
<th>MT, dB SPL</th>
<th>Depth, μm</th>
<th>Latency, ms</th>
</tr>
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<tbody>
<tr>
<td>SP</td>
<td>16</td>
<td>25.1 ± 9.2 (15.7–45.5)</td>
<td>42 ± 20 (3–57)</td>
<td>611 ± 363 (180–1,485)</td>
<td>12.1 ± 4.2 (3.5–18.5)</td>
</tr>
<tr>
<td>BP</td>
<td>13</td>
<td>31.3 ± 11.5 (15.5–60.0)</td>
<td>37 ± 15 (11–57)</td>
<td>606 ± 322 (213–1,422)</td>
<td>18.1 ± 4.5 (11.0–26.0)</td>
</tr>
<tr>
<td>LP</td>
<td>5</td>
<td>25.3 ± 11.6 (16.4–44.4)</td>
<td>35 ± 19 (5–64)</td>
<td>589 ± 309 (184–1,046)</td>
<td>12.2 ± 3.1 (7.0–15.5)</td>
</tr>
<tr>
<td>BR and MP</td>
<td>9</td>
<td>23.0 ± 9.3 (14.0–44.4)</td>
<td>44 ± 9 (30–54)</td>
<td>488 ± 387 (106–1,331)</td>
<td>12.3 ± 3.1 (8.5–17.0)</td>
</tr>
<tr>
<td>Nonsensitive</td>
<td>52</td>
<td>27.0 ± 11.9 (7.5–75.1)</td>
<td>45 ± 14 (5–70)</td>
<td>648 ± 409 (201–1,981)</td>
<td>13.4 ± 4.0 (5.5–23.0)</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>26.4 ± 11.1 (7.5–75.1)</td>
<td>43 ± 16 (3–70)</td>
<td>599 ± 377 (180–1,981)</td>
<td>13.5 ± 4.3 (3.5–26.0)</td>
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</table>

Values are means ± SD; n, number of neurons. Two of the LP neurons lost their duration tuning at higher stimulus intensities and are thus, in a strict, not considered to be LP tuned. BF, best frequency; MT, minimum threshold.

**Effects of CF-FM stimulation on duration selectivity**

In addition to testing the duration sensitivity to bursts of pure tones, we also examined how the presentation of a more natural, echolocation call-like signal affected the duration tuning. For this purpose, we added an FM component at the end of the CF bursts, creating CF-FM pulses. To examine possible intensity effects on duration tuning, we changed the intensities of the CF and the FM components independently (see following text). To maintain compatibility between our data sets for CF and for CF-FM stimulation, we limited the spike counts for CF-FM stimulation to the responses to the CF-component alone (see METHODS).

Of the total of 95 IC neurons, we were able to test 65 neurons using both CF and CF-FM stimuli. In general, we found that CF-FM signals increased the number of neurons exhibiting duration sensitivity. Whereas for CF stimulation, 45% of all neurons tested (43 of 95 units; Fig. 2A) showed some form of duration tuning, at the same intensity levels, 57%
TABLE 2. Discharge patterns of duration-sensitive IC neurons in response to a CF burst delivered at the unit’s BF and 10 dB above MT

<table>
<thead>
<tr>
<th>Tuning Characteristic</th>
<th>Discharge Pattern</th>
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<tbody>
<tr>
<td></td>
<td>Phasic–burst</td>
</tr>
<tr>
<td>SP</td>
<td>13 (14%)</td>
</tr>
<tr>
<td>BP</td>
<td>13 (14%)</td>
</tr>
<tr>
<td>LP</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>BR and MP</td>
<td>8 (8%)</td>
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<tr>
<td>Nonsensitive (AP)</td>
<td>43 (46%)</td>
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<tr>
<td>Total</td>
<td>82 (87%)</td>
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</tbody>
</table>

The increased number of duration-sensitive neurons for CF-FM stimulation was recruited mostly from neurons that exhibited no duration tuning to CF tone bursts (i.e., AP neurons; Table 3). Seven to 15 neurons that were classified as AP when stimulated with CF bursts became duration sensitive when exposed to CF-FM stimulation. Depending on stimulus intensity, between 4 and 8 became SP, 1–3 turned into BP, 1–2 into LP, and 1–3 into BR (Table 3). Figure 4 illustrates the detailed changes in relative spike counts with varying CF-FM duration for 4 representative AP neurons that became duration tuned to each of these filter types, i.e., SP (Fig. 4A), BP (Fig. 4B), LP (Fig. 4C), and BR (Fig. 4D). Interestingly, for each of these neurons (Fig. 4, A–D), the changes were virtually independent of the stimulus intensities for either the CF or FM component (see following text for details).

In addition, several neurons that were duration sensitive to CF signals changed their filter characteristics when stimulated with a CF-FM pulse (Fig. 2, Table 3). The greatest changes occurred among neurons that exhibited BP tuning to CF bursts. When those neurons were stimulated with a CF-FM pulse, 2 turned into SP, ≤2 into MP, and 2 became duration insensitive (at higher stimulus intensities; Table 3). Two of the SP neurons (for pure-tone stimulation) also changed their filter characteristics and responded as BP to CF-FM pulses. Finally, only 1 of 6 neurons remained BR, whereas 3 of them became duration insensitive and 1 neuron each switched to SP and BP, respectively (Table 3).

How did adding a terminal FM component to the CF burst alter the responses of the previously non-duration-sensitive neurons shown in Fig. 4? For this purpose, we plotted their response latency (“first spike latency”) against the duration of the CF component for different stimulus combinations (Fig. 5). This allowed us to determine whether the neurons responded to the onset or offset (CF part) of the CF-FM stimulus. The diagonal hatched line in Fig. 5 indicates where response latencies were equal to stimulus durations (CF bursts or CF component of CF-FM signal). Therefore if dots in our curves were to the right of this line, the neurons fired during the stimulus in response to its onset. In contrast, if latencies increased with increasing stimulus durations and dots were located to the left of the diagonal line, the response latency was larger than the stimulus duration and the neurons fired in response to the stimulus offset.

Interestingly, we found that adding a terminal FM component did not have a uniform effect but instead differed between neurons (Fig. 5). Whereas two neurons (Fig. 5, A and D) responded only to stimulus onsets, irrespective of the stimulus combination, the two other neurons (Fig. 5, B and C) responded to stimulus offset for short stimulus durations of ≤20 ms and to the stimulus onset for longer durations ≥20 ms (Fig. 5, B1, B2, C1, and C2). These differences may have implications for the synaptic mechanisms creating this duration sensitivity and are discussed in the following text.

TABLE 3. Changes of filter characteristics of duration-sensitive neurons in response to stimulation with a natural echo mimic (CF-FM stimulus)

<table>
<thead>
<tr>
<th>From (for CF)</th>
<th>To (for CF-FM)</th>
<th>FM at MT + 10 dB (n = 63)</th>
<th>FM at MT + 20 dB (n = 60)</th>
<th>FM at MT + 30 dB (n = 53)</th>
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<tr>
<td>AP</td>
<td>AP</td>
<td>26</td>
<td>23</td>
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<td></td>
<td>SP</td>
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One MP neuron (the only MP neuron tested for all combinations) did not alter its duration sensitivity and is therefore not listed.
Effects of CF stimulus intensity on duration sensitivity

We tested the effects of stimulus intensity on duration selectivity in all 95 IC neurons by presenting CF bursts at their BFs at 10, 20, and 30 dB above MT. Overall, we found that 54% of all IC neurons tested (51/95) showed at least one form of duration sensitivity to pure tone signals at one or more intensity levels, which is a higher percentage than that when testing CF bursts only at 10 dB above MT (Fig. 2A). For any intensity level tested (Fig. 2, A–C), SP neurons were by far the most common type, accounting for 15–17%, followed by BP neurons (7–14%), LP neurons (5–6%), BR neurons (2–6%), and MP neurons (2–3%).

When changing the stimulus level of the CF burst, we found that in 71% of all neurons tested (67/95), the filter characteristics remained unaffected. Among the 43 duration-sensitive neurons, the filter characteristics of 53% (23/43) were not affected by increases in CF stimulus level from 10 to 30 dB above MT and they maintained the same type of duration tuning. This is illustrated in Fig. 6, which shows the duration-tuning curves for all three intensity levels tested for representative examples of each of the six types. Although the overall spike count for each duration tested increased with increasing intensity, the duration tuning remained independent of the intensity level. Of those neurons, 16% (7/43) were SP (Fig. 6A), 12% (5/43) were BP (Fig. 6B), 12% (5/43) were LP (Fig. 6C), 9% (4/43) were BR (Fig. 6D), and 5% (2/43) were MP (Fig. 6E). Most neurons that were not duration sensitive to CF bursts (AP type: 85%, 44/52) were also unaffected by variations in sound level (Fig. 6F).

In the remaining IC neurons (29%, 28/95), the percentage of duration-sensitive responses decreased significantly for each type with increasing intensity of the CF burst (Fig. 2, A–C; P < 0.05, one-way ANOVA). On the other hand, of the 7 neurons that showed LP behavior at the lowest stimulus intensity, 2 lost their duration selectivity, which may indicate that their apparent LP behavior for low-intensity CF signals merely reflects the fact that soft, short-duration tone bursts did not contain sufficient stimulus energy to drive these cells. Similarly, of the 11 BP neurons, 1 became SP at higher CF levels, which may also reflect that the SP behavior at higher stimulus levels may also have been caused by an increase in stimulus energy for short-duration signal. Conversely, however, a lack of stimulus energy did not explain its BP tuning at lower levels.

Figure 7 demonstrates how various parameters of the duration tuning changed with intensity levels. On average, BP neurons exhibited a slight but not very significant trend for their mean bandwidth to increase from 36.2 to 44.3 ms (P > 0.05, one-way ANOVA; Fig. 7A) when CF burst levels rose from 10 to 30 dB above MT. In one instance, however, the bandwidth decreased from 68 to 55 ms for the same increase in intensity. In the same BP neurons, the mean BD decreased significantly with an increase in the intensity of the pure-tone burst, shifting on average from 36.4 ms at 10 dB to 17.5 ms at 30 dB above MT (P < 0.01, one-way ANOVA; Fig. 7B). For both, SP (Fig. 7C) and LP neurons (Fig. 7D), the cutoff duration decreased systematically with an increase in CF burst intensity (P > 0.05, one-way ANOVA).

Effects of CF-FM stimulus intensity on duration selectivity

Figure 2 illustrates and Table 3 lists how the various types of duration-sensitive neurons changed their filter characteristics for different stimulus intensities. The most evident change caused by increases in the intensity of both pure-tone bursts (i.e., no FM present; Fig. 2, A–C, Table 3) and CF portion within a CF-FM stimuli (Fig. 2, D–H, Table 3) was an increase in the number of AP neurons, mostly because some BP neurons lost their duration sensitivity. When the amplitude of the FM component in a CF-FM burst was increased, the number of SP neurons increased slightly (Fig. 2, D and F and E and G, Table 3).

A more detailed analysis of the changes in duration tuning for BP and SP neurons as a function of stimulus intensity is given in Fig. 8, which shows the average bandwidth and BD of BP neurons (Fig. 8, A and C) and cutoff duration of SP neurons (Fig. 8C) for different FM intensities and contrasts them with the response to pure-tone bursts (i.e., no FM present). The average bandwidths of BP neurons (Fig. 8A) were 32.8 ± 12.0 ms for pure-tone bursts delivered at 10 dB above MT, 30.2 ± 11.4 ms for CF-FM stimuli with FMs at 10 dB above MT, and 25.5 ± 0.7 ms for 20 dB above MT. For the same stimulus conditions, their average BDs (Fig. 8B) were 36.7 ± 8.2.
43.3 ± 8.2, and 40.0 ± 0.0 ms, respectively. Although the sample size is very limited, there was no significant difference in bandwidth or BDs in response to these different stimulus conditions (paired t-test, all P > 0.05).

In contrast, for seven SP neurons (Fig. 8C), the average cutoff duration exhibited a significant change in response to changes in stimulus condition, which decreased from 41.3 ± 19.8 ms for CF bursts to 24.1 ± 16.2 ms for CF-FM stimulation with FMs at 20 dB above MT (paired t-test, P < 0.05). The difference between pure-tone stimulation and CF-FM stimuli with FMs at 10 dB above MT was not significant.

In two LP neurons tested, we also found a significant shift in their cutoff durations (paired t-test, P < 0.05; data are not displayed graphically due to the small sample size). In both neurons, the cutoff durations increased from 21.0 and 6.0 ms, respectively, for pure-tone bursts, to 26.0 and 12.0 ms, respectively, for CF-FM stimulation with FMs at 10 dB above MT, to 26.0 and 19.0 ms, respectively, for FMs at 20 dB above MT.

**DISCUSSION**

Duration-sensitive neurons have been described in the IC or auditory cortex of several species of mammals, including bats (Brand et al. 2000; Casseday et al. 1994, 2000; Chen 1998; Ehrlich et al. 1997; Fremouw et al. 2005; Fuzessery and Hall 1999; Jen and Feng 1999; Jen and Schlegel 1982; Pérez-González et al. 2006; Pinheiro et al. 1991; Xia et al. 2000;
Zhou and Jen (2001) as well as in the frog midbrain (Feng et al. 1990; Gooler and Feng 1992). The present study, however, focused on examining duration sensitivity in a hearing specialist, the horseshoe bat, by using a natural, behaviorally relevant stimulus paradigm: we tested the differential effects of the two components of the CF-FM echolocation calls of horseshoe bats, i.e., pure-tone (CF) burst and terminal FM component. Our results showed that more than half of all IC neurons tested in the least horseshoe bat (68%, 65/95) exhibited at least one form of duration tuning to either a CF burst alone or to a combined CF-FM stimulus. Approximately two thirds of all duration-sensitive neurons in the horseshoe bat IC were either SP (37%) or BP (30%), and only 12% had LP characteristics (Fig. 2A, Table 1). These ratios of the different response types correspond well with those described for other bats (Eptesicus fuscus: ~36%, Ehrlich et al. 1997; Faure et al. 2003; Perez-Gonzalez et al. 2006; Antrozous pallidus: 53%, Fuzessery and Hall 1999; and Molossus molossus: 41%, Mora and Kössl 2004).

Mechanisms underlying duration selectivity

What is the neural basis for such duration sensitivity? Two different models are currently used to explain this effect: coincidence (Casseday et al. 1994; Ehrlich et al. 1997; Narins and Capranica 1980) and anticoincidence mechanisms (Fuzessery and Hall 1999). The coincidence model contains three components (Casseday et al. 1994): sustained inhibition, offset excitation (i.e., postinhibitory rebound excitation), and delayed onset excitation. It is based on synaptic delay lines generating a wide range of spike latencies and convergence onto a duration-tuned neuron. The coincidence model provides an effective mechanism for BP duration tuning. The neuron fires maximally when the delayed onset excitation arrives after the end of the sustained inhibition, which lasts as long as the stimulus, and thus coincides with the postinhibitory rebound excitation. This coincidence occurs for only a particular stimulus duration. If the sound signal is too long, the delayed onset excitation is reduced or even eliminated by the still ongoing
sustained inhibition. If the stimulus is too short, the delayed excitation arrives after the postinhibitory rebound excitation and the net response is also lower than when the two coincide. This model has been used successfully to explain the large percentage of offset responders found in the IC of various FM bats, such as E. fuscus (Covey et al. 1996; Erlich et al. 1997), A. pallidus (Fuzessery and Hall 1999), and M. molossus (Mora and Kössl 2004). The anticoincidence model, on the other hand, was proposed to explain SP tuning and also involves sustained inhibition and delayed onset excitation (postinhibitory rebound excitation can also be present but does not play a role in generating the duration tuning; Fuzessery and Hall 1999). A neuron fires maximally only when the two events do not coincide, i.e., when the sustained inhibition is over before the delayed excitation arrives, which is the case for short-duration stimuli. If the stimuli are too long, the still ongoing inhibition reduces or eliminates the delayed excitation.

In the horseshoe bat IC, however, most types of duration-sensitive neurons exhibited on-responses for all durations tested (Fig. 1, A1, B1, D1, and E1). Only in some neurons was the response latency locked to sound offset, but only up to some maximum duration, beyond which the neurons switched to on-responses (Figs. 1C1 and 5, B1, B2, C1, and C2).

On-responses in duration-sensitive neurons within the IC of the big brown bat, where they represent only a small portion, have been explained by adjusting the magnitude of any of the three components of the coincidence model (Ehrlich et al. 1997). The authors assumed that the two excitatory components are sufficient to elicit spikes occasionally even when the other excitatory input is not coincident. If the delayed onset excitation is stronger than offset excitation, the response would have predominantly an onset pattern because the leading inhibition is insufficient to completely suppress the delayed onset excitatory input. The onset response pattern observed in our SP neurons (Fig. 1A) may accordingly be based on a strong delayed onset excitation that was strong enough to elicit a response, whereas the offset excitation was subthreshold (e.g., due to short stimulus durations and thus short-lasting inhibition). For short-duration stimuli, the offset excitation would then coincide with the delayed onset excitation and produce strong discharges. With increasing stimulus durations, however, the sustained inhibition would encroach on the delayed onset excitation to reduce the onset discharges. Alternatively, as discussed earlier, such onset neuronal response patterns in SP neurons can also be explained by an anticoincidence model.

LP neurons found in our study showed off-responses for short-duration stimuli (≤20 ms) and on responses for longer durations (Figs. 1C and 5, C1 and C2). This switch in response patterns with stimulus duration requires a more complicated interaction between coincident and anticoincident synaptic inputs. If transient inhibition was evoked by the offset of the stimulus, it could eliminate responses to sounds with durations shorter than the latency of the delayed onset excitatory input, while permitting responses to sounds at all longer durations (Pérez-González et al. 2006). Therefore we postulate that the responses of these LP neurons may be based on a coincident mechanism for shorter durations and an anticoincidence between delayed onset excitation and offset inhibition for longer durations.

With respect to the onset neurons with multi-peaked duration tuning (Fig. 1E), Mora and Kössl (2004) suggested that an anticoincidence mechanism might create one of the peaks and the other peaks could reflect strong excitatory inputs from other duration-sensitive neurons. Conversely, for BR neurons, we propose that the insensitive durations in these neurons may reflect strong inhibitory inputs from other duration-sensitive neurons with BDs matching the range of durations to which BR neurons were insensitive.

Duration tuning in BP neurons with offset responses may be based on a simple coincidence mechanism. But how can we explain the duration tuning of BP neurons with onset responses that we observed in our study? One possible mechanism underlying such a response pattern may involve a combination of coincident and anticoincident synaptic inputs. Similar to the situation in an anticoincidence mechanism, a BP neuron with onset responses may receive an early inhibitory input that persists while the sound is on and a delayed excitatory input triggered at stimulus onset. In addition, we assume that there is an excitatory rebound from the early inhibitory input. Thus at short stimulus durations, inhibition and excitatory rebound are over before the arrival of the delayed excitatory input, which can still cause the neuron to fire, yet at submaximal rates. With increasing durations, the excitatory rebound coincides with the excitatory input and the neuron responds maximally. As the duration increases further, the inhibitory is still ongoing while
the delayed excitatory input arrives, which reduces or even eliminates the response.

**Effect of FM on duration selectivity**

Previous work on duration tuning in the big brown bat showed that the filter characteristics of IC neurons examined with both pure tones and FM stimuli were similar (Casseday et al. 1994; Jen and Zhou 1999). However, we found that in the horseshoe bat, 7–15 neurons that did not exhibit any duration sensitivity to pure-tone stimuli (AP neurons) became duration sensitive to the same CF burst when a terminal FM component was added to form a CF-FM stimulus. Two alternative synaptic mechanisms may explain the duration sensitivity to the natural echo signal of these bats. First, the FM portion of the stimulus may have introduced a sustained inhibition in these neurons by evoking an inhibitory postsynaptic potential (IPSP). The particular latency, duration, and strength of this IPSP then changed this neuron’s responsiveness causing the excitatory inputs to coincide, thus making the neuron duration sensitive. Another possible explanation could involve convergence of two (or more) purely excitatory inputs onto those IC neurons (Mora and Kössl 2004). One input would arise from CF-tuned neurons not sensitive to particular stimulus durations. The other input(s) would originate from neurons tuned to the FM range and would involve neurons that are duration sensitive. This duration-sensitive input would presumably represent either an intrinsic connection within IC or a descending input from diencephalon or cortex because thus far no duration-sensitive neurons have been found below the level of IC in any vertebrate examined (Brand et al. 2000; Casseday et al. 1994; Chen 1998; Condon et al. 1996; Covey and Casseday 1999; Ehrlich et al. 1997; Faure et al. 2003; Feng et al. 1990; Fremouw et al. 2005; Fuzessery and Hall 1999; Galazyuk and Feng 1997; Gooler and Feng 1992; He et al. 1997; Mora and Kössl 2004; Narins and Capranica 1980; Pérez-González et al. 2006; Pinheiro et al. 1991). Activation of both inputs by presenting a combined CF-FM stimulus would then induce duration sensitivity in neurons that otherwise exhibit AP characteristics when stimulated only with a pure-tone burst.

**Duration selectivity and stimulus intensity**

It appears that, depending on the species examined, changing sound levels had different effects on the selectivity of auditory neurons for particular sound durations. In the IC of the big brown bat, Eptesicus fuscus, the majority of duration-tuned neurons were tolerant within 50 dB above MT, although the selectivity appeared to be somewhat reduced at high sound levels (Fremouw et al. 2005; Zhou and Jen 2001). Most neurons retained overall duration selectivity with only minor changes in BD and exhibited stable first spike latencies. On the other hand, Zhou and Jen (2001) also found that about one third of all duration-sensitive IC neurons lost their duration selectivity at higher stimulus intensities or changed their filter characteristics.

In the horseshoe bat, we found that only about 53% (23/43) of all duration-sensitive neurons [71% (67/95) of all IC neurons tested] remained unaffected by changes in intensity levels of CF bursts, whereas about 26% (7/27) of duration-tuned neurons were unaffected by changes in the intensity of CF-FM stimuli. In the remaining neurons, increasing sound levels generally lowered the percentage of duration-tuned neurons to 33% for CF (Fig. 2, A–C) and 37% for CF-FM stimulation (Fig. 2, F–H). In those that remained duration tuned (53% of all duration-sensitive neurons with CF, 26% of all duration-sensitive neurons with CF-FM), the duration tuning, BDs, and cutoff durations changed with stimulus intensity (Figs. 7 and 8). Louder CF bursts decreased BDs in BP neurons and cutoff durations in SP and LP neurons (Fig. 7), whereas louder CF-FM stimuli reduced only the cutoff durations in SP neurons (Fig. 8). Bandwidths of BP neurons were not significantly affected by any stimulus configuration, with only a slight trend for increasing bandwidths for louder CF bursts (Fig. 7A) but not CF-FM stimuli (Fig. 8A). In addition, an increasing number of duration-sensitive neurons became nonsensitive (AP) when we raised the intensity of a CF burst alone (Fig. 2, A–C) or of the CF component within a CF-FM stimulus (Fig. 2, D–H; Table 3). Interestingly, however, when only the intensity of the FM component in a CF-FM stimulus was raised, the ratio of duration-tuned neurons, especially SP neurons, increased slightly (e.g., Fig. 2, D and F).

In two other bat species, even larger increases in sound levels ≤80–90 dB above MT were tested. As a result, and perhaps also due to species differences, a much larger percentage of neurons changed their responses with variations in sound level. In the IC of M. molossus, ≤82% (36/44; Mora and Kössl 2004) and in auditory cortex of Myotis lucifugus ≤69% of neurons (68/99; Galazyuk and Feng 1997) eventually became duration sensitive with increasing sound levels. In mice, a change in sound intensity sometimes also altered and even abolished duration-tuning characteristics of IC neurons (Brand et al. 2000).

Galazyuk and Feng (1997) compared the effects of varying sound level on duration selectivity in IC neurons and AC neurons in M. lucifugus in more detail. They showed that cortical neurons with onset phasic responses were affected by the varying intensities but that IC neurons were not. In addition, response latencies of IC neurons became progressively longer with increasing stimulus duration, whereas in AC neurons, latencies were independent of stimulus duration. Thus the coincidence mechanism (Casseday et al. 1994) that created duration selectivity in the IC was transformed along the colliculo-geniculo-cortex axis to account for the duration selectivity in the AC. Galazyuk and Feng (1997) suggested that long recovery cycles in neurons tuned to longer durations and high thresholds in those tuned to shorter durations account for the duration selectivity of AC neurons.

**Behavioral significance**

The function most commonly attributed to duration selectivity in auditory neurons is the processing of biologically important signals such as communication calls in frogs and mammals and echolocation calls in bats. In several bat species that have been studied, the range of BDs correlates with the range of echolocation calls used by these species. Neurons in the IC of E. fuscus and M. lucifugus have BDs of ≤20 ms, which correspond to the range of call durations emitted by these species during different stages of hunting (Ehrlich et al. 1997; Galazyuk and Feng 1997; Moss and Surlykke 2001; Surlykke and Moss 2000). In the pallid bat, which emits even
shorter echolocation pulses that range from 1 to 6 ms, BDs are all <7 ms (Fuzessery et al. 1993). While searching for prey, *M. molossus* broadcasts pulses with durations around 11 ms (Mora et al. 2004). Although duration coding in the IC of this species was not unambiguous, with approximately 30% of the SP and BP neurons responding best to two different stimulus durations, one of their BDs was always confined to the range of stimulus durations between 10 and 16 ms (Mora and Kössl 2004).

In the horseshoe bat, we also found that most BDs in BP neurons and cutoff durations for SP neurons covered the range of pulse durations emitted during echolocation. *R. pusillus* emits echolocation pulses with durations varying between 16.8 and 58.0 ms during free flight indoors (Luo et al. 2006). This is similar to findings in other horseshoe bat species for pulses produced in a confined room or in the wild (Jones and Rayner 1989; Ma et al. 2007; Neuweiler et al. 1987; Schnitzler 1968; Smotherman and Metzner 2005; Tian and Schnitzler 1997). In parallel with the longer pulse durations in horseshoe bats, the cutoff durations and BDs found here are also generally longer than those reported in the other bats. We found that the BDs in BP neurons were 20–40 ms when using a CF stimulus and 10–60 ms using a CF-FM stimulus. For SP neurons, cutoff durations were distributed from 8.0 to 56.0 ms for CF stimulation and between 7.5 and 59.0 ms for CF-FM stimuli. Only two SP neurons had cutoff durations above the normal range of echolocation calls (80 and 88 ms, respectively), although horseshoe bats have recently been reported to occasionally emit echolocation pulse-like calls that exceed 200 ms, which in addition to potentially increasing the duty cycle of echolocation pulses may also possess a communication function (Ma et al. 2006). Thus although duration tuning in echolocating bats—especially when restricted to a particular range of durations, such as in BP, SP, and MP neurons—most likely evolved in the context of enhancing echolocation performance, it may of course still have retained its original function of processing communication signals.

We found that when we presented a CF-FM stimulus, which mimics the bats’ echolocation signal more naturally, the percentage of duration-tuned neurons increased to 57 and 60% compared with 45% for stimulation with pure-tone bursts, which was largely based on an increase in the ratio of SP and BP neurons (Fig. 2, A, D, and F). In addition, we found that the cutoff durations of SP neurons decreased significantly from 41.3 ms when no FM was presented to 21.1 ms for a CF-FM signal delivered at 20 dB above MT (Fig. 8C). However, in BP neurons, BDs and bandwidths were not significantly altered by changes in FM intensity. Finally, raising only the intensity of the FM component in a CF-FM burst slightly increased the overall ratio of duration-tuned neurons, whereas increasing the intensity of the CF component alone had no such effect (e.g., Fig. 2, D and F vs. Fig. 2, D and E or F and G). Thus it appears that presentation of a natural CF-FM stimulus improved the duration tuning in IC neurons by increasing the ratio of duration-tuned neurons and making them less susceptible to changes in signal intensity.

The behavioral significance of duration tuning in horseshoe bats becomes apparent when considering that as a horseshoe bat approaches its target, the pulses not only shorten, mostly because the CF component shortens (Smotherman and Metzner 2005; Tian and Schnitzler 1997), but the FM component also becomes more prominent, covering a broader frequency range and occupying a larger proportion of the total call duration (Jones and Rayner 1989; Neuweiler et al. 1987; Tian and Schnitzler 1997). Although the former adjustment in call design prevents pulse-echo overlap, the latter gives the bat more information about the position and nature of the target (Schnitzler and Kalko 2001). Thus the increased percentage of SP neurons and their shortened cutoff durations may enhance the detection of target echoes at short ranges.

Nine neurons in our sample had responses that we classified as BR or MP, and similar types of neurons have been described in *E. fuscus* (Pinheiro et al. 1991), chinchilla (Chen 1998), rats (Pérez-González et al. 2006), and *M. molossus* (Mora and Kössl 2004). Multipeaked units may be suited for detecting the echo of paired pulses such as in *M. molossus* (Mora and Kössl 2004). *R. pusillus*, similar to other horseshoe bats (Jones and Rayner 1989; Smotherman and Metzner 2005; Smotherman et al. 2006; Tian and Schnitzler 1997), often emits sequences of two or more shortened echolocation pulses as doublets or multiplets, giving rise to a bimodal distribution of call durations (Smotherman and Metzner 2005; Smotherman et al. 2006), which may be reflected in the tuning of BR and MP neurons.

**Acknowledgments**

We thank Dr. Jia Tang, X. Wang, and A. A. Li of the College of Life Sciences, Central China Normal University, Wuhan, China, for technical assistance. We thank two anonymous reviewers for reading an earlier version of the manuscript.

**Grants**

This work was supported by National Natural Science Foundation of China Grants 30470564, 3051120058, 90208012 to Q. Chen, a special grant from East China Normal University to S. Zhang, and National Institute on Deafness and Other Communication Disorders Grant DC-5400 to W. Metzner.

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