Leading Inhibition to Neural Oscillation Is Important for Time-Domain Processing in the Auditory Midbrain

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Galazyuk, Alexander V., Wenyu Lin, Daniel Llano, and Albert S. Feng. Leading inhibition to neural oscillation is important for time-domain processing in the auditory midbrain. J Neurophysiol 94: 314–326, 2005. First published March 16, 2005; doi:10.1152/jn.00056.2005. A number of central auditory neurons exhibit paradoxical latency shift (PLS), a response characterized by longer response latencies at higher sound levels. PLS neurons are known to play a role in target ranging for echolocating bats that emit frequency-modulated sounds. We recently reported that early inhibition of unit’s oscillatory discharges is critical for PLS in the inferior colliculus (IC) of little brown bats. The goal of this study was to determine in echolocating bats and in nonecholocating animals (frogs): 1) the detailed characteristics of PLS and whether PLS was dependent on sound level, frequency, and duration; 2) the time course of inhibition underlying PLS using a paired-pulse paradigm. We found that 22% of IC neurons in bats and 15% in frogs exhibited periodic discharge patterns in response to tone pulses at high sound levels. The firing periodicity was unit specific and independent of sound level and duration. Other IC neurons (28% in bats; 14% in frogs) exhibited PLS. These PLS neurons shared several response characteristics: 1) PLS was largely independent of sound frequency and 2) the magnitude of shift in first-spike latency was either duration dependent or duration tolerant. For PLS neurons, application of bicuculline abolished PLS and unmasked the unit’s periodic firing pattern that served as the building block for PLS. In response to paired sound pulses, PLS neurons exhibited delay-dependent response suppression, confirming that high-threshold leading inhibition was responsible for PLS. Results also revealed the timing of excitatory and inhibitory inputs underlying PLS and its role in time-domain processing.

INTRODUCTION

Animals that echolocate, such as bats and dolphins, use a sonar system to orient in 3-dimensional space and to detect and track moving prey. For this, they must assess the static and dynamic characteristics of each target in their surroundings, such as the target range, size, shape, azimuth, elevation, direction, and speed of target movement. Target ranging is especially important for tracking a moving prey and has been extensively studied behaviorally and physiologically. Physiological studies have shown that central auditory neurons of echolocating bats are tuned to echo delay, showing response facilitation to pulse–echo pairs at particular echo delays (Feng et al. 1978; Mittmann and Wenstrup 1995; O’Neill 1995; O’Neill and Suga 1979; Sullivan 1982a,b). Although delay-tuned response characteristics have been described in detail, the underlying mechanisms are not well understood.

In the auditory cortex of little brown bats, a species that uses frequency-modulated (FM) ultrasonic pulses for echolocation, Sullivan (1982a,b) proposed that delay-tuned responses are attributed to a coincidence-detection mechanism. For this, the input neurons must show a delayed response to a loud sonar signal such that this response can align temporally with the units’ response to a weak and late arriving echo. Neurons exhibiting paradoxical latency shift (PLS), i.e., longer response latencies for intense sounds than for weaker sounds, are therefore well suited for construction of delay-tuned responses. There are 2 lines of evidence suggesting that PLS plays a role in target ranging. First, behavioral experiments in bats suggested that neurons exhibiting PLS may be involved in target ranging (Denzinger and Schnitzler 1994). Second, Sullivan (1982b), and later Berkowitz and Suga (1989), found that for the majority of neurons showing PLS in the little brown bat auditory cortex, the magnitude of the latency shift for each neuron corresponded to the unit’s best echo delay to which this neuron is tuned.

Sullivan proposed 2 models that may account for the PLS in the cortex of little brown bats (the Delay Line model and the Timed Inhibitory model), but neither of these models was experimentally validated. Recently Galazyuk and Feng (2001) reported that, for the inferior colliculus in the same species, PLS can be created by high-threshold early inhibition of neural oscillatory discharges. Their result provides support for the Timed Inhibitory model of Sullivan. Early inhibition (which shall be referred to as “leading inhibition”) that precedes an excitatory response therefore represents a key building block for the PLS. At this time, our understanding of the basic characteristics of leading inhibition and its role in PLS is limited. The goal of the present study is to fill this knowledge gap. Because the inferior colliculus is the first station along the central auditory pathway where PLS neurons are found (Berkowitz and Suga 1989; Klug et al. 2000), our study was focused there. Additionally, because PLS appears to be a common phenomenon among animals (cats: Rose et al. 1963; gerbils: Klug et al. 2000; and insects: Krahe et al. 2002), the present study will investigate PLS phenomena in the IC of the little brown bats (an echolocating species for which PLS is known to have a role in delay-tuned response and target ranging) and the northern leopard frog (a nonecholocating vertebrate).

METHODS

Surgical and recording procedures

BATS. Experimental subjects constituted 23 little brown bats, Myotis lucifugus. Details of experimental methods are given in Galazyuk and Feng (2001). For surgery, the animal was anesthetized by halothane...
inhalation (4% halothane administered by a precision vaporizer). After incision of the skin and clearing of the tissues above the skull, a small metal rod was glued to the skull using glass ionomer cement. After the surgery, animals were allowed to recover for 2–4 days in individual holding cages.

Recordings were made from awake bats. During the recording session the animal was placed inside a sound-attenuating chamber. The metal rod on the bat’s head was secured to a small holder for restraining the animal’s headatraumatically, leaving the ears unobstructed for free-field acoustic stimulation. A small hole (about 50 μm) was then made in the skull overlying the IC through which a recording electrode was inserted to reach the IC. Throughout the recording session, the animal was offered drinking water periodically and monitored for signs of discomfort. After a recording session of 6–8 h, the exposed skull was covered with sterile bonewax, and the animal was returned to its holding cage. Such experiments proceeded every 2–3 days for a maximum of 3 wk.

Extracellular single-unit recordings were made with glass microelec-
one micropipettes (10–20 MΩ, 2- to 3-μm tip) filled with horseradish peroxidase (5% in 0.2 M Tris buffer). Such electrodes produce stable single-unit recordings over long durations and thus are preferred over other types of recording electrodes. The electrode was positioned above the IC by means of a precision (1 μm) digital micromanipulator and lowered to the dorsal brain surface. The relative position of each electrode was monitored from the readouts of digital micrometers using a common reference on the skull. Vertical advancement of the electrode was made by a precision piezoelectric microdrive from outside the sound-attenuating chamber. Recorded action potentials were amplified (Dagan 2400 preamplifier), monitored audiovisually, stored on the computer hard drive, and processed off-line (100-μs bin width) using data-acquisition software/hardware from RC Electronics.

**FROGS.** Experimental methods generally adhered to those described in Lin and Feng (2003). Briefly, 32 Northern leopard frogs (\textit{Rana pipiens}), weighing 12–32 g, were used in this study. For surgery, anesthesia was induced with 25% above the background spontaneous firing). The spontaneous firing rate was the average response over a 50-ms window before each stimulus epoch.

**Acoustic stimulation**

**BATS.** Acoustic stimuli, consisting of tone pulses at the unit’s characteristic frequency (CF), were delivered to the bat by a free-field ultrasonic loudspeaker located 60 cm in front of the bat. Tone pulses had a constant rise–fall time of 0.5 ms. The duration of tone pulses ranged from 2 to 8 ms. Sound pulses of a wide range of sound levels, from 10 to 90 dB SPL, were presented at a rate of 1/s in increments of 2–4 dB. The range of sound level was extended to lower values if the unit’s response threshold was <10 dB SPL. The absolute sound pressure level (dB re: 20 μPa) was measured with a 1/4-in. microphone (Bruel & Kjær 4135) situated near the concha of the ear opposite to the recording side. Typically we presented 10–20 tone pulses at each sound level.

**FROGS.** Acoustic stimuli, consisting of tone bursts at the unit’s CF having a rise–fall time of 5 ms and duration of 20–80 ms, were presented once every 2.5 s. The stimulus parameters were controlled by a PC-based Tucker Davis System-2 hardware and software. The frog was stimulated by a free-field loudspeaker (ADS 200 LC) located 55 cm on the frog’s right side (90° from the frog’s longitudinal axis, which corresponds to the best azimuth of most IC units). The absolute sound pressure level (dB re: 20 μPa) was calibrated at the location of the frog’s left tympanum using a free-field condenser microphone (Bruel & Kjær 4165). Ten tone bursts were presented at each sound level; the sound level ranged from 20 to 80 dB SPL.

**Data analysis**

Dot raster histograms were used to depict a unit’s temporal discharge pattern for each epoch at each level. Each dot in a raster histogram indicated a spike at the relative time instant with respect to the stimulus onset. Dot raster histograms from different sound levels were combined to create a unit’s composite dot histogram for visualization of how the response latency and the firing pattern changed globally with sound level. To quantify the response latency, we determined the average and the SD of latency of the first spike of a unit’s responses to 10–20 epochs within the “response window” (defined as the window wherein the spike count and/or firing rate is >25% above the background spontaneous firing). The spontaneous firing rate was the average response over a 50-ms window before each stimulus epoch.

**Iontophoretic drug injection**

For a number of neurons in the bat and frog IC showing PLS, we applied bicuculline [i.e., a γ-aminobutyric acid-A (GABA\_A) receptor blocker] iontophoretically to assess the role of GABAergic inhibition in unit’s temporal firing pattern and/or PLS. For this, the glass micropipette that was used for extracellular single-unit recording was piggybacked to a 5-barrel glass pipette with a tip diameter of about 10 μm (Havey and Caspary 1990); the tip of the former protruded about 20 μm beyond the tip of the latter. Two of the 5 barrels were filled with bicuculline methiodide (BIC, 10 mM, pH 3.0; Sigma), a GABA\_A receptor blocker. The central barrel was filled with 0.64% sodium chloride (pH 7.4) for maintaining electrical balance. The 5-barrel pipette was connected to a 6-channel iontophoresis current generator (Dagan 6400). A retaining current of ~15 nA (or ~20 nA) was used to prevent drug leakage at all times except during drug injection. To apply BIC, we used a current of +5 to +20 nA. A unit’s response was evaluated before, during, and after drug application.

**RESULTS**

Extracellular responses were obtained from 186 neurons in the IC of \textit{M. lucifugus} and 202 neurons in the IC of \textit{Rana pipiens}. Data from the bat IC will be described first,
followed by data from the frog IC. Results from a smaller sample in the bat IC have been described briefly in a short report (Galazyuk and Feng 2001).

In agreement with previous reports (Condon et al. 1994; Galazyuk et al. 2000), IC neurons in little brown bats had CFs ranging from 7 to 80 kHz. Thresholds at CF ranged from 2 to 68 dB SPL, with a modal value of 49 dB SPL. Units with low CFs were represented in the dorsal aspect of the IC and higher frequencies progressively more ventrally in the IC.

For the majority of IC neurons (132 units, or 71%), an increase in sound level elicited a monotonic increase in spike count and a concomitant decrease in the first spike latency. The increase in spike count and the decrease in latency shift were most pronounced at low sound levels, within 20 dB of the threshold at the unit’s CF; the spike count and the latency did not change appreciably with a further increase in sound level. Similar level-dependent latency changes have been described by others (Heil and Irvine 1997; Park and Pollak 1993).

A subset of the total population (40 units, or 22%) exhibited periodic discharge patterns at high sound levels. An increase in sound levels prolonged the firing duration of these neurons as a result of the addition of later spikes at a fixed period. A notable feature was the constancy of their firing periodicity. Responses of 2 representative neurons exhibiting periodic discharge patterns are shown in Fig. 1. Unit GGF6 in Fig. 1A fired on average 1–2 spikes at 10–20 dB SPL, and it consistently fired 4–5 spikes at regular firing intervals from 24 to 90 dB SPL. The firing periodicity of this neuron was level independent and remained constant at 1.9 ms at 24–90 dB SPL, as evidenced by the tight distribution of the interspike interval histogram (Fig. 1B). Unit GK12 in Fig. 1C fired one cycle at 10–30 dB SPL, and this grew to 2 firing cycles at 34–60 dB SPL. Further increase in sound level led to a progressive increase in the number of firing cycles, ending up with 4 at 80–90 dB SPL. Its firing periodicity remained the same (about 4.8 ms) over a wide range of sound levels (Fig. 1D). The firing periodicity was unit specific, ranging from 1.3 to 8.4 ms (Fig. 1E). Figure 1E shows that the firing periodicity was not evenly distributed in this range; the majority of 40 units had firing periodicities in the range from 1 to 3 ms.

For 26 neurons exhibiting periodic discharges, we evaluated whether the unit’s firing periodicity depended on sound duration ranging from 1 to 8 ms. We did not use longer sound durations for this test because the majority of IC neurons did not respond to tone bursts having longer sound durations. For 25 of 26 units, a change in sound duration did not change the unit’s firing periodicity. Although the unit’s firing periodicity was unchanged, the firing duration was in some cases (12 of 25) dependent on stimulus duration (Fig. 2C), and in other cases (13 of 25) independent of the stimulus duration (Fig. 2A). Unit GGY4 in Fig. 2A discharged one spike at low sound levels (<34 dB SPL) but gave 3 spikes at higher sound levels at a constant interspike interval (about 1.7 ms) for 1- to 8-ms tone pulses, as shown by interspike interval histograms at different sound durations (Fig. 2B). The above neuron was therefore duration independent, both in terms of its firing periodicity and duration. In contrast, for unit GGT9 (Fig. 2C), its firing periodicity also remained constant but its response duration increased progressively with an increase in stimulus duration (Fig. 2D).

Paradoxical latency shift (PLS)

For 52 of 186 IC neurons (28%), an increase in sound level elicited an increase in the first spike latency (i.e., these neurons exhibited PLS). A large majority of these neurons (48/52) exhibited latency shifts in quantum steps that were unit specific (Fig. 3, A and B). For example, unit AGO5 in Fig. 3A gave a single spike to tone pulses at 20–60 dB SPL with average latency of 13 ms; this response latency was shifted in a quantum step from 13 to about 18 ms when the sound level was increased to >60 dB SPL (Fig. 3C). For unit AGG3 in Fig. 3B, the average first spike latency changed from 8 to 9 ms at low sound levels to 12–13 ms at intermediate sound levels, to 16–17 ms at high sound levels (Fig. 3D). Sometimes the spike count was sparse in the zone of latency transition (Fig. 3A). Within each response range, the latency was relatively stable with variations of <6% from the average latency (see error bars in Fig. 3, C and D). The latency shift appeared to be attributable to the occurrence of early inhibition at high sound levels.
The distribution of shifts in the first spike latency of PLS neurons, in response to 2-ms tone pulses, is shown in Fig. 3E (range 0.9–10.2 ms). The majority of PLS neurons showed a shift of 4 to 7 ms, 12 units exhibited a latency shift of 0.9–3 ms, and 5 units had a shift of 7–10.2 ms.

Effect of BIC on PLS

The extensive overlap in the distributions of firing periodicities and of PLS indicated that the 2 classes of neurons might be functionally related (compare Figs. 1E and 3E). The PLS response pattern of unit AGG3 (Fig. 3B) additionally suggested that PLS might be attributed to high-threshold early inhibition of periodic discharges. To test this hypothesis directly, in 21 of the IC neurons exhibiting PLS, we studied each unit’s responses to tone pulses before, during, and after iontophoretic application of BIC, a GABA_A blocker. Drug application not only consistently increased the unit’s spike count as expected, but also abolished PLS. More important, administration of BIC transformed the unit’s firing pattern at high sound levels into periodic discharges (Fig. 4), indicating that the drug unmasked the unit’s periodic firing pattern. Before drug application, the first spike latency of unit GK10 (Fig. 4A, top panel) showed a shift from 12.6 to 19.5 ms in quantal steps of approximately 2.3 ms (see arrowheads in top panel) when the sound level was raised to >48 dB SPL. Similarly, before drug application unit GL26 (Fig. 4B, top panel) also showed a level-dependent shift of 5.3 ms in its first spike latency. Under BIC, these units responded to tone pulses over a broad range of sound levels with evidence of periodic discharges having multiple firing cycles (middle panels of Fig. 4, A and B). After recovery both neurons once again showed a distinct PLS (bottom panels of Fig. 4, A and B).

For the 21 PLS neurons tested, the average firing periodicity observed during drug application approximated the average quantal shift in first spike latency during the predrug period (Fig. 4C). These 2 values were tightly correlated (correlation coefficient = 0.93).

Effect of sound frequency on PLS

In our pilot study (Galazyuk and Feng 2001), based on a sample of 4 units, we reported that the magnitude of the PLS...
seemed to be independent of sound frequency. The more extended study on 17 units confirmed this finding. When sound frequency was varied within 10% of a unit’s CF, the amount of latency shift showed little or no change, as shown by a representative example in Fig. 5. This unit exhibited PLS in response to tone pulses at the unit’s CF, that is, 48 kHz (middle panel of Fig. 5A). PLS was also evidenced at 49 and 47 kHz, and the magnitude of latency shift was unchanged. When the sound frequency was further shifted to higher or lower frequencies (to 50 and 46 kHz), PLS was still evidenced in spite of the fact that the spike count was much weaker. Importantly, the magnitude of latency shift was stable for these sound frequencies, as shown by the dashed lines across the composite poststimulus time (PST) histograms in Fig. 5B.

Effect of sound duration on PLS

For 33 neurons exhibiting PLS, we studied the effects of stimulus duration on the PLS pattern. Sound duration affected the unit’s response in one of 2 ways. For 19 of 33 units (58%), the PLS was duration tolerant. Increasing sound duration did not appreciably alter the magnitude of the PLS, although it often suppressed the unit’s responses to tone pulses at high sound levels, and to a lesser extent the unit’s responses at low sound levels (Fig. 6A). Unit GGN5 in Fig. 6A exhibited a distinct PLS, with a shift of 5.1 ms in response to 1-ms tone pulses. When the sound duration was increased to 2 or 4 ms, the PLS remained distinct and the amount of latency shift remained 5.1 ms. For 8-ms tone pulses, this neuron stopped responding to sound above 66 dB SPL.

Latency plots for 19 neurons exhibiting duration-tolerant PLS are shown in Fig. 6B. For these neurons, the correlation between sound duration and amount of latency shift was very low (correlation coefficient $r = 0.24$), suggesting that stimulus duration had little effect on latency shift.

For the remaining 14 neurons (42%), the PLS was duration dependent, as exemplified by unit GGO1 in Fig. 6C. This neuron showed a stepwise 3-ms latency shift in response to 1-ms tone pulses. Increasing the sound duration to 2, 4, and 8 ms
The functional significance of PLS, we studied the response patterns of IC neurons to paired sound pulses ("pulse" and "echo") at the unit's CF, at interstimulus intervals of ±10 ms (in 1-ms steps). Of the 26 neurons tested, 19 were PLS neurons and 7 were control neurons. Faure et al. (2003) recently used a similar stimulus paradigm and analytical method to successfully elucidate the timing of synaptic inhibition underlying duration tuning.

Because a unit’s response to high-level sound (the pulse) was often complex, characterized by early inhibition followed by excitation, whereas a unit’s response to low-level sound (the echo) was strictly excitatory, we used a unit’s response to the echo to determine the timing of pulse-induced inhibition. A unit’s responses to the echo (in terms of spike counts) were measured for different pulse–echo intervals, and the unit’s responses to the pulse were ignored. Spike count to the echo was measured over a time window during which this response was >50% above the unit’s spontaneous rate.

We observed that the unit’s response to the echo was consistently suppressed when the echo coincided (or with slight lead) and immediately followed the pulse. In response to 2-ms tone pulses, the unit in Fig. 7A showed a PLS of 4.9 ms; the unit’s response to the echo (tone pulse at 40 dB SPL) had a latency of 7.5 ms but to the pulse (tone pulse at 80 dB SPL) the latency was 12.4 ms (Fig. 7A). In response to pulse–echo pairs (i.e., tone pulses at 40 and 70 dB SPL, respectively), its response to echo was uninhibited when the echo led the pulse by ≤0.8 ms (Fig. 7D). When the echo delay was 0.2 ms, the unit’s response to echo was completely inhibited. When the echo delay was >2.5 ms, there was a partial release from inhibition, and even stronger recovery thereafter (Fig. 7G). The above result revealed that this unit’s response to pulse at 70 dB SPL was preceded by a leading inhibition. The paired-pulse data thus corroborated the BIC experiments in revealing the existence of high-threshold leading inhibition for PLS neurons.

The latency and the duration of the inhibition could be estimated. In response to pulse–echo pairs we first observed response suppression when the echo led the pulse by ≥0.8 ms. This means that inhibition occurred 6.7 ms after the onset of pulse (response latency to echo 7.5 ms minus 0.8 ms, the interpulse interval when we first observed suppression). This suppression lasted 3.4 ms, as indicated by dashed vertical lines in Fig. 7G.

For 7 control neurons showing a short (Fig. 7B) or long (Fig. 7C) burst of spikes to sound pulses at the unit’s CF throughout 10–90 dB SPL without evidence of PLS, we also studied their responses to paired-tone pulses. Because their responses to CF at 40 and 70 dB SPL (or 50 and 80 dB SPL) were similar, both pulse and echo were presented at the same intensity (70 dB SPL). We found that a subgroup of control neurons (n = 4) displayed postexcitatory inhibition, which was evidenced only from their paired-pulse responses, as exemplified by unit AGN2 in Fig. 7E. This unit showed scattered responses to single-tone pulses <34 dB SPL, but for tone pulses at 34 to 90 dB SPL it gave periodic discharges over a duration of about 3 ms that was level independent (Fig. 7B). In response to paired-tone pulses, when the echo delay was ≥3 ms, its echo response was severely inhibited (Fig. 7E), suggesting the existence of postexcitatory inhibition. Postexcitatory inhibition occurred 2.3 ms after response onset as indicated by the vertical dashed line in Fig. 7H.

Responses of IC neurons to paired sound pulses with various interpulse intervals

The BIC experiments (Fig. 3) revealed that high-threshold early inhibition is critical for creation of the PLS. To determine the latency and duration of this inhibition and to evaluate the functional significance of PLS, we studied the response patterns of IC neurons to paired sound pulses with various interpulse intervals.

FIG. 5. Effects of stimulus frequency on PLS for a representative IC neuron (unit GGO1). Shown are composite dot raster histograms (A) and poststimulus time (PST) histograms (B) in response to tone pulses at the unit’s CF (48 kHz) and neighboring frequencies at sound levels from 10 to 90 dB SPL. See legend of Fig. 1 for explanation of composite dot histograms. Vertical dashed lines in B show that the magnitude of latency shift is more or less independent of the tone frequency. Sound stimuli are shown by black horizontal bars below each of the dot raster histograms (same timescale as dot raster histograms).

ms increased the latency shift to 3.9, 6.1, and 12.4 ms, respectively. The latency plots for 14 neurons (Fig. 6D) showed that the change in PLS was closely correlated with the change in sound duration (correlation coefficient r = 0.98); the magnitude of latency shift was a monotonically increasing function of stimulus duration.

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Three of the control neurons showed progressively longer periodic discharges with an increase in the sound level (Fig. 7C). In response to paired-tone pulses at 70 dB SPL, this neuron gave a more or less constant spike count, independent of the interpulse interval (Fig. 7F). Such IC neurons showed no evidence of inhibition in their responses to single- or paired-tone pulses (Fig. 7F).

Frog inferior colliculus

Recordings in the frog IC were made primarily from the magnocellular nucleus and the lateral region of the principal nucleus (Endepols et al. 2000; Feng and Lin 1991). Unit CF ranged from 200 to 1700 Hz and distributed around 3 clusters: 300–700 Hz (low-frequency), 900–1,300 Hz (mid-frequency), and 1,400–1,600 Hz (high-frequency). Unit thresholds at CF ranged from 21 to 47 dB SPL.

The majority of frog IC neurons (174 of 202 neurons, or 86%) showed a systematic decrease in the first spike latency when sound level was increased. Of these, a subset of neurons (n = 30; 15%) showed overt periodical discharge patterns at high sound levels, as exemplified by the composite dot histogram of the unit shown in Fig. 10C. Similar to the corresponding units in the bat IC, the unit’s firing periodicity, ranging from 5.8 to 60 ms, was largely independent of the absolute sound level.

Interestingly, 28 IC neurons (14%) exhibited PLS (Figs. 8A, 9A, 9B, and 10A). For all but 2 of these PLS neurons, the first spike latency shifted in quantum step(s), as exemplified by the units shown in Figs. 8A, 9A, 9B, and 10A; 2 PLS neurons showed a gradual increase in the response latency with sound intensity (not shown). For IC unit in Fig. 8A, in response to 20-ms sound pulses at the unit’s CF, it fired a burst of spikes over the range of sound levels tested. The first spike latency changed from about 39 ms at 20 dB SPL to about 55 ms at 82 dB SPL in quantal steps of about 7 ms (shown by open arrows on the top panel).

There was a notable difference in the magnitude of latency shift between the bat and frog IC. In bats, the latency shift ranged from 1.2 to 10.1 ms (mean = 5.6 ms), whereas the range was 6 to 76 ms (mean = 48 ms) in the frog IC.

Similar to bats, PLS in the frog IC was attributed to high-threshold leading inhibition. We evaluated the PLS before, during, and after application of BIC in 5 PLS neurons. Drug
application increased the unit’s spike count and abolished the PLS that was evidenced during the predrug condition (Fig. 8B). The unit’s PLS fully recovered after the drug application was withdrawn (Fig. 8C). However, in contrast to the bat IC, the PLS in the frog IC was seldom completely abolished with local application of BIC, even with application of a stronger iontophoretic current (20–30 nA).

PLS in the frog IC was also unit specific and reliably reproducible with repeated measurements of a unit’s response over time (compare Fig. 8A and 8C). A change in sound frequency also had little effect on the magnitude of shift in the unit’s first spike latency (not shown). When the tone frequency was moved away from the unit’s CF by ≲10%, PLS neurons showed the same PLS pattern and amount of latency shift as they did in response to tone bursts at the unit’s CF.

Similar to the bat IC, a change in stimulus duration had 2 different consequences. For 11 of PLS neurons (39%), the magnitude of the latency shift was duration independent. For example, unit 7/16–7 in Fig. 9A showed a distinct PLS in response to 10-ms tone bursts (top panel); its phasic response at lower sound levels was delayed by about 46 ms when the sound level reached 80 dB SPL. For 20- and 40-ms tone pulses (2nd and 3rd panels in Fig. 9A), the unit’s PLS remained unchanged, and there was a latency shift of about 48 ms when the sound level reached >84 dB SPL. At 80 ms, the unit’s response to tone pulses at >82 dB SPL was completely abolished and so was the unit’s PLS (bottom panel, Fig. 9A).

Seventeen PLS neurons (61%) in the frog IC showed duration-dependent PLS. As shown for unit W751 in Fig. 9B, increasing the stimulus duration from 10 to 20, 40, and 80 ms increased the latency shift progressively from 25 to 50, 81, and 98 ms, respectively. The magnitude of the latency shift was roughly proportional to the change in stimulus duration.

For 33 IC neurons (25 PLS neurons and 8 non-PLS neurons), we studied their responses to paired-tone pulses at the unit’s CF with interpulse intervals of from 0 to ±100 ms (in 10-ms steps). We found that PLS neurons and non-PLS neurons behaved similarly to the corresponding neurons in the bat IC (see Fig. 7 for comparison). The PLS neuron in Fig. 10A was stimulated with a pair of sound pulses (pulse at 70 dB SPL; echo at 40 dB SPL). This neuron showed inhibitory interactions, suggesting the presence of leading inhibition (Fig. 10D); it exhibited response suppression to echo when the pulse–echo interval was −20 to 45 ms (Fig. 10G). Thus with the unit’s response latency to echo of 32 ms (Fig. 10A), using simple calculations (32 − 20 = 12; 20 + 45 = 65), we deduced that inhibition must have begun 20 ms after the pulse onset and lasted 65 ms.

Non-PLS neurons in the frog IC similarly showed 2 different response patterns (Fig. 10, B, E, H and Fig. 10, C, F, I), as did non-PLS neurons in the bat IC. For 3 of 8 non-PLS neurons, there was evidence of postexcitatory inhibition, as exemplified by the unit in Fig. 10B. This unit showed tonic discharges in response to 20-ms tone bursts (Fig. 10B). Unlike the PLS unit
in Fig. 10A, this unit’s discharge terminated rather abruptly, with little or no delay response after the initial burst (Fig. 10B). In response to paired-tone pulses at 70 dB SPL, there was a marked response suppression to echo for pulse–echo interval of 20–100 ms (Fig. 10, E and H). As before, because the unit’s response latency to echo had a latency 26 ms, we estimated that this unit’s postexcitatory inhibitory period must have begun 13 ms after the response onset, as indicated by the vertical dashed line on the bottom panel of Fig. 10B.

For 5 of 8 non-PLS neurons, there was no evidence of inhibitory interaction over the entire range of interpulse intervals, as exemplified by the paired-pulse responses of a representative unit in Fig. 10C. This unit gave tonic and periodic discharges in response to tone pulses presented at all sound levels, followed by a delayed nonperiodic firing (Fig. 10C). In response to paired pulses at 70 dB SPL, there was no sign of inhibition across all interpulse intervals (Fig. 10, F and I).

DISCUSSION

Oscillatory discharges

A sizable number of IC neurons in frogs (n = 30; 15%) and bats (n = 40; 22%) show evidence of oscillatory discharges in response to tone pulses at the unit’s CF at high sound levels. The firing periodicity is essentially constant, unit specific, and independent of stimulus intensity, frequency, or duration (Figs. 1, 2, and 10C). Whereas the firing periodicity is invariable with a change in stimulus duration, a unit’s firing duration can be either independent of (Fig. 2B) or dependent on (Fig. 2D) the stimulus duration. In all cases, the duration of periodic firing is prolonged with an elevation of sound level (Figs. 1 and 2). Together, these results suggest that periodical firing likely

FIG. 8. Effects of BIC on a PLS neuron in the frog IC. Shown are unit’s composite dot raster histograms before (A), during (B), and after (C) iontophoretic application of BIC (30 nA). To construct the dot histogram, tone pulses (duration = 20 ms) at unit’s CF were presented at different sound levels, from 10 to 90 dB SPL. For each sound level, 10 tone pulses were presented. See legend of Fig. 4 for other details. Open arrows in A represent the times of first spike latency at different sound levels. Sound stimuli are shown by black horizontal bars below each of the dot raster histograms (same timescale as dot raster histograms).

FIG. 9. A: example from a representative PLS neuron (unit 7/16–7) showing that stimulus duration has little or no influence over the PLS of 46 ms at around 80 dB SPL. Shown are composite dot raster histograms in response to tone pulses at the unit’s CF having different durations (10 ms [top panel] to 80 ms [bottom panel]). Increase in sound duration from 10 ms (top panel) to 80 ms (bottom panel) reduced the spike count to sounds at high levels (>80 dB SPL) but did not change the amount of PLS. B: example from a representative PLS neuron (unit W751) showing duration-dependent PLS. This unit showed a latency shift of 25 ms in response to 10-ms tone pulses. When the sound duration was increased to 20, 40, and 80 ms this unit increased the latency shift to 50, 81, and 98 ms, respectively.
arises from an endogenous origin (e.g., intrinsic oscillation) and that such oscillation is damped over time.

Additionally, pharmacological results from 21 PLS neurons in the bat IC and 5 PLS neurons in the frog IC show that the periodic discharge at high sound levels is often masked and evidenced only when GABAergic inhibition is suppressed, i.e., when BIC is administered (Figs. 4 and 8). As before, after the oscillatory discharge is unmasked, its periodicity is independent of sound intensity and its firing duration is longer at higher sound levels—these again suggest that its origin is endogenous and the intrinsic oscillation is damped. In patch-clamp recordings from the IC of the big brown bat, Covey et al. (1996) observed damped oscillatory potentials in neurons having different CFs, across a wide range of stimulus amplitudes; membrane oscillations occur after sound offset, as observed in the present study. The rate of oscillations reported by these authors is lower than what we observed in the little brown bat but longer than that in the leopard frog, however.

The maximum firing duration of bat IC neurons exhibiting periodic discharge patterns could be as long as 26 ms, which was an order of magnitude longer than the stimulus duration of 2 ms. Therefore these neurons differ from that of chopper cells in the brain stem auditory nuclei in a fundamental way (Rhode and Greenberg 1992); the chopper firing pattern in the cochlear nucleus is usually observed within the period of stimulation. However, it is noted that studies of chopper cells typically used long tone bursts at low suprathreshold levels.

Level-dependent latency change and occurrence of PLS

The response latency of sensory neurons generally shortens with an increase in stimulus intensity, a phenomenon commonly known as “level-dependent latency change.” Because many auditory functions critically depend on precise timing of neural inputs (reviewed by Covey and Casseday 1999; Oertel 1999), one would expect that level-dependent latency change would have an especially significant impact on auditory processing. In the auditory system, level-dependent latency change has been observed at every level investigated, including the cochlea (guinea pig: Deatherage et al. 1959), cochlear nucleus (cat: Goldberg and Brownell 1973; Kitzes et al. 1978; rat: Miller 1975), superior olivary complex (gerbil: Sanes and Rubel 1988), lateral lemniscus (cat: Aitkin et al. 1970; bat: Haplea et al. 1994), inferior colliculus (cat: Hind et al. 1963; Irvine and Gago 1990; bat: Bodenhamer and Pollak 1981; Haplea et al. 1994), medial geniculate body (cat: Aitkin and Dunlop 1968), and auditory cortex (cat: Brugge et al. 1969).

The mechanisms underlying level-dependent latency change in the auditory system are not completely understood. The extensive studies by Heil and Irvine (Heil 1997a,b; 1998; Heil and Irvine 1996, 1997, 1998) show that the average level-dependent latency shift is essentially the same at the auditory
nerve and at the auditory cortex, thus suggesting that the effect is established at the periphery. These studies also show that the first spike latency depends more on the maximum acceleration of peak pressure during the rise time than on the steady-state peak pressure per se (Heil 1997a; Heil and Irvine 1997).

PLS, as observed in the present and previous studies, is characterized by a level-dependent increase (instead of a decrease) in response latency. In contrast to the common level-dependent latency change, PLS appears to have a central origin (see following text on mechanisms of PLS). A series of studies revealed that PLS is more prevalent and overt at the level of the midbrain and above, especially in bats (Aitkin et al. 1970; Berkowitz and Suga 1989; Covey 1993; Klug et al. 2000; Rose et al. 1959; Suga 1970; Sullivan 1982b).

Among vertebrates other than bats, PLS neurons have been observed in the auditory system of cats (Rose et al. 1963) and gerbils (Klug et al. 2000). The finding that some frog IC neurons also exhibited PLS suggests that the occurrence of this response characteristic is not limited to mammals or to animals that echolocate. Because frogs and mammals evolve along separate lines of evolution, this feature must have evolved independently.

**Neural mechanisms underlying PLS**

On the basis of a preliminary study, we previously hypothesized a possible neural mechanism for construction of PLS (Galazyuk and Feng 2001). In this hypothesis, we assume that IC neurons showing unit-specific oscillatory discharges are the basic building blocks for PLS. When a unit’s oscillatory discharge is subjected to high-threshold leading inhibition, the inhibition suppresses the early response components of a unit’s oscillatory discharge at high sound levels, one at a time, thereby delaying the unit’s response in quantum steps; quantum PLS would have been difficult to create without oscillatory discharge as a building block. The results from a more extended study herein support this hypothesis as one of the mechanisms for creation of PLS, i.e., duration-independent PLS (see following text). We found that about one-half of PLS neurons in the bat and frog IC exhibit duration-independent PLS (Figs. 6 and 9A) for which a change in sound duration does not change the unit’s PLS. Because application of BIC abolishes PLS and unmasksthe unit’s oscillatory discharge of these neurons (Figs. 4 and 8), we conclude that the underlying mechanism for duration-independent PLS is high-threshold inhibition of the unit’s oscillatory discharge.

The other half of PLS neurons, however, exhibit duration-dependent PLS. These neurons exhibit a greater shift in the first spike latency with an increase in sound duration (Figs. 6 and 9B). We believe duration-dependent PLS must be attributed to a different neuronal mechanism. One possible mechanism involves simple switching of the unit’s firing pattern (e.g., responding to stimulus onset at low sound levels) and to stimulus offset at high levels, and as such, an increase in stimulus duration would produce a delayed offset response. A response switch at low versus high sound levels has been observed in intracellular recordings from the cat auditory cortex (Volkov and Galazyuk 1991) and extracellular recordings from the guinea pig auditory thalamus (He 2002). However, whether the response switch is responsible for duration-dependent PLS in the IC requires direct empirical validation.

An alternative mechanism is having an inhibition whose duration lengthens with the stimulus.

**Interplay of excitation and inhibition in processing of time-varying information**

The paired-pulse experiments reported here allow us to quantify the time course of inhibition without involving drug application or intracellular recordings. Our results show that the majority of IC neurons in both groups of animals receive both excitatory and inhibitory inputs. The timing of these inputs can be used to generate various paired-pulse response functions. For example, when an IC neuron receives an excitation that is followed by inhibition a few milliseconds later, it produces a short-lasting firing activity (Figs. 7B and 10B). Such neurons show suppressed paired-pulse responses at long echo delays. In contrast, as described previously, high-threshold leading inhibition of periodic excitatory discharge can create PLS. PLS neurons exhibit suppressed paired-pulse responses at short echo delays (including short echo leads). For units that do not show evidence of inhibition, their paired-pulse responses are characterized by simple summation of responses to the individual pulses (Figs. 7C and 10C).

As noted above, temporal combination of excitatory and inhibitory inputs has also been previously shown to be responsible for sound-duration tuning in the IC of big brown bats (Casseday et al. 1994). Interestingly, during whole cell patch-clamp recordings (Covey et al. 1996), other combinations of excitatory and inhibitory effects have been observed although their functional significances are not clarified. For example, sound-evoked excitatory postsynaptic currents are often preceded, or followed, by an inhibitory postsynaptic current, or both. Our present studies suggest that such combinations are useful for construction of PLS as well as non-PLS with short-burst firing pattern, which in turn produce distinct suppression patterns in paired-pulse responses.

**Inferior colliculus versus auditory cortex**

In the auditory cortex of the little brown bat, Sullivan (1982b) and later Berkowitz and Suga (1989) reported that one-half of PLS neurons in the cortex show delay-tuned responses and that the magnitude of a unit’s PLS is correlated with the unit’s best delay. Surprisingly, although it was not a major aim of our research, in response to paired-tone pulses, PLS neurons in the bat IC do not exhibit delay-dependent facilitation. Instead, PLS neurons in the IC exhibit paired-pulse inhibition almost exclusively (Figs. 7A and 10A). There are several plausible explanations for the disparity in experimental results between the IC and auditory cortex. First, there are some differences in the experimental paradigms between the various studies. Paired-pulse stimuli in our experiments involved pure-tone pulses presented at the unit’s CF, whereas the earlier studies used frequency-modulated (FM) sweeps from 80 to 20 kHz. FM sounds include both units’ CF and a wide spectrum of non-CF frequencies. It is possible that side band inhibition (that has been shown to be evoked by non-CF tones) may be influential in shaping a unit’s pulse–echo response pattern. Future research that compares pulse–echo response patterns of PLS neurons to CF and FM sounds might clarify this point. Second, paired-pulse processing in the IC can be
basically different from that in the auditory cortex. It is possible that PLS is first created in the IC to generate delay-dependent inhibition—this can in theory be used to create delay-dependent facilitation at the levels of the auditory thalamus and cortex, e.g., by means of disinhibition. In the mustached bat, Portfors and Wenstrup (2001) showed that the organization of delay-tuned neurons in the IC is different from that in the auditory cortex. Delay-tuned neurons are topographically organized in the auditory cortex but not in the IC. These authors suggested that the creation and organization of delay-tuned neurons occur at different stages in the ascending auditory system.

**Behavioral significance of PLS**

In echolocating bats, the existing evidence suggests that PLS neurons are important for target ranging. Behaviorally, ranging has been shown to involve analysis of the elapsed time between the bat’s ultrasonic emission and its echo, or *echo delay* (Simmons 1973). PLS neurons showing delay-dependent facilitation (or delay-tuned response), i.e., showing maximal response to a best delay, are therefore well suited for determining target range (Feng et al. 1978). Indeed, as described above, one-half of PLS neurons in the auditory cortex give delay-tuned responses with the unit’s best delay matching the magnitude of a unit’s PLS (Berkowitz and Suga 1989; Sullivan 1982b). To evaluate the role of PLS neurons behaviorally, Denzinger and Schnitzler (1994) studied the ranging performance of big brown bats at different relative sound levels of echoes with a fixed difference in target range. They reasoned that if PLS underlies ranging, the bat’s performance in target ranging would be compromised when the echo level approaches the sound level of bat’s emitted signal. This is exactly what these authors observed—this result is consistent with the involvement of PLS in target ranging, although other explanations cannot be ruled out.

That PLS occurs widely in vertebrates suggests that it must have general relevance for processing sounds. Indeed, PLS neurons in the IC of bats and frogs display delay-dependent inhibition in response to *pulse–echo* pairs. Such inhibition can be used to suppress weak echoes, allowing animals to extract sound with minimal interference. In bats, delay-dependent inhibition in the IC may be used to create delay-dependent facilitation in the cortex for the purpose of target ranging as well as processing of communication sounds (Esser et al. 1997). In contrast, delay-dependent inhibition may be the final product of signal processing in frogs and nonecholocating mammals such as gerbils and cats.

In the insect auditory system, Krahe et al. (2002) proposed that PLS neurons might be involved in transmitting the information on onset steepness, a sound attribute that is important for sexual recognition. The empirical evidence in support of this hypothesis, however, is lacking. Nonetheless, the result of this study suggests that the auditory system may use PLS for yet a different listening task. Clearly, further studies are necessary to determine the actual functional significance of PLS for the various nonecholocating animals.

**Functional significances of duration-dependent and duration-independent PLS in bats**

During echolocation when a bat approaches a target, it systematically adjusts many different parameters of its ultrasonic cries: it increases the emission rate and concomitantly decreases the duration and intensity of its ultrasonic pulses, most notably during the final approach to an insect prey (Griffin et al. 1960; Kalko 1995; Kalko and Schnitzler 1989; Schnitzler et al. 1987). In the auditory cortex of the little brown bats and mustache bats, there are 2 types of delay-sensitive neurons (Chittajallu et al. 1995; O’Neill and Suga 1979, 1982; Tanaka et al. 1992; Wong et al. 1992). The first, the more common type (so-called *delay-tuned neurons*), is sharply tuned to specific echo delays regardless of the repetition rate and duration of the stimuli. The second type (so-called *tracking neurons*) shows changing best delays according to the repetition rate and duration of sound pulses. With increasing pulse repetition rate or decreasing sound duration, these neurons shift their best delays to smaller values. It has been hypothesized that delay-tuned neurons presumably respond only to echoes when the target is at a particular target distance (corresponding to the best delay) and are thus activated over a limited period during a target pursuit, with different neurons activated during different periods. In contrast, “tracking neurons” would be activated during the entire period of target pursuit, regardless of the target distance (O’Neill and Suga 1979).

The present findings from the bat IC provide additional support for the above hypothesis. In response to sounds having different durations we found 2 different populations of PLS neurons. Over 1/2 of PLS neurons (58%) show duration-independent PLS and the remainder of PLS neurons (42%) exhibit a greater shift with an increase in sound duration. Neurons exhibiting duration-independent PLS are useful for constructing delay-tuned responses, neurons showing duration-dependent PLS can be used to construct tracking neurons.

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