Chronotopically Organized Target-Distance Map in the Auditory Cortex of the Short-Tailed Fruit Bat

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Hagemann C, Esser KH, Kössl M. Chronotopically organized target-distance map in the auditory cortex of the short-tailed fruit bat. J Neurophysiol 103: 322–333, 2010. First published November 11, 2009; doi:10.1152/jn.00595.2009. Topographic cortical representation of echo delay, the cue for target range, is an organizational feature implemented in the auditory cortices of certain bats dedicated to catching flying insects. Such cortical echo-delay maps provide a calibrated neural representation of object spatial distance. To assess general requirements for echo-delay computations, cortical delay sensitivity was examined in the short-tailed fruit bat Carollia perspicillata that uses frequency-modulated (FM) echolocation signals. Delay-tuned neurons with temporal specificity comparable to those of insectivorous bats are located within the high-frequency (HF) field of the auditory cortex. All recorded neurons in the HF field respond well to single pure-tone and FM-FM stimulus pairs. The neurons respond to identical FM harmonic components in echolocation pulse and delayed echo (e.g., FM2-FM2). Their characteristic delays (CDs) for low echo amplitudes range between 1 and 24 ms, which is comparable to other bat species. Maps of the topography of FM-FM neurons show that they are distributed across the entire HF area and organized along a rostrocaudal echo-delay axis representing object distance. Rostrocaudally located neurons tuned to delays of 2–8 ms are overrepresented (66% of CDs). Neurons with longer delays (≥10 ms) are located throughout the caudal half of the HF field. The delay-sensitive chronotopic area covers ~3.3 mm in rostrocaudal and ~3.7 mm in dorsoventral direction, which is comparable or slightly larger than the size of cortical delay-tuned areas in insectivorous constant frequency bats, the only other bat species for which cortical chronotopy has been demonstrated. This indicates that chronotopic cortical organization is not only used exclusively for precise insect localization in constant frequency bats but could also be of advantage for general orientation tasks.

INTRODUCTION

Insectivorous Microchiroptera navigate and locate small prey insects using echolocation. The most common type of echolocation signal is a short FM sweep used for both precise localization and spectral classification of targets. The cue for target-ranging is echo delay. This has been discovered in the inferior colliculus, auditory thalamus, and auditory cortex (AC) in the form of combination-sensitive neurons. They are strongly facilitated when an FM component of the echolocation pulse is presented together with a delayed FM echo (Feng et al. 1978; Mittmann and Wenstrup 1995; O’Neill and Suga 1979; Portfors and Wenstrup 1999; Suga 1984). The response properties of such neurons, which enable the bat to compute target distances, are created by temporal interaction of inhibitory and excitatory mechanisms (Nataraj and Wenstrup 2006; Sanchez et al. 2008). In constant frequency bats, the echolocation signals of which contain constant frequency components in addition to FM components, delay-tuned neurons were studied most extensively, and several areas were identified where echo delay is mapped chronotopically on the cortical surface. Neurons with short best delays are located further rostrally than neurons tuned to longer delays (Edamatsu and Suga 1993; O’Neill and Suga 1982; Schuller et al. 1991; Suga and Horikawa 1986; Suga and O’Neill 1979). To date, a chronotopic organization of delay-tuned neurons has been found neither in the inferior colliculus of the mustached bat (Portfors and Wenstrup 1999, 2001) nor in the AC of bats that only use FM echolocation signals (Dear et al. 1993; Wong and Chen 2004; Wong and Shannon 1988).

All FM and constant frequency bats, which so far have been investigated with respect to neuronal delay tuning, have in common that they are insectivorous and—when zooming in on their target—they use terminal buzzes, i.e., echolocation call sequences at very high repetition rates during the final phase of insect catching. The preference of their cortical FM-FM neurons for short best delays could be an adaptation to close-range information processing demands in the final phase of insect hunting.

Bats that do not catch flying insects might be expected to have different demands in terms of target-range processing while obtaining information about the distance of objects for orientation. Delay-tuned neurons have been found in the AC of the frugivorous bat Carollia perspicillata (Esser and Eiermann 2004). Carollia uses short multiharmonic FM echolocation signals with main energy at the 2nd and 3rd harmonic (Fig. 1). Its behavioral hearing threshold has two broad minima close to 25 and 70 kHz (Koay et al. 2003). The AC of C. perspicillata can be divided into six auditory fields (Fig. 2) (Esser and Eiermann 1999). Tonotopy is found in the primary AC (AI), the anterior auditory field (AAF), and the secondary AC (AII). The dorsal areas (dorsoposterior field, DP; high-frequency fields I and II, HFI and HFII) contain neurons responsive to pure tones ≥50 kHz, but a strict tonotopical organization is absent (Esser and Eiermann 1999).

The present study focuses on the topography of delay-sensitive neurons in the AC of Carollia to assess if different requirements for echolocation, dedicated insect detection versus general orientation, are reflected in response properties and the topographic arrangement of such neurons. We show that neurons in a large dorsal cortical area that is responsive to high-frequency pure-tones are facilitated by FM pulse echo combinations and are organized chronotopically comparable to...
monic range of 42.1–28.2 kHz. The frequency ranges of the higher harmonics are multiples of the 1st level (in relative units) is associated with the 2nd and 3rd harmonics of the call. The power spectrum shows that peak sound pressure harmonics of the call. The power spectrum (\( B \)) gives the 4 FM/HFII flat frequency response that varies less than 3 dB for 20–120 kHz. The measured gradients of neuronal characteristic frequencies allowed to define the recording area according to published cortical frequency maps of \( C. \) perspicillata (Esser and Eiermann 1999).

At the end of each recording session, a lethal dose of pentobarbital was injected. Subsequently, the animal was decapitated and the head fixed by immersion in 4% paraformaldehyde. The experiments comply with the Principles of Animal Care, publication 86–23, revised 1985, of the National Institutes of Health, with the Declaration of Helsinki and also with German federal regulations.

**Recording procedures and acoustic stimulation**

Carbon electrodes (Carbostar-1, Kation Scientific, 0.4–0.8 M\( \Omega \)) were used to record action potentials extracellularly from single units and multiunits (see following text) from the left AC. Electrode penetrations were made orthogonal to the cortical surface using a piezo manipulator (PM 10/1, Science Products GmbH, Hofheim, Germany). Multi-unit recordings were obtained at an intracortical depth of 210–490 \( \mu \)m (layers III and IV). Neuronal properties at a given recording site within this depth range were investigated in detail with the priority to obtain multiple penetration sites for mapping purposes. In the present study, no attempt was made to measure multiple recording sites along one penetration to obtain information about processing in cortical columns. The distance between neighboring penetrations was 150–200 \( \mu \)m in both rostrocaudal and dorsolateral directions.

The electrical signal from the recording electrode was amplified, band-pass filtered (0.3–5 kHz, TDT PCI, Tucker-Davis Technologies) and fed into a D/A converter of a data acquisition board (DAP 5200a board, Microstar Laboratories, Bellevue). Pure-tone stimuli (10-ms duration, 0.5-ms rise/fall time) and FM stimuli (2-ms duration, 0.5-ms rise/fall time) were generated by the DAP 5200a board at a sampling rate of 385 kHz and fed through attenuators (PA5, Tucker-Davis Technologies, Alachua, FL) and an amplifier (Stereo Power Amplifier RB 580, Rotel Electronics, Tokyo, Japan) into the calibrated loudspeaker (ScanSpeak Revelator R2904/7000, Avisoft Bioacoustics, Berlin, Germany). The loudspeaker was positioned contralateral in the right auditory hemi field of the bat (45° from the midline) at about 30 cm distance to the bat. The maximum speaker level decreased with increasing sound frequency by 21 dB in the range of 10–140 kHz.

The loudspeaker calibration curve was used to adjust the attenuators so that known pure-tone sound pressure levels (dB re 20 \( \mu \)Pa) were applied. For FM stimuli, the center frequency of the FM was...
used for sound pressure level adjustment according to the calibration curve. The level of the different frequencies within the presented FM stimuli varied according to the changes of calibration curve within the respective FM frequency range (FM\textsubscript{1}; 5 dB; FM\textsubscript{2}; 8 dB; FM\textsubscript{3}; 12 dB). In case of the FM\textsubscript{4} stimulus that extended up to 168 kHz, which was above the range of reliable calibration (140 kHz), the measured calibration curve variation of 10 dB up to 140 kHz is probably an underestimation. Harmonic distortions of the system were ≥55 dB below the used pure-tone stimulus levels. To obtain neuronal frequency response areas and calculate frequency-tuning curves (FTCs), pure tones of various frequencies and levels were randomly presented at a repetition period of 250 ms. Each frequency level combination was presented five times, and the resulting responses were averaged.

To establish echo-delay sensitivity of the units, the center frequency and range of downward FM sweeps were adjusted to mimic components of the pulse of the biosonar signal (FM\textsubscript{p}) und a delayed echo (FM\textsubscript{e}). The used frequency and bandwidth values were in accordance with echolocation call parameters recorded from bats of our colony and with published data (Sterbing 2002). The following FM stimuli with 2-ms duration were used: first: shown harmonic FM with a center frequency of 33 kHz (42.1–28.2 kHz), second harmonic FM with a center frequency of 66 kHz (84.2–56.4 kHz), third harmonic FM with a center frequency of 99 kHz (126.3–84.6 kHz), and fourth harmonic FM with a center frequency of 132 kHz (168.4–112.8 kHz). At a given level of the presented echolocation pulse (70–80 dB SPL), both the delay and the level of the echo FM were varied randomly to obtain delay-tuning curves (DTCs) that display response strength against echo delay and level. Each delay level combination was presented eight times. In a subpopulation of units, in addition to the downward-FM stimulus pairs, upward-FM stimulus pairs were used as stimuli to assess selectivity of the delay-tuned neurons to the direction of the FM stimuli.

Data analysis

For spike sorting of single-unit and neuronal cluster (multiunits, 2–3 units) responses, the first three principle components of all spike waveforms were calculated (Lewicki 1998) using routines designed by C. Abel (Abel and Kössl 2009). To separate the spikes originating from different neurons, an automatic clustering algorithm “Klustakwik” (Harris et al. 2000; http://klustakwik.sourceforge.net/) based on the expectation-maximization algorithm, was fed with the first three principle components of each spike and then appropriate clusters were selected manually (Harris et al. 2000).

For analysis of frequency response areas obtained with pure-tone stimuli of 10-ms duration, the neuronal activity was measured within a time window of 20–80 ms, starting before any conspicuous increase in neuronal activity assessed with poststimulus time histograms (PSTHs). From the neuronal responses to pure-tone frequency/level combinations, FTCs were calculated for a threshold criterion of 30% of maximum activity and used to obtain the minimum threshold (MT) and the frequency at MT (characteristic frequency, CF). In addition, the best frequency (BF), defined as the stimulus frequency that evoked maximum neuronal response at any tested level, was determined. Neuronal tuning curves with two separate pure-tone response areas that had respective MT values within 20 dB of each other were defined as double-peaked.

DTCs were calculated from the responses to the FM pulse echo combinations based on a criterion of 50% of maximum activity of the unit. The echo-delay at maximum response strength (best delay, BD) and at lowest echo level that produced a response of 50% of maximum activity (characteristic delay, CD) was determined. For analysis of DTCs, the neuronal activity was measured within a time window of 20–70 ms that was adjusted individually to cover all neuronal responses to the different call echo combinations. As a measure of temporal specificity of delay tuning, we determined the range of the DTC in milliseconds at 5 and 10 dB above the CD level and used the maximum of both values as bandwidth of delay tuning.

Construction of composite maps of delay tuning

During each experiment, the location of the recording sites was measured in relation to dominant cortical blood vessels. After the experiments, the skull with the fixed brain, and the still visible blood vessels were aligned with the help of a model (brain imprint casted by dental acrylic), the location of the recording sites remeasured, and the entire recording area drawn by using a drawing mirror (Wild Drawing tube M5A, 10-time magnified, Heerbrugg, Switzerland). A composite map of BDs and CDs was constructed from 27 aligned maps of individuals of \textit{C. perspicillata}.

RESULTS

Responses to pure tones

In 27 individuals of \textit{C. perspicillata}, a total of 212 sound-responsive neurons were recorded across the HF fields and, more ventrally located, in parts of the AI and/or AAF. With the exception of one unit that only responded to FM-FM stimulus pairs, all neurons were responsive to single pure-tone stimuli within a tested stimulus-level range of up to 80 dB SPL. The CFs of the units ranged from 50 to 100 kHz, and MTs from 10 to 71.4 dB SPL. In our data set, it was not possible to distinguish between a low- and high-threshold region (HFI and HFII). It may be necessary to obtain a large number of penetrations from a single animal to be able to define HFI and HFII (Esser and Eiermann 1999). In our study, the number of penetrations per animal was restricted because we focused on detailed measurement of delay-tuning properties at each recording site (see METHODS). Consequently, when referring to our own data in the following, we use the comprehensive term HF area. The distribution of the CFs shows a bias for frequencies within the range of 70–95 kHz with a maximum at 70 kHz (Fig. 3) and a mean of 80.9 ± 11.7 kHz (SD). This preferred CF-range covers the upper part of the 2nd harmonic (84.2–56.4 kHz) and the lower part of the 3rd (126.3–84.6 kHz) harmonic of the echolocation call. In addition to a high-frequency response area, most units also exhibited a secondary response area at frequencies below ~50 kHz (see examples in Fig. 4, A and C). In 97 (46%) of the 211 units with a pure-tone response, the threshold sensitivity of the secondary low-frequency response area was within 20 dB of the more

![Fig. 3. Histogram of the characteristic frequencies (CFs; n = 211) of the units from the HF areas of the AC of \textit{C. perspicillata}.](http://jn.physiology.org/DownloadedFrom)
sensitive high-frequency minimal threshold. Such FTCs were defined as double-peaked. The secondary CF of double-peaked neurons ranged from 10 to 65 kHz with a mean of 22.5 kHz. A harmonic ratio between the secondary low-frequency CF and the high-frequency CF could not be established.

**Delay-tuned neurons in the HF area of the AC**

The 212 recorded neurons responded preferentially to specific echo delays when stimulated with pairs of FM stimuli that mimicked echorlocation pulse and echo. In all cases, the delay-tuned responses could be evoked by using the same FM harmonic for both pulse and echo (e.g., FM₂-FM₂, FM₃-FM₃). In Fig. 5, A, C, and E, examples of responses of three units to FM-FM pairs at best echo-delay are shown (BDs of 6, 10, and 20 ms) and compared with the response at echo delays smaller and larger than BD. For the same units, the response to the pulse FM alone is displayed (Fig. 5, B, D, and F). The units are most strongly facilitated by the presentation of FM-FM pairs at BD compared with presentations of single FM sweeps or FM-FM stimulus pairs at other echo delays. To derive BD and CD, we chose levels of 70–80 dB SPL for the FM echorolocation pulse and then presented the echo at varying delays and levels. The resulting response matrix [examples in Fig. 4, B, D, and F, with PSTH responses (left) and a contour plot of total spike number (right)] shows a clearly defined response area (see METHODS). It was used to determine the delay-tuning curve (DTC; Fig. 4, B, D, and F, white dashed lines), BD and CD. For the level adjustment of both stimuli, the center frequency of the FM in relation to the calibration curve of the speaker was used as reference. This implies that the actual FM level could vary according to the calibration curve variation within the range of the used FM-frequencies (see METHODS).

The maximum response rates of delay-tuned neurons to FM-FM stimulation were compared with single pure-tone stimulation (10-ms duration). In 69% of the units, the maximum pure-tone response rate was higher than the maximum FM-FM

FIG. 4. Examples for delay-sensitive units in the HF area of the AC. Each row shows 1 unit. Contour plots of the pure-tone responses (A, C, and E) are compared with responses to 2 FM stimuli mimicking pulse and echo (B, D, and F). A, C, and E: from the neuronal activity in spikes/s (see gray-scaling of sidebar) measured within a specific time window (see METHODS), a frequency-tuning curve (FTC) was calculated using a criterion of 30% of maximal activity (white dashed line; cross gives CF). The FTCs are characterized by a sensitive high-frequency component at the frequency ranges of FM₂ or FM₃ (indicated by stippled lines) and a secondary low-frequency response area. B, D, and F: for each unit, the tested FM components (inset left) the poststimulus time histogram (PSTH) responses for different echo levels and delays (middle: x axis: echo delay; y axis: echo level) and the corresponding activity contour plots (right) are shown. Pulse level was 70 dB SPL. The x axis in each of the individual PSTH extends from 0 to 84 ms, the y axis from 0 to 8 spikes [binwidth: 1 ms (B), 1.5 ms (D), 2 ms (F)]. From the contour plots, a delay-tuning curve (DTC) is calculated using a threshold criterion of 50% of maximal activity [white dashed line; cross: characteristic delay (CD), star: best delay (BD)]. The CDs and BDs of the shown units are between 4 and 8 ms. Please note that the echo-delay response range (maximum bandwidth of DTC) is narrower in B and D in comparison with F. In each unit, the frequency range of the preference FM for delay tuning (either FM₂ or FM₃) extends into the FTC.
of the FM2-preference neurons, the FM2-frequency range overlaps with the FM1 range. In the 12 FM4-preference neurons, the pure-tone response areas were below the FM4 range. In summary, in 191 units (90.1%), a relationship between the maximum pure-tone response range and the preferred harmonic of the delay-tuned response has been observed (see examples in Fig. 4, A and C).

**Distribution of preferred delays and response bandwidth**

The CDs of the 212 delay-sensitive neurons ranged from 1 to 24 ms (Fig. 6) with a median of 6 ms. The echo levels at CD covered a wide range between 10 and 73.7 dB SPL with a mean of 45.1 ± 10.2 dB SPL. The BDs were measured between 1 and 30 ms with a median of 6 ms, and at BD, the echo levels ranged from 30 to 80 dB SPL with a mean of 59.1 ± 9.2 dB SPL, which on average was 17.8 dB below pulse level. Short CDs were overrepresented. To assess the delay preference of units the delay range was subdivided into delay intervals (0–4, 5–9, 10–14, 15–19, and ≥20 ms; Table 1). The largest subpopulation of units had CDs of 5–9 ms (94 units, 44.3%). When using BDs to describe delay-tuning characteristics, the distribution is similar to that of CDs.

In general, delays between 2 and 8 ms were clearly overrepresented both in regard to CD (140 units, 66.0%) and BD (145 units, 68.4%). The temporal bandwidth of a DTC was measured as the maximum delay-range within the particular curve (Fig. 7). The bandwidth values are rather variable at each CD. In the linear regression analysis, the bandwidth significantly increases with an increasing CD at a slope of 0.42 ms/ms.

**Selectivity to different FM harmonics and direction of FM modulation**

Many neurons (67.1%) responded to more than one FM harmonic pair. The FM component that produced the most sensitive DTC was defined as the preference FM (see preceding text). To quantify FM directional selectivity across the population of neurons, a neuron was defined as responsive to more than one FM harmonic if the sensitivity difference between the preference FM and an alternative harmonic (which is a multiple of this preferred harmonic) did not exceed 20 dB.

![Diagram](http://jn.physiology.org/ by 10.22033:33 on September 24, 2016)
In a total of 140 delay-sensitive units, all possible FM harmonics (1–4) were tested. In these tests, only stimulus pairs of equal FM harmonics were used (e.g., FM₂-FM₂), and the level of the first stimulus (i.e., call) was fixed at 70 dB SPL. The largest subpopulation of neurons (51 units, 36.4%) responded to both FM₂ and FM₃ pairs of stimuli (Fig. 8A). The example unit shown in Fig. 8C is most sensitive to FM₂, whereas its FM₃ response is less sensitive (~4.6 dB). The unit did not respond to FM₁ (not shown here) and FM₄ stimuli. Units responding exclusively to FM₂ constitute the second largest subpopulation (38 units, 27.1%; Fig. 8A). Sixteen units (11.4%) responded to FM₂, FM₃, and FM₄ combinations (example in Fig. 8D). Apparently, the majority of delay-sensitive neurons (67.1%) does respond to more than one FM component. Twenty units that were delay-tuned to homoharmonic combinations were additionally tested using hetero-harmonic stimuli pairs of different FM harmonic components (e.g., FM₁-FM₂). From this subpopulation, 14 units (70.0%) were delay-tuned during heteroharmonic stimulation.

In addition, selectivity of neuronal responses regarding the direction of the FM stimuli was assessed (Fig. 9). From 49 units, where both “natural” downward-FM stimuli (FM-down, FM) and upward-FM stimuli (FM-up, FM) were tested, 85.7% had a delay-tuned response to both FM directions (Fig. 9A). For the majority of these units (n = 27, 55.1%), FM-down and -up sensitivity was similar (with the echo level differences at CD for FM-up and -down not exceeding 10 dB). The examples in Fig. 8, B–E, shows DTCs of two units, tuned to short and long delays, respectively, that had comparable responses to both FM directions. In 13 units (26.5%), the responses to FM-up were even more sensitive than to stimulation with FM-down. Seven units (14.3%) were defined as not responsive to FM-up stimuli, i.e., the echo level for the FM-up CD exceeded the FM-down CD echo level by more than 20 dB. Based on these results, it can be concluded that FM-neurons in C. perspicillata do not have a significant preference for the direction of the FM modulation.

**Representation of echo-delay in AC**

In the following, we focus on echo-delay representation based on CD. We have calculated separate maps for the individual animals and aggregated these to obtain a composite map of echo-delay tuning. We did not attempt to derive maps based on maximal activity during stimulation with a fixed echo level because this could distort mapping topography due to different minimal thresholds of DTCs. We present two data sets on the topography of CD representation. In the first data set, each unit was extensively tested to derive detailed response properties, and hence the number of penetrations per animal was restricted (examples in Fig. 10A). From the pooled data of 27 animals, we constructed a composite map (Fig. 10, B and C). To reconfirm the results of this composite map, we separately mapped three additional animals using a reduced stimulation paradigm with only three averages per stimulus condition to obtain a higher number of penetrations covering larger parts of AC.

**Composite spatial map of echo delay in AC (data set 1)**

After alignment of the recording areas of the individual bats, using brain blood vessels and a model brain cast (see methods), a composite map of delay-tuning in the dorsal AC of C. perspicillata was constructed. The map included a total of 212 delay-sensitive neurons from 27 individual bats (Fig. 10, B and C; color-coded symbols) and an additional 14 neurons that were not delay-sensitive and had pure-tone CFs below 45 kHz (gray symbols).

To illustrate AC delay tuning and, in particular, the representation of CD along the rostrocaudal axis in exemplary animals, individual cortical maps of two animals out of the population of 27 used to derive the composite map are shown in Fig. 10A. In bat T53, 15 multiunits were mapped within a narrow rostrocaudal corridor of the AC. In this bat, the measured CDs increased from 4 to 14 ms. The recorded region can be clearly separated into two areas: neurons with CDs ≤6 ms were located in the rostral part, while neurons with CDs between 10 and 14 ms were located in the caudal part (Fig. 10A). In bat T56, 12 multiunits were recorded at slightly more ventral positions than in the previous animals. CDs ranged from 2 to 24 ms. The six neurons in the rostral part of the recording area had CDs up to 8 ms, whereas the six neurons located in the caudal part had CDs above 10 ms (Fig. 10A).

Using a dorsolateral viewpoint on the standardized brain of Carollia, approximately perpendicular to the surface of the HF area (comparable to cortical field reconstruction of Esser and Eiermann 1999), the delay-tuned area is caudal to a prominent blood vessel located within the depression of the pseudocentral...
Each polygon in the composite map represents one recording site and displays the corresponding CD based on a color code (Fig. 10C). Results for the two measures of delay tuning, BDs and CDs, varied only slightly, and overall proved to be very consistent (BD data not shown). The recorded area of \( \text{HF area} \) extends 3.28 mm in rostrocaudal and 3.77 mm in dorsoventral direction. It covers the HF area and ventrally extends into the AAF and AI mapped by Esser and Eiermann (1999: Fig. 2). Based on chronotopic characteristics, the dorsal AC of Carollia can be subdivided into parallel rostrocaudal zones. In the most rostral zone, units with short CD between 0 and 4 ms (red color) are dominant. More caudally, units tuned to echo delays between 5 and 9 ms (orange color) prevail. Neurons with longer delays between 10 and 19 ms are located throughout the caudal half of the recording area (green color: delays between 10 and 14 ms, blue color: delays between 15 and 19 ms). Neurons tuned to delays of more than 20 ms (magenta color) are located closer to the caudal border of the recording area than the units tuned to shorter delays.

A large posterior part of a tonotopically organized field, the AI and AAF (Fig. 2) (defined by Esser and Eiermann 1999) are located ventrally to the HF area. These areas were not systematically mapped for echo-delay tuning in this study. The CFs of 14 nondelay-tuned units recorded in this area were between 10.3 and 45 kHz. Five units that had CFs between 70 and 100 kHz were located either at the rostral border of the high-frequency part AI or the caudal AAF and all were delay-tuned to short echo-delays.

Cortical maps from individual bats covering large areas of AC (data set 2)

For verification of the composite map, from three animals we obtained 66–77 penetrations per animal using a reduced data-acquisition paradigm. These individual maps cover nearly the entire extension of AC (Fig. 10D, bats T113, T114, T115).

In bat T113 (total of 72 penetrations) throughout the entire AC, 40 delay-sensitive neurons were recorded in the HF area. For testing of the units, only homomorphic FM pairs were used (FM, FM, FM, FM, FM, and FM). A: distribution of response types. The harmonics a neuron responded to with delay-tuning are indicated by numbers below the x axis. As criterion for a “response” to a FM harmonic, the sensitivity of DTCs is used: a response is considered valid, if the minimum threshold of the 50% contour of the corresponding FM-delay-tuning curve is within 20 dB of the minimum at the preferred FM harmonic (lowest minimum threshold for all tested harmonics). The largest subpopulations of units respond either to FM alone (DTCs of an example unit shown in B), to FM and FM (example unit in C), or to FM, FM, and FM (example unit in D). For further explanation of B–D see Fig. 4. Please note that the CD and BD (indicated by cross and star, respectively) can slightly vary in dependence of the used FM harmonic.
with CDs increasing rostrocaudally from 2 to 28 ms. Twenty neurons were recorded in the low-frequency areas (DP, AI, and AAF) and were not delay-sensitive. Twelve units recorded in peripheral regions of AC were responsive neither to pure-tones nor to FM-FM stimuli. The HF area can be separated into three zones: neurons with short delays were delay-tuned with CDs increasing from 2 to 32 ms in the rostral part; further caudally adjacent to this region are neurons with CDs between 10 and 14 ms (n = 8); neurons with longer delays between 15 and 26 ms are located throughout the caudal half of the HF area.

In bat T114 (total of 66 penetrations), 41 recording sites were delay-sensitive. In the dorsal part of the recording area, 17 rostrally located neurons had CDs of ≤9 ms, whereas 14 caudally located neurons were tuned to CDs between 10 and 21 ms. In addition, 10 units tuned to short echo delays (1–5 ms) were located more rostroventrally, presumably at the border between AI and AAF, where high-frequency pure-tone responses are typical. At 12 sites located in the DP and the AI, the units were not tuned to echo delay but responded to low-frequency pure tones (CF < 50 kHz). In peripheral regions of AC, 13 units responded neither to FM-FM nor to pure-tone stimulation.

In bat T115 (total of 77 penetrations), 32 recording sites were delay-tuned with CDs increasing from 2 to 32 ms in rostrocaudal direction. The HF area can be clearly separated into three zones: CDs of short delays <8 ms (n = 14) are located rostrally, neurons with longer delays between 12 and 14 ms (n = 5, with 1 exception of 16 ms) are arranged further caudally. Twelve units with the longest CDs between 15 and 32 ms are located in the third most caudal section the HF area.

In addition, 29 neurons located in low frequency areas (DP and AI) were not delay sensitive, and 16 units were responsive neither to pure tones nor to FM-FM stimuli.

The topographic representation of delay tuning in these animals is consistent with the composite map (Fig. 10, B and C). However, differences between the three individual animals were observed. In T113 and T115, relatively long CDs >8 ms occupy a larger area than short CDs. In animal T114, short CDs are overrepresented, and the corresponding cortical zone extends further ventrally into the border region between AI and AAF, which is also evident in the composite data set (Fig. 10C).

**Relationship between CD and distances from rostral to caudal along the cortical surface**

To assess the chronotopic organization of the delay-tuned units, their CDs were plotted as a linear function of their location in rostrocaudal direction (Fig. 11). Such functions derived from individual animals (Fig. 11A: data set 2, B: 3 bats from data set 1) have slopes between 4.46 and 7.72 ms/mm, and the regression coefficient varies between 0.48 and 0.91. For data set 1 (lower number of penetrations per animal), the regression coefficients are slightly higher, and hence the data spread is slightly lower than for data set 2. This may reflect the fact that the recordings for data set 1 were concentrated in a narrow rostrocaudal zone (see Fig. 10A), whereas for data set 2 covered nearly the entire AC.

When assessing chronotopic organization across all 212 delay-tuned units in the 27 bats of data set 1, a significant rostrocaudal distance-dependent increase in CDs (Fig. 11C)
and BDs was determined based on a regression analysis (CDs: $n = 212$, $R^2 = 0.32$, slope $= 3.46$, $T = 10.0$, $P < 0.0001$; BDs: $n = 212$, $R^2 = 0.33$, slope $= 3.68$, $T = 10.19$, $P < 0.0001$).

There was no significant correlation between CD (and BD) and the dorsalventral direction (CD: $n = 212$, $R^2 = 0.002$, slope $= 0.23$, $T = 0.57$, $P = 0.5681$; BD: $n = 212$, $R^2 = 0.001$, slope $= 0.24$, $T = 0.56$, $P = 0.5903$). For the overall population (data set 1: Fig. 11C; $n = 27$), the slope was slightly smaller than for the six individual data sets (A and B). Both the difference in slope and the lower regression coefficient in the composite data set compared with the individual data sets may be due to inter-individual variability.

To quantify echo-delay-tuning characteristics of neurons along the rostrocaudal dimension, the rostrocaudal axis was divided into four sections of 1-mm length each (Fig. 11C). In the most rostral section (0–1 mm, 31 neurons), 51.6% of the units had CDs between 0 and 4 ms, and 41.9% had CDs between 5 and 9 ms. In the second, further caudally located section (1–2 mm, 93 neurons) 49.5% of the units were tuned to delays between 5 and 9 ms, while 32.3% were tuned to short delays (0–4 ms) and 18.3% were tuned to delays between 10 and 20 ms. In the third section (2–3 mm, 65 neurons), 43.1% of neurons were responsive to delays between 5 and 9 ms, 33.8% were tuned to delays between 9 and 14 ms. In the fourth, most caudal section (3–4 mm, 23 neurons), 39.1% of the units were tuned to CDs between 10 and 14 ms, 30.4% to delays between 5 and 9 ms and 30.4% to delays $>15$ ms.

**DISCUSSION**

**General properties of delay-tuning**

**CHRONOTOPY.** Cortical delay-tuning in the form of FM-FM combination-sensitive neurons is a surprisingly robust phenomenon in *C. perspicillata*. All neurons recorded within the dorsal HF area are delay-tuned (Fig. 4), and the delay-tuned area most likely reaches ventrally into the high-frequency domains of tonotopically organized AI and/or AAF (Fig. 10). From our data set, we were not able to resolve a low-threshold HFI and a high-threshold HFII field in *C. perspicillata* as it was possible in the study by Esser and Eiermann (1999). This difference may be due to the larger number of penetrations obtained in individual bats by Esser and Eiermann (1999). Consequently, we cannot attribute certain echo-delay ranges to HFI versus HFII. Within our mapping resolution and the variability between individuals, the cortical echo-delay map in *C. perspicillata* is continuous, and its area covers both HFI and
As in constant frequency bats, the delay-tuned area in C. perspicillata is chron topically organized with short delays represented rostrally. In comparison to data from Pteronotus parnaellii and Rhinolophus rouxi (Schuller et al. 1991; Suga and Horikawa, 1986; Suga and O’Neill, 1979), the chronotopy is more variable in the combined data set from all 27 bats. In the individual maps, regularity of delay-tuning is higher; however, there are still a number of units that slightly deviate from a strictly rostrocaudal chronotopic gradient. Insectivorous FM bats differ from Carollia not only in their absence of chronotopy, but delay-tuned neurons in these species are encountered more sparsely in the respective cortical areas (Dear et al. 1993). In the constant frequency bat Rhinolophus, delay-tuned neurons are also intermingled with nonfacilitated neurons (O’Neill 1995; Schuller et al. 1991). Irrespective of functional reasons, chronotopic cortical characteristics may also represent an evolutionary homologous feature between Pteronotus and Carollia because their respective families, Mormoopidae and Phyllostomidae, are closely related (Jones and Teeling, 2006).

DISTRIBUTION OF PREFERRED ECHO DELAYS. When using the CD at minimal threshold or the BD (delay at the echo level that evokes maximum neuronal response) to define preferred echo delay, a delay range of about 2–8 ms is overrepresented for both measures and the delay distributions peak at 4 ms (BD) or 6 ms (CD). Longest cortical neuronal delays are at 25 ms (CD) or 30 ms (BD; Fig. 6). There is no obvious bimodal distribution of preferred delays as it has been found in other FM bat species (Dear et al. 1993; Shannon-Hartman et al. 1992). In constant frequency bats, the distribution of CDs also is unimodal with preference of short delays (O’Neill and Suga 1982; Schuller et al. 1991). In C. perspicillata, peak CDs are comparable to those found in the FM-FM and dorsal fringe areas of Pteronotus (O’Neill and Suga 1982; Suga and Horikawa, 1986) (3–6 ms) and slightly higher than in Rhinolophus (Schuller et al. 1991) (2–4 ms). If the delay data are interpreted in terms of target range, this may imply that Carollia is adapted for short-range echolocation although it does not use specialized terminal buzzes (Thies et al. 1998).

RESPONSE SPECIFICITY. The fact that in Carollia, the delay-tuned neurons strongly respond to single pure tones within the frequency-range of the species’ biosonar signal makes them clearly less stimulus-specific than in Pteronotus where pure-tone responses are absent or weak (Suga et al. 1983). In this respect, Carollia resembles insectivorous FM bats where a substantial portion of the cells also seems to respond well to pure tones (Dear et al. 1993; Wong and Shannon, 1988).

A large subpopulation (85.7%) of FM-FM neurons in Carollia responds equally well at similar thresholds when stimulated with paired upward-FMs (Fig. 9). In contrast, selectivity for FM direction is higher in the FM-FM and dorsal fringe region of Pteronotus, although, to obtain a delay-tuned response, here it often is sufficient when one of the two FMs of a pair is in the “natural” downward direction (Suga et al. 1983; Taniguchi et al. 1986).

Comparable to insectivorous FM bats and different from the constant frequency bats, Pteronotus and Rhinolophus (review: O’Neill, 1995), facilitation in Carollia is homoharmonic and delay-tuned neurons respond best to FM pairs of the same harmonic order. Most neurons, however, can be driven by
more than one FM harmonic, usually the 2nd or 3rd harmonic are best suited, which correlates with the strength of the harmonics in the echolocation calls (Figs. 1 and 7). In a subset of neurons tested for hetero-harmonic facilitation, 70.0% can be stimulated with different harmonics in a pair (e.g., FM\textsubscript{1}-FM\textsubscript{2}). The neurons are responsive to hetero-harmonic stimulation as long as the respective harmonics can be also used as effective stimuli in homo-harmonic stimulation.

Both homophase facilitation for more than one FM harmonics and heterophase facilitation require a convergence of different frequency channels. This could already be established on the level of the midbrain, if in Carollia, similar to Pteronotus combination-sensitive neurons are found in the inferior colliculus (e.g., Portfors and Wenstrup 1999). The amount of frequency convergence required to account for combination sensitive neurons in the cortex, surely is larger in Pteronotus because in this species a low-frequency FM\textsubscript{1} is always essential for facilitation and the relevant combinations cover FM\textsubscript{1}-FM\textsubscript{2-4}. The fact that in Pteronotus homophase facilitation is absent (see O’Neill 1995) could, e.g., indicate a much stronger or longer lasting inhibition following stimulation with higher FM frequencies used as pulse. To compare the relevant mechanisms, a detailed study on the interplay of inhibitory versus facilitatory interaction in the different species is required.

**BANDWIDTH OF DELAY-TUNING.** The temporal bandwidth of delay-tuned neurons increases with CD at an average slope of 0.42 ms/ms (Fig. 7). The bandwidth increases stronger at short CDs than at high CDs, which is similar to the situation in the insectivorous FM bat Myotis lucifugus (Sullivan 1982). In the FM-FM and dorsal fringe area of Pteronotus, bandwidths increase at a larger average slope (Suga and Horikawa 1986) (slope close to 1).

**Functional implications**

The computational advantage of chronotopic echo-delay maps can be interpreted in terms of providing the substrate for integration of different echo parameters as they are important, for instance, in tracking an object and in enhancing acuity of target-range calculation, in particular for small targets in highly cluttered habitats (Altes 1989). In Carollia, it is unclear, if this species requires high precision and clutter-resistant target-range calculation for navigation or location of food. After a primarily odor-oriented detection of ripe fruit, when approaching the fruit target, Carollia changes echolocation call parameters and shortens the duration of the call while slightly increasing the pulse repetition rate (Thies et al. 1998) However, Carollia lacks a distinct terminal phase such as is found in aerial insectivores where the bats emit very short signals at high repetition rates of 150–200 Hz (see Thies et al. 1998). Such final buzzes, which are typical for sophisticated close-range echolocation in the terminal phase of catching flying insects, are not reported for Carollia. The diet of Carollia predominantly consists of fruit, nectar, pollen, and flower parts (Fleming 1988; Nowak 1994) but can also contain arthropods (Fleming 1991; Whitaker and Findley 1980; Willig et al. 1993). It is unclear if these are taken up coincidentally with fruit or are actively pursued by the bat.

The delay-tuned neurons in the dorsal AC of Carollia also could be employed for tasks different from echolocation. In Pteronotus, it has been shown that the same FM-FM neurons that are facilitated by pulse echo pairs also respond vigorously to species-specific communication calls and could be vital for recognition of the temporal sequence of different communication syllables (Esser et al. 1997; Ohlemiller et al. 1996; communication calls see Kanwal et al. 1994). Comparable dual function neurons also have been reported for the inferior colliculus of Pteronotus (Holmstrom et al. 2007). Carollia lives in harems with complex social interaction, where highly developed neuronal computation of communication calls should be of advantage. This, of course, per se, would not explain the necessity of chronotopy. It remains to be studied if the chronotopic cortex enables temporal grouping of communication call sequences. The fact that the delay-tuned neurons in Carollia are not specific in terms of FM direction selectivity and are strongly driven by single pure tones could imply that they generally would respond to communication calls. However, detailed data on communication calls and their behavioral context in Carollia are presently not available and would be required to study respective cortical processing mechanisms in the HF area.

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**REFERENCES**


