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Research paper

Properties of echo delay-tuning receptive fields in the inferior colliculus of the mustached bat

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ABSTRACT

One role of the inferior colliculus (IC) in bats is to create neuronal delay-tuning, which is used for the estimation of target distance in the echolocating bat's auditory system. In this study, we describe response properties of IC delay-tuned neurons of the mustached bat (*Pteronotus parnellii*) and compare it with those of delay-tuned neurons of the auditory cortex (AC). We also address the question if frequency content of the stimulus (pure-tone (PT) or frequency-modulated (FM) pairs stimulation) affects combination-sensitive interaction in the same neuron. Sharpness and sensitivity of delay-tuned neurons in the IC are similar to those described in the AC. However, in contrast to cortical responses, in collicular neurons the delay at which the neurons show the maximum response does not change with changes in echo level. This tolerance to changes in the echo level seems to be a property of collicular delay-tuned neurons, which is modified along the ascending auditory pathway. In the IC we found neurons that showed a facilitated delay-tuned response when stimulated with FM components and did not show any delay-tuning with PT stimulation. This result suggests that not only is echo delay-tuning generated in the IC but also its FM-specificity observed in the cortex could be created to some extent in the IC and then topographically organized at higher levels.

1. Introduction

Echolocating bats emit ultrasonic pulses and listen for the returning echoes reflected by surrounding objects (Griffin, 1958). One component of this imaging process is the estimation of target range. This task is achieved by auditory neurons that compute the time interval between the call and its echo (defined as pulse-echo delay) and provide a direct measure of the distance of reflecting surfaces (O'Neill and Suga, 1982; Simmons, 1971; Sullivan, 1982). In the mustached bat, *Pteronotus parnellii*, delay-tuned neurons respond to the combination of frequencies within the first harmonic frequency-modulated (FM1) sweep from the emitted sound and frequencies within a higher harmonic FMx (FM2, FM3 or FM4) component of the echoes (O'Neill and Suga, 1979, 1982; Suga and O'Neill, 1979; Suga et al., 1983). Although delay-tuned neurons were first described in the auditory cortex (AC), they have also been found in the medial geniculate body (MGB) (Olsen and Suga, 1991) and inferior colliculus (IC) of echolocating bats (Mittmann and Wenstrup, 1995; Dear and Suga, 1995; Yan and Suga, 1996; Portfors and Wenstrup, 1999).

There is strong evidence that certain response properties (hetero-harmonic delay-sensitivity, range of best facilitatory delays and sharpness) are features of delay-tuned neurons that undergo little modification between IC and AC (Portfors and Wenstrup, 1999, 2004; Wenstrup, 1999). One feature that has been systematically studied in the AC but not in the IC is the tilting of the delay-tuning curves. Cortical neurons often show tilted delay-tuning curves, such that the neuron at lower echo levels responds to longer delays than at higher echo levels (O'Neill and Suga, 1982; Hagemann et al., 2011).

Quantitative comparisons that may highlight additional processing of target distance information in auditory cortex are not straightforward (Wenstrup and Portfors, 2011). The general impression is that cortical delay-tuned neurons may be more likely to display preferences for FM sweeps rather than to tonal stimuli (Taniguchi et al., 1986), while this does not seem to be the case in the IC (Wenstrup and Portfors, 2011). However, most of IC experiments focused on pure-tone related response properties

Abbreviations: IC, inferior colliculus; AC, auditory cortex; PT, pure-tone pair stimulation; FM, frequency modulated; FTC, frequency tuning curve; FRA, frequency response area; PSTH, post-stimulus-time-histogram; MT, minimum threshold; ChF, characteristic frequency; DTC, delay-tuning curve; DRA, delay response area; CD, characteristic delay; CF, constant frequency.

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while the cortical experiments typically emphasized responses to sonar elements (Portfors and Wenstrup, 1999, 2004; Wenstrup and Portfors, 2011; Suga and O'Neill, 1979; O'Neill and Suga, 1979; Suga et al., 1983; O'Neill, 1995). In intracellular recording experiments, Peterson et al. (2008) suggested that the distribution and types of combination-sensitive interactions in the IC neuronal population are not dependent on tonal or FM stimulation. However, both types of stimulation have not been tested in the same neuron.

In this paper we describe properties of delay-tuned neurons in the IC of the mustached bat using randomized stimulus presentation and identical analysis techniques as those used in quantitative studies on cortical delay-tuning (Hagemann et al., 2011). We also address the question of how combination-sensitivity is affected by the presentation of tonal or FM stimuli.

2. Materials and methods

2.1. Animals

The study was conducted on the IC of nine adult *P. parnellii* (four males and five females). Animals were captured at the entrance of their cave roost during their evening exodus and kept in captivity in a room with temperature, humidity and photoperiod conditions similar to those of the bat natural environments. The experiments were performed at the Goethe University of Frankfurt am Main, Germany. The animal use in this study was authorized by the Centre for the Inspection and Control of the environment, Ministry of Science, Technology and Environment, Cuba and is covered by German animal experimentation licenses.

2.2. Surgical procedures

Bats were prepared for surgery by anesthetizing them with sodium pentobarbital (0.05 mg/g body weight) via subcutaneous injection in the neck. A longitudinal midline incision was made through the skin overlying the skull, and the underlying temporal musculature was reflected from the incision along the midline. Wound surfaces were treated with a lidocaine solution applied topically. A custom-made metal rod was then glued to the skull using dental cement. We let the animals rest for 24 h before starting the electrophysiological recordings. After recovery, during the experiment, the awake bats were placed in a body mold made of plastic foam. The head was tightly held by the rod fixed in a metal holder. Using skull and brain-surface landmarks, a small hole ($<$ of $\sim$1 mm diameter) was made over the IC with a scalpel blade. The hole was covered with saline solution during the experiments, and care was taken to prevent desiccation.

A microelectrode (see following text) was then inserted through the hole in the skull. The experiments were conducted inside a soundproof room (temperature: 27–32 °C) for less than 6 h. After a recording session, the exposed skull was covered with sterile bone wax, and the animal was returned to its individual cage. Bats could be studied for several consecutive days. All experiments were in accordance with the Declaration of Helsinki and also with German federal regulations.

2.3. Acoustic stimulation and recording

Acoustic stimuli were delivered from a ScanSpeak Revelator R2904/7000 speaker (Avisoft Bioacoustics, Berlin, Germany). There was a decrement of $\sim$20 dB SPL in the speaker level when increased the frequency from 10 to 120 kHz. The intensity of the presented stimuli was online corrected in accordance with the calibration frequency response curve of the speaker. The calibration curve was obtained with a Brüel&Kjaer sound recording system (¼-inch Microphone 4135, Microphone Preamplifier 2670) connected to a conditioning microphone amplifier (Nexus 2690). We made sure that speaker distortions were at least 50 dB below the desired stimulus intensities. For all measurements, the speaker was positioned 10 cm away from the ear contralateral to the recording site.

Extracellular neuronal recordings were made with carbon electrodes (Carbostar 0.4–0.8 MΩ). The depth of the recording electrode was controlled and adjusted by a piezo micro stepper (PM 10-1, Maerzhaeuser). After amplifying (Differential amplifier EX1, Dagan Corporation) and band-pass filtering between 200 Hz and 5 kHz, the neuronal signal was digitized by a Microstar DAP board (sampling rate 33 kHz), processed by the computer program, and stored for further analysis.

The analysis of the measured electrophysiological data was performed by MatLab 6.5 (Mathworks) scripts originally designed by Cornelius Abel. The recorded multi-unit activity was amplitude filtered so that for subsequent analysis we selected only those spikes whose amplitude was at least three standard deviations above the recording baseline. The selected spikes were sorted using the first three principle components of all spike waveforms (Lewicki, 1998). To separate the spikes originated from different neurons, an automatic clustering algorithm "KlustaKwik" (Harris et al., 2000; http://klustakwik.sourceforge.net/) was fed with the first three principle components of each spike. This method has been used to discriminate single from multi-unit activity in other studies (Abel and Kössl, 2009).

Once a neuronal response was detected, the unit's frequency tuning curve (FTC) was determined using randomly presented pure-tone bursts (10-ms duration with 0.5 ms rise/fall time presented at a repetition period of 300 ms) with variable frequency and level combinations (usually 20–95 kHz and 0–80 dB SPL). Each frequency level combination was presented 16 times. For analysis of the FTC obtained with pure-tone stimuli of 10 ms, the neuronal activity was measured within a time window of 20–70 ms, starting before any conspicuous increase in neuronal activity assessed with Post-Stimulus-Time-Histograms (PSTH). From the neuronal responses to tone-burst frequency/level combinations, frequency response areas (FRAs) were calculated for a threshold criterion of 25% of maximum activity and used to obtain the minimum threshold (MT) and the frequency at minimum threshold (characteristic frequency, ChF). We also determined the Q10-dB (calculated as ChF divided by the bandwidth measured 10 dB above MT).

Neurons were tested for combination-sensitivity by presenting both a low- and a high-frequency signal. Sensitivity to delay between the low-frequency (pulse) and high-frequency (echo) signals was assessed by varying the delay in steps of 2 ms. Level of the high-frequency signal (echo level) was also varied. At a given level of the presented pulse (70–80 dB SPL), both the delay and the level of the echo were varied randomly to obtain delay-tuning curves (DTCs) that display response strength against echo delay and level. Each delay level combination was presented 16 times. Two types of DTC were obtained for each neuron: (i) using pairs of 3 ms pure tones (PT), since in most of the cases the neurons showed two separated FRAs at the frequency range of the first and one of the higher harmonics, frequency of the pure tones corresponded to ChFs of the two FRAs; and (ii) using 3 ms FM signals in which the pulse was always the FM component of the 1st harmonic (from 22.5 to 30.5 kHz) and the echo was fixed in the 2nd (from 45 to 61 kHz) or the 3rd (from 67.5 to 91.5 kHz) harmonic, depending on the FRA of the neuron. Pairs of stimuli, both PT and FM, were presented at a repetition period of 250 ms.

In each DTC, delay response area (DRA) was calculated from the responses to pulse-echo pairs based on a criterion of 50% of maximum response activity of the unit. The lowest echo level that