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Recovery cycle of inferior collicular neurons in *Hipposideros pratti* under behavior-related sound stimulus and the best Doppler-shift compensation conditions

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HIGHLIGHTS

- Inferior colliculus (IC) involves in processing Doppler-shift compensation (DSC) signal.
- Neurons with different recovery cycle types play roles in different phases of predation.
- DSC rearranges the different types of recovery cycle of IC neurons.
- DSC could help bats enhance the processing of rapid returning echo.
- DSC could avoid echo interferences produced by vegetation in complex habitats.

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ABSTRACT

The Doppler-shift compensation (DSC) behavior of constant frequency – frequency modulation (CF-FM) bat (*Hipposideros pratti*) is vital for extraction and analysis of echo information. This type of behavior affects the recovery cycles of sound-sensitive neurons, but their precise relationship remains unclear. In this study, we investigated the effects of DSC on the recovery cycles of inferior collicular (IC) neurons in *H. pratti*. We simulated the pulse-echo pair in bats by changing the emitted pulse frequency and keeping the echo frequency constant during DSC in echolocation. The neuronal recovery cycles of IC neurons are categorized into four types: unrecovered, monotonic, single-peak, and multi-peak. The recovery cycle of IC neurons shortens after DSC; moreover, the amount of neurons with multi-peak recovery cycle increases and concentrates in the short recovery area. This paper also discusses the possible neural mechanisms and their biological relevance to different phases of bat predation behavior.

1. Introduction

Constant frequency-frequency modulation (CF-FM) bats use sonar to guide flight and find prey in the dark. During flight, motion relative to the target induces differences in frequency between echo and emitted pulses, CF-FM bats adjust the frequency of emitted pulses by Doppler-shift compensation (DSC) to maintain the echo frequency within the narrow range wherein they can hear best [1,9,10,13,28,30]. DSC may be positive (decreasing the frequency of the emitted pulse) or negative (increasing the frequency of the emitted pulse). Positive compensation occurs when bats approach the target to compensate for the increasing frequency shift of echo, and negative compensation may occur in "somersault landings" or to compensate for "overcompensation" in the terminal phase of bat predation behavior [14]. DSC is important in bats for the following reasons: a) by changing the frequency of the emitted pulse, bats can avoid the sound signal interference of other bats and easily distinguish their own echo signal [21]; b) by keeping the echo carrier frequency in a relatively constant level, bats can detect frequency and amplitude modulation by insect fluttering and thus classify insect species [23]; c) bats can also obtain flying speed information on insects by calculating frequency differences between the emitted pulse and echo [33]; d) active control of their own calls plays a key role in bat movement and spatial memory, except for perception [38].

Abbreviations: BF, Best frequency; CF, Constant frequency; DSC, Doppler-shift compensation; FM, Frequency modulation; IC, Inferior colliculus; IPI, Inter-pulse interval; MT, Minimal threshold; LR, Long recovery; MR, Moderate recovery; SR, Short recovery.

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Previous studies on CF-FM bats [8,27] showed that the DSC behavior is vital for extraction and analysis of echo information. This compensation behavior is consistent with the function of bat auditory nuclei, which are highly specialized in processing the CF components in the stable second harmonics (i.e., the dominant frequency) of echoes [23]. For example, the auditory cortex can be divided into DSCF (DSC processing), CF-CF, FM-FM, and dorsal fringe areas [15,40]. The DSCF area significantly over-represents the second harmonic frequencies of the CF portion of the sonar pulse of CF-FM bat; thus, neurons in the DSCF area are extremely well-tuned to second harmonic frequencies. Therefore, the DSCF neurons of the auditory cortex can represent very small Doppler shifts [32]. Bats cannot make fine frequency shift judgments when the DSCF area is impaired [34]. These findings suggest that the DSCF area of the auditory cortex may regulate the DSC behavior of CF-FM bats.

The role of the subcortical auditory nucleus in processing DSC signals remains unclear. Schuller reported that four inferior collicular (IC) neurons of *Rhinolophus ferrumequinum* respond weakly to simulated pulse-echo pairs when the echo frequency does not shift [26]. However, the number of impulses significantly increases when the positive shifts of the echo frequency exceed 800 Hz. IC neurons are sensitive to echo frequency shifts and are thus considered to be related to the DSC behavior of *R. ferrumequinum*. Other studies also find that most of the recorded IC neurons, whose best frequencies (BFs) are near reference frequency, exhibit sharp frequency tunings [16,17]. These neurons are located in the region similar to the DSCF area of auditory cortex; hence, IC neurons are also involved in processing DSC signals. Therefore, the mechanism through which IC neurons respond to the pulse-echo pairs during DSC in CF-FM bat flight must be elucidated.

Recovery cycle is a basic property of sound-sensitive neurons and is the neural basis for bats to extract echo information accurately and rapidly [11]. The recovery cycle determines the ability of neurons to restore responses to the second sound impulse during short inter-pulse intervals [22] and is important for echolocation bats. Bats analyze information about target distance, shape, and texture by using returned echo information; hence, the recovery cycle of neurons directly determines the target perception ability of bats. Considering that the DSC behavior is vital for extraction and analysis of echo information, we hypothesize that the recovery cycles of sound-sensitive neurons are affected by the DSC behavior of CF-FM bats. However, changes in neuronal recovery cycles during DSC remain unknown. In this study, we simulated the pulse-echo pair of bats during DSC in echolocation by changing the frequency of the emitted pulse and maintaining the echo frequency constant. We then investigated the recovery cycle properties of IC neurons in Pratt's roundleaf bat (*Hipposideros pratti*) under different compensation conditions. We also discussed the biological relevance of the recovery cycle properties of IC neurons in bat predation behavior.

2. Material and methods

2.1. Animal preparation and surgery

CF-FM bat (*H. pratti*) caught in the Hubei Province of China was used in this study. We terrecorded the echolocation signals of *H. pratti* (D980, Petterson Elektronik AB, Sweden). Surgical procedures for neurophysiological study of seven bats [42.5–51.6 g (48.3 ± 3.3 g) body weight] were performed using methods reported in our previous works [4,5]. Briefly, the flat head of a 1.8 cm nail was attached onto the exposed skull of each Nembutal-anesthetized bat (45–50 mg/kg b.w.) 1 or 2 d before the recording session by using acrylic glue and dental cement. The exposed tissues were treated with an antibiotic (neosporin) to prevent inflammation and given with the neuroleptanalgesic Innovar-Vet (fentanyl, 0.04 mg/kg b.w.; droperidol, 2 mg/kg b.w.). The subject was then placed in a wire mesh bat holder suspended from an elastic sling inside a custom-made, double-walled, soundproof room (temperature 28 °C–30 °C). The ceiling and interior walls of the room were covered with 8 cm convoluted polyurethane foam to reduce echo.

2.2. Acoustic stimulation and recording of neuronal responses

After fixing the bat's head with a set screw, small holes (200–500 μm) were bored in the skull above the IC for orthogonal insertion of 2 M NaCl glass pipette electrodes (tip diameter: <1 μm, impedance: 5–10 MΩ) to record sound-activated responses. Additional doses of Innovar-Vet were administered during the later phases of recording when the bats showed signs of discomfort. A local anesthetic (lidocaine) was applied to the open wound area to reduce any possible pain. Recording depth was read from the scale of a microdrive (David Kopf Instrument, Model 640, USA). A common indifferent electrode (silver wire) was placed at nearby temporal muscles. Each bat was used in one to five recording sessions on separate days, with each recording typically persisted for 2–6 h. This study used the minimum number of bats to obtain data.

For acoustic stimulation, continuous sine waves from a function generator (GFG-8016G, Good Will Inst. Co., Ltd., Bayan Lepas, Penang, Malaysia) were formed into pure-tone pulses by a custom-made tone burst generator (electronic switch) driven by a stimulator (Model SEN-7203, Nihon Kohden Co, Shinjuku, Tokyo, Japan). The tone pulses were amplified (custom-made amplifier) after passing through a decade attenuator (LAT-45, Leader, Kohokuku, Yokohama, Japan), then fed into a small loudspeaker (AKG model CK 50, 1.5 cm in diameter, 1.2 g, frequency response of 1–100 kHz). The loudspeaker was placed 30 cm away from the bat ear and 30° contralateral to the recording site. The loudspeaker was calibrated with a 1/4 in. microphone (4939, B&K, Denmark) placed at the bat's ear with a measuring amplifier (2610, B&K, Denmark). Loudspeaker output was expressed in decibel sound pressure level (dB SPL) in reference to 20 μPa root mean square. The frequency response curve of the loudspeaker was plotted to determine the maximum available sound amplitude at each frequency. The maximum stimulus amplitude ranged from 110 dB to 125 dB SPL between 10 and 80 kHz but decreased sharply to 80 dB SPL at 100 kHz thereafter. CF-FM sounds ($5_{CF} + 2_{FM}$ ms with 0.5 ms rise-decay times) were generated via a linear voltage ramp from the function generator (Agilent 33220A) driven by the same electronic switch. Briefly, 7 ms CF sound was generated. The onset of the linear voltage ramp was adjusted and synchronized at 5 ms of CF sounds, such that the amplitude was not changed at the boundary between the CF and FM components. By contrast, the CF sound frequency decreased by 20% within the FM portion; thus, the end frequency of the 2 ms FM component was 80% of the CF frequency. Upon isolation of a neuron with 7 ms CF sound, the sound-activated response was amplified (ISO-DAM WPI) and sent to an oscilloscope (TDS210 Tek) and audio monitor (AM9 Grass). The threshold at each responsive frequency was audio-visually determined by changing the sound amplitude, and the result showed 50% response probability on average. BF was defined as the sound frequency that elicited neuronal responses at the lowest amplitude. The minimal threshold (MT) was defined as the threshold at the BF. All sounds were delivered at 2 pulses per second. CF frequency was delivered at neuronal BF.

Sound-sensitive neurons were detected by 7 ms single CF sounds. BF, MT, and recording depth of neurons were also recorded. The response of IC neurons to CF-FM sound (5 + 2 ms) was determined, and the number of impulses was set as the control. The recovery cycles of IC neurons were measured by two CF-FM sounds with constant duration (5 + 2 ms). The difference of intensities of the first and second sounds was 10 dB (MT + 30 dB & MT + 20 dB, or MT + 20 dB & MT + 10 dB). The CF frequencies of the first sound (emitted pulse) were changed into BF + 4, BF + 3, BF + 2, BF + 1, BF + 0.5, BF, BF − 0.5, BF − 1, BF − 2, BF − 3, and BF − 4 kHz (i.e., the compensation values were +4, +3, +2, +1, +0.5, 0, −0.5, −1, −2, −3, and −4 kHz), and the CF frequency of the second sound (echo) was set as neuronal BF to simulate pulse-echo pairs during DSC. We defined the intervals of the emitted pulse and echo as inter-pulse intervals (IPI, onset–onset). IPI values of 1, 2, 4, 10, 20, 30, 50, 75, and 100 ms for each pair of stimuli were randomly presented to the investigated neuron. A recovery