

Laryngeal Nerve Activity During Pulse Emission in the CF-FM Bat, *Rhinolophus ferrumequinum*

I. Superior Laryngeal Nerve (External Motor Branch)

Gerd Schuller* and Rudolf Rübsamen

Arbeitskreis Neuro- und Rezeptorphysiologie, Fachbereich Biologie der J.W. Goethe-Universität, D-6000 Frankfurt/Main,
Federal Republic of Germany

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Summary. The activity of the external (motor) branch of the superior laryngeal nerve (SLN), innervating the cricothyroid muscle, was recorded in the greater horseshoe bat, *Rhinolophus ferrumequinum*. The bats were induced to change the frequency of the constant frequency (CF) component of their echolocation signals by presenting artificial signals for which they Doppler shift compensated. The data show that the SLN discharge rate and the frequency of the emitted CF are correlated in a linear manner.

Introduction

Bats are nocturnal animals which use an echolocation system for the pursuit of prey and avoidance of obstacles. The echolocation sounds are produced in the larynx, modified and filtered in their spectral composition by transmission through the mouth (e.g. vespertilionids) or nose (e.g. rhinolophids). The echolocation pulses have frequencies that range from about 10 kHz up to about 150 kHz and they are emitted at intensities up to 110 dB SPL, when measured 15 cm in front of the bat's head.

The spectral structure of the echolocation signals in different bats are characteristic for the species but they also may be adapted to meet specific behavioural situations (e.g. see Schnitzler 1978). Most bats use broad band pulses consisting of frequency modulations with or without several harmonics (e.g. vespertilionids) whereas each echolocation pulse of the species considered in this paper (*Rhinolophus ferrumequinum*) consists of a long (10–80 ms) constant frequency (CF) portion (ca. 83 kHz) terminated by a 1–3 ms down-

ward frequency sweep of 15–20 kHz. The echolocation pulse of this species at 83 kHz is in fact the second harmonic of a harmonically structured signal produced in the larynx, having components at about 41.5 kHz, 83 kHz and 124.5 kHz. Vocal tract filtering is responsible for suppression of the fundamental and the third harmonic and transmission of the second harmonic component (Roberts 1973).

During flight the frequency of the CF-portion of the echo is shifted relative to the frequency in the emitted sound due to the Doppler effect and this raises the echo frequency in accordance to the relative speed between the bat and its target. *Rhinolophus ferrumequinum* has a control system that holds the CF of the echo at a reference frequency of about 83 kHz. This is independent of the relative speed between the bat and its target and is accomplished by lowering the frequency of the emitted sound according to the echo frequency information received (Schnitzler 1968; Schuller et al. 1974). This so called Doppler shift compensation system (DCS) involves a fine laryngeal control that typically has an accuracy of ± 50 Hz over a shift range of about 5 kHz (Schuller et al. 1974).

Denervation experiments in different species of FM-bats (Novick and Griffin 1961; Suthers and Fattu 1973) and in *Rhinolophus ferrumequinum* (Schuller and Suga 1976) have shown that the frequency control in the larynx is mainly accomplished by the cricothyroid muscles which alter the tension of the vocal cords. These muscles are innervated by the external (motor) branch of superior laryngeal nerve (SLN). Sectioning both superior laryngeal nerves was found to lead to dramatic decreases of the emitted frequency and to the appearance of harmonics, whereas the sectioning of one recurrent laryngeal nerve (RLN), innervating the internal laryngeal muscles, only caused minor frequency changes in the emitted sound (Schuller and Suga 1976).

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Abbreviations: SLN Superior laryngeal nerve; RLN Recurrent laryngeal nerve; DCS Doppler compensation system; CF Constant frequency; FM Frequency modulation

This paper describes the activity of the superior laryngeal nerve during the emission of echolocation pulses and during Doppler shift compensation.

Materials and Methods

The discharge activity of the motor branch of the superior laryngeal nerve was recorded in six greater horseshoe bats, *Rhinolophus ferrumequinum*. Before surgery the Doppler shift compensation behaviour of the bat was tested with the electronic Doppler shift simulation device described by Schuller et al. (1974). This yielded information on the sound parameters of the echolocation pulses of the individual bat, the range of frequency within which the bat could alter its emission frequency and the constancy with which the CF was maintained during sound emission.

For the recordings the bat was prepared under ether anaesthesia. The top of the skull was exposed and cleaned and a rod was mounted on the bone with dental acrylic (Paladur). The rod was locked into a rigid bar and the bat was positioned ventral side up to allow access to the larynx. By making a small incision in the skin over the larynx the ventral part was exposed, yielding access to the SLN near its entrance to the cricothyroid muscle. During the recordings the wound margins were continuously treated with a local anaesthetic (Novocain).

The recording electrodes were very flexible silver wires of 0.1 mm diameter. The flexibility of the electrodes prevented a large displacement of the electrode tip from the SLN when the larynx moved during vocalization. The electrode tip was positioned 0.5 to 1 mm proximal to the entrance of the SLN into the cricothyroid muscle. The indifferent electrode was fixed in the neck muscles.

The nerve potentials were amplified by conventional methods, passed through a window discriminator to reduce background discharges and stored on magnetic tape (Philips Analog-7). The echolocation pulses were picked up with a Brüel & Kjaer microphone (Type 4136), amplified and fed to a frequency to voltage converter (custom made with IC 9400) which had a better than 50 Hz frequency resolution in the 83 kHz range. The frequency to voltage converter signal was stored in parallel to the nerve activity on an analog tape recorder and thus the temporal correlation between nerve activity and frequency of the emitted CF could be assessed. During recording the bats spontaneously emitted echolocation sounds.

In order to manipulate the frequency of the emitted sound, the Doppler shift compensation behaviour of this bat was used: the echolocation pulses picked up with the microphone were electronically shifted to higher frequencies and played back to the bat's ear, so that the animal adjusted its emitted frequency in subsequent sounds to a lower frequency in order to compensate for the frequency shifts in the echoes (for details of the method see Schuller et al. 1974).

In this way the emitted frequency could be shifted between the resting frequency of each bat (near 83 kHz) and frequencies up to 4 kHz lower than the resting frequency. The frequency in the emitted echolocation pulses could either be kept constant at a lowered level or the emitted frequency could be induced to change periodically following a sinusoidal waveform modulating the Doppler shifts in the playback echoes (Schuller et al. 1975).

The recordings on analog tape were processed off-line with a PDP-12 computer. The number of SLN discharges was counted within 100 ms prior to the beginning of each echolocation pulse until the end of the echolocation pulse and then correlated to the emitted CF-frequency. The mean number of SLN-discharges for a distinct CF-frequency was established averaging over a large number of echolocation pulses at this CF-frequency. The computer program to store the analog data from tape in a digital form on DEC-tape was provided by Hans Zöller.

Results

The gross activity of the superior laryngeal nerve was closely tied to the emission of echolocation pulses, which were spontaneously emitted by the bats. It started between 30 and 50 ms prior to the onset of vocalization, and continued during sound emission. The mean discharge rate stayed roughly constant throughout this period. The end of activity corresponded to the end of the CF component.

The duration of the orientation sounds was fairly constant (ca. 50 ± 5 ms) throughout the experiments. Thus the number of discharges of the SLN per echolocation sound could be taken as a measure ($\pm 10\%$) of the electrical activity of the nerve.

The SLN-activity was studied in conjunction with the frequency of the emitted CF-component during Doppler shift compensation, i.e., its dependence on frequency over a range between 0 and typically 3 kHz below the ca. 83 kHz resting frequency emitted by the roosting bat. Figure 1 shows two examples of the spike count versus frequency function measured for emitted sounds between 82.5 kHz (resting frequency) and 79.5 kHz. During the emission of the resting frequency the SLN shows its maximum discharge activity of 30 to 40 spikes per echolocation sound, corresponding to discharge rate of $600\text{--}800\text{ s}^{-1}$. The number of discharges per sound decreases gradually when the emitted frequency decreases and is lowered to about 8 to 10 spikes per sound (corresponding discharge rate: $160\text{--}200\text{ s}^{-1}$) for an emitted CF-frequency of 3 kHz below the resting frequency.

The correlation of the spike count and the emitted CF-frequency can be fitted with linear curves that have high correlation coefficients of 0.96 (upper graph) and 0.95 (lower graph). Depending on the recording situation the spike count versus frequency functions can be less steep but can always be fitted with linear functions at high correlation coefficients. A linear change of SLN activity consequently entrains a linear change of frequency of the CF-portion in the emitted orientation pulse. It was not possible in our experiments to demonstrate any function of SLN-activity in the control of the final FM-sweep of the sound.

The linear relationship between emitted CF-frequency and the SLN-activity could further be confirmed in experiments where the dynamic properties of Doppler shift compensation and the SLN-response were studied.

In this case the emitted frequency was not kept at a constant level throughout a sequence of echolocation pulses but the bat was induced to change its emitted frequency periodically. For this purpose the frequency shifts introduced into the simulated echoes

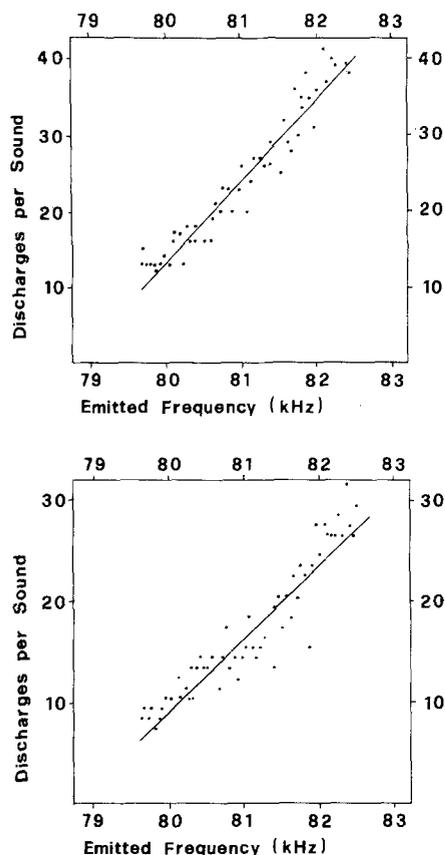


Fig. 1. Discharges of the superior laryngeal nerve (SLN) of the greater horseshoe bat as a function of the CF frequency component emitted in the echolocation pulses recorded from two individual horseshoe bats. The SLN-activity is represented as the number of discharges per echolocation pulse (ordinate); the emitted CF-frequency of the echolocation pulse is given on the abscissa. The reference frequency for both bats lies at about 82.5 kHz. The correlation coefficients are 0.96 (upper) and 0.95 (lower) for fitting straight lines

were sinusoidally modulated at different modulating frequencies. The bat compensated for these periodical frequency shifts in the echo by decreasing its emitted CF-frequency below the resting frequency, so that the echo frequency was always near or at the resting frequency (see Schuller et al. 1975). The sinusoidal frequency shifts introduced in the playback echoes range from 0 to 3 kHz with modulating waveforms at frequencies between 0.05 and 0.5 Hz.

Accordingly the emitted CF-signals also change between 82.5 kHz and about 79.5 kHz and also the number of the SLN-discharges per echolocation sound changed. In Fig. 2 the emitted CF-frequencies and the corresponding SLN-discharges are represented in response to sinusoidal frequency shifts.

The respective modulating frequency for each graph is indicated at the right side of each pair of emitted frequencies and SLN-discharges in response to the sinusoidal frequency shifts. Each dot in the

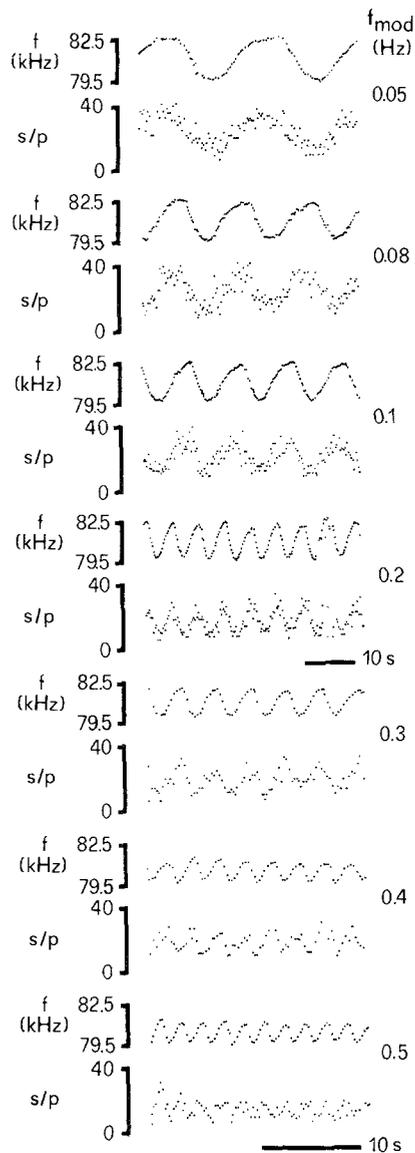


Fig. 2. Dynamic properties of SLN-discharges for sinusoidally modulated Doppler shifts at modulating frequencies between 0.05 and 0.5 Hz. The Doppler shift modulation cycles through 0 to 3 kHz. Each pair of curves represents the emitted CF-frequency (upper, in kHz, each point represents one individual echolocation sound) and the corresponding SLN-discharges per echolocation sound (lower, spikes per pulse). Modulating frequency indicated on the right for each pair of curves. Note the different time scales (upper four pairs and lower three pairs of traces)

graph gives the value of the CF-frequency or SLN-discharge per pulse for a single pulse emission. For low modulating frequencies (0.05 Hz; 0.08 Hz and 0.1 Hz) the emitted frequencies changed in accordance with the sinusoidal frequency shift variations. The compensation covered approximately the range of the introduced 3 kHz frequency shift which drove the emitted frequency between 82.5 and 79.5 kHz. Variations of the discharge count between 40 and

10 discharges per pulse were correlated with the resulting changes in the emitted frequency and the discharge rate changes mirrored the waveform of the emitted frequency changes. Due to the movement of the larynx during recording there were some fluctuations in the SLN discharge function.

As the modulating rate was increased above 0.1 Hz the frequency-shifts in the echo were no longer completely compensated for and the emitted frequency did not reach the resting frequency when the modulation cycle drove the frequency shift through zero Hz.

The SLN-discharges showed the same properties at all modulating rates above 0.1 Hz and did not return to the initial 40 discharges per echolocation pulse which corresponded to an emitted CF at the resting frequency (i.e., when the modulation rates were below 0.1 Hz). Thus the SLN-discharge counts and the CF were closely tied. The correlation is independent of the rate with which the frequency shifts were modulated. The relation between SLN-discharges and the emitted CF-frequency remained linear in the range of modulation frequencies from 0.05 Hz to 0.5 Hz.

The mechanism which limits the dynamic response of the Doppler shift compensation system (Schuller et al. 1975), and therefore introduces the nonlinearity in the control system, must be of neural origin and be activated before the control signal reaches the SLN.

Discussion

The Doppler shift compensation system of the greater horseshoe bat keeps the frequency of the returning echo CF in a narrow band around the 'reference' frequency (the frequency to which the ear is sharply tuned) (Schnitzler 1968; Schuller et al. 1974; Simmons 1974). This feedback system involves an information transmission path between the auditory system and the vocalization system for the adjustment of the outgoing CF within accuracy limits of 50 to 200 Hz (Schuller et al. 1974).

Earlier denervation experiments have shown the predominant role of the cricothyroid muscle in the fine adjustment of the CF of echolocation signals of *Rhinolophus ferrumequinum* (Schuller and Suga 1976) and for frequency control in other species (Novick and Griffin 1961; Suthers and Fattu 1973). The cricothyroid muscle therefore is the structure concerned with frequency control in the larynx. The recordings from the nerve innervating the cricothyroid muscle showed a close relationship to the frequency that is emitted in the constant frequency portion of the echolocation pulse. This functional relationship

of the activity in the nerve to the frequency is preserved also in the EMG-activity of the cricothyroid muscle (Schuller and Suga 1976). Thus the emitted CF is clearly controlled by the activity of the SLN. The precision of the frequency emission is determined by the range of variability of SLN-discharge rate relative to the emitted frequencies. The discharge rate changes about 600 Hz for an emitted frequency span of 3 kHz in the CF, corresponding to a 10 Hz SLN-discharge rate change for 50 Hz CF-frequency change. Consequently the emitted CF-control by the SLN-activity can be theoretically achieved within the behaviourally measured accuracy of ± 50 Hz.

There are two methodological factors that could not be eliminated that might have introduced variations into the measurements of the spike counts per emitted echolocation pulse and thus in the discharge rate: 1) the recordings are from a moving structure so that the movements of the larynx during sound emission vary the electrode-SLN configurations slightly and thus influence the amount of activity picked up with the electrode, and 2) the durations of echolocation pulses vary slightly from the mean and consequently the spike count per echolocation pulse varies accordingly. This variance of the control signal is larger than the actual variance of the frequency in the emitted sounds. This fact is clearly due to the recording situation described above.

The nerve activity starts 30–50 ms prior to the emission of the echolocation pulse and is maintained during the emission of the constant frequency portion. The cricothyroid muscle exerts tension on the vocal cords, which is of importance for the fundamental frequency produced in the larynx and therefore the frequency of the transmitted echolocation signal. The activity in the superior laryngeal nerve builds up the tension of the cricothyroid muscle and therefore the tension on the vocal cords prior to the emission of the echolocation pulse. Less activity in the SLN means a weaker tension of the cords and a lower emitted frequency. This may help to maintain the amplitude of the changing emitted CF, whereas it will of course not control the fundamental period of the signal. This hypothesis gets support from Roberts (1972) who provided evidence for a variable resonance that can be tuned between 75–83 kHz. He proposed that such a resonance could emphasize the FM component of the signals, which would also hold for the CF-frequencies emitted by a Doppler compensating horseshoe bat. On the other hand the activation of the cricothyroid muscle could change the volume of the space below the vocal folds. If this subvocal space of the sound production system of the CF-FM-bats works similar to a volume resonator as proposed by Griffiths (1978) for a mormoopid bat, a concurrent

increase of the subvocal fold volume with a decrease of the cricothyroid tension would tune the resonating frequency of the cavity down in parallel to the emitted CF-frequency. There are no data available in *Rhinolophus* to support or refute this hypothesis at the moment. As the discharge of the SLN ceases shortly before or with the termination of the echolocation signal a concurrent release in tension of the vocal folds may lead to the decrease in emitted frequency during the final frequency modulated sweep. The extent of downward frequency modulation may be determined and limited by the variable resonance of the subvocal fold volume resonator (Roberts 1975) and the transmission properties of the vocal tract.

Nerve cell bodies of the SLN lie within the nucleus ambiguus. The Doppler shift compensation system in the greater horseshoe bat involves the relay of frequency information that finally adjusts the output of the nucleus ambiguus. The dynamic properties measured for the Doppler shift compensation system in *Rhinolophus ferrumequinum* reveal strong nonlinearities that limit the speed of the compensation process and lead to different time constants for the two directions of CF changes (lowering or raising) (Schuller et al. 1974). When the SLN-discharges in response to sinusoidal Doppler shift modulations were observed at different modulation frequencies it was found that the same nonlinearities occur for higher modulation rates. The relationship between SLN-discharge rate and emitted CF stays linear over the whole modulation rate range. Therefore it can be concluded that the nonlinearity must be of neural origin. 'Subsystems' for the Doppler shift compensa-

tion system, consisting of SLN, cricothyroid muscle and acoustical sound transmission path, cannot account for the observed nonlinearities.

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