Laryngeal Mechanisms for the Emission of CF-FM Sounds in the Doppler-Shift Compensating Bat, *Rhinolophus ferrumequinum* [*]

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Summary. 1. The laryngeal mechanisms for the production of CF-FM orientation sounds in the horseshoe bat, *Rhinolophus ferrumequinum*, were studied with 3 different methods: a) denervation of laryngeal muscles, b) recordings of electrical activity of the cricothyroid muscle during vocalization and Doppler-shift compensation, and c) electrical stimulation of the cricothyroid muscles.

2. Unilateral section of the inferior laryngeal nerve (i.e. denervation of the intrinsic laryngeal muscles) had no effect on the sound pattern, the frequency of the orientation sounds and the Doppler-shift compensation. Bilateral denervation led always to suffocation.

3. Unilateral section of the superior laryngeal nerve (i.e. the denervation of the cricothyroid muscles) did not change the sound pattern, but caused a decrease in the frequency of the emitted CF by 4–6 kHz. Doppler-shift compensation was possible, but it was unstable and was not accurate.

4. Bilateral denervation of the cricothyroid muscles introduced several strong harmonics in the orientation sounds. The fundamental frequencies changed considerably between 12 and 42 kHz after surgery. The frequency pattern in each harmonic is the same as in normal orientation sounds (Fig. 1).

5. The spike number per vocalization of cricothyroid muscle fibres was proportional to the frequency of the CF component of the orientation sound in a range between the resting frequency and 5 kHz below it (Fig. 2).

6. Electrical stimulation of the cricothyroid muscles increased the frequency of the emitted CF. The maximum frequency change due to electrical stimulation was about 45 Hz per ms when measured during the CF component of the orientation sound (Fig. 3).

7. Tetanus fusion frequency of the cricothyroid muscle was about 200 Hz for continuous stimulation and 400 Hz for short stimulation applied during vocalization.

Introduction

The echolocation system consists of the vocalization system for production of orientation sound and the auditory system for reception and processing of echoes. In microchiropterans the echolocation signals are produced in the larynx and modified in their spectral composition by the transmission through the mouth (e.g. Vespertilionids) or the nostrils (e.g. Rhinolophids). Vespertilionids commonly use frequency-modulated (FM) sounds for echolocation, while Rhinolophids use signals each of which consists of a long constant frequency (CF) component and short FM components. The functions of the different laryngeal muscles and nerves have been studied in different species of "FM" bats, and it has been found that denervation of the cricothyroid muscles causes prominent decreases in the frequency of the emitted sounds and the range of the frequency sweeps (Novick and Griffin, 1961; Suthers and Fattu, 1973). No experiments, however, have yet been performed on the laryngeal mechanisms of "CF-FM" bats.

In terms of acoustic behaviour the greater horseshoe bat (*Rhinolophus ferrumequinum*) is the best studied animal among "CF-FM" bats. When *Rhinolophus ferrumequinum* hears positively Doppler-shifted echoes it lowers the frequency of subsequent orientation sounds in order to compensate the Doppler-shift and stabilizes the echoes at a certain preferred frequency (Schnitzler, 1968). This interesting acoustic behaviour called "Doppler-shift compensation" is shown even when the bat is held in a plastic batholder and is listening to electronically produced Doppler-shifted echoes triggered by its orientation sounds, which are commonly emitted at a rate of 4–5 per s (Schuller et al., 1974). *R. ferrumequinum* is probably one of the best suited animals for studies on the mechanisms in sound production. Thus experiments with *R. ferrumequinum* on the peripheral mechanisms for the emission of CF-FM sounds and Doppler-shift compensation were performed. The present paper describes the effect of selective denervation of the laryngeal muscles on orientation sounds and the activity of the laryngeal muscles in relation to Doppler-shift compensation.

Material and Methods

Denervation of Laryngeal Muscles. Denervations of laryngeal muscles were performed in 5 horseshoe bats, *Rhinolophus ferrumequinum*. Under ether anesthesia superior and inferior (recurrent) laryngeal nerves on the left and right sides were cut in different combinations. The length of the nerve cut out was 1–2 mm long in order to prevent quick reinnervation. The wound was desinfected with sulfathiazole and than sewed together. All bats recovered quickly, so the postoperational tests were started 3 days after the surgery and lasted for 3 weeks to 2 months. In 3 bats an additional denervation was performed 3 weeks after the first surgery.

Recording of Electromyograms from Cricothyroid Muscles. The skull of the bat was exposed under ether anesthesia and a screw was mounted on it with dental cement. Than the screw was locked into a metal bar in order to immobilize the head. The animal was placed ventral side up and the ventral part of the larynx was exposed after cutting the sternohyoid muscles. The impairment of the sternohyoid muscles had no noticeable effect on the vocal performance of the bat. Action potentials of the cricothyroid muscles were recorded with chlorided silver electrodes of 0.1 mm

diameter which were placed in the ventrolateral part or the ventromedial part of the cricothyroid muscle. The electrodes were 10 cm long and very flexible, so that their tips could freely move as the larynx moved during vocalization. Action potentials of a few muscle fibres were amplified and passed through a window discriminator which was set to pass only the largest action potentials. Pulses from the window discriminator were recorded on magnetic tape in parallel to the frequency of the CF-component of the orientation sounds picked up 15 cm in front of the bat's head. The number of impulses per orientation sound and the frequency of the CF-component of an orientation sound were processed off-line with the PDP-12 laboratory computer.

Electrical Stimulation of the Cricothyroid Muscles. A 0.1 ms long electrical stimulus was bilaterally applied at different rates to the cricothyroid muscles with a pair of silver wires of 0.1 mm in diameter. The frequency of the CF-component or orientation sounds was simultaneously recorded with the electrical stimuli on a magnetic tape and was processed with the laboratory computer (PDP-12).

Analysis of Orientation Sounds. In order to study the effect of denervation or electric stimulation of the laryngeal muscles on vocalization, orientation sounds emitted by the bats during the delivery of artificial echoes with or without Doppler-shifts were detected with a 1/4'' microphone (Bruel & Kjaer 4135) placed 15 cm in front of the animal. The sounds were recorded on a magnetic tape, if necessary, and were analyzed with a Kay sonagraph. The frequency of the CF-component in an orientation sound (here after the emitted CF) was determined with a frequency to voltage converter and a sample could be picked up by the computer (PDP-12) 15 ms after the onset of the sound. The methods for the production of electronically frequency-shifted echoes and the measurement of the frequency of the emitted CF had been described in detail elsewhere (Schuller et al., 1974).

Results

Denervation of Laryngeal Muscles

When the inferior laryngeal nerve was cut unilaterally in 3 bats, no change was observed in the orientation sound and the rate of sound emission. Doppler-shift compensation was not affected at all. The bilateral transection of the inferior laryngeal nerves always caused suffocation, even if atropin sulphate was administered to the bat prior to the transection. In one bat the transection was performed after tracheotomy. Than the bat survived without any problem in respiration. It is known from human surgery that bilateral impairment of the inferior laryngeal nerve intails an adduction of the vocal folds (King and Gregg, 1948).

In 5 bats the cricothyroid muscles were denervated by cutting the superior laryngeal nerve on one of both sides. The unilateral transection of the superior nerve caused no drastic change in the basic pattern of the orientation sounds emitted. Each sound consisted of a long CF component followed by a short FM component as the normal orientation sounds did. The frequency of the emitted CF, however, was 1.5 to 4 kHz lower than the resting frequency of the intact animals (Fig. 1 A, middle sonagram), and faint harmonics were noticed in the sounds emitted by some of these bats only within a few days after the surgery. These bats responded to Doppler-shifted echoes with an irregular lowering of the frequency of the emitted CF. The amount of frequency compensation was not strictly proportional to the Doppler-shift introduced in the echoes,
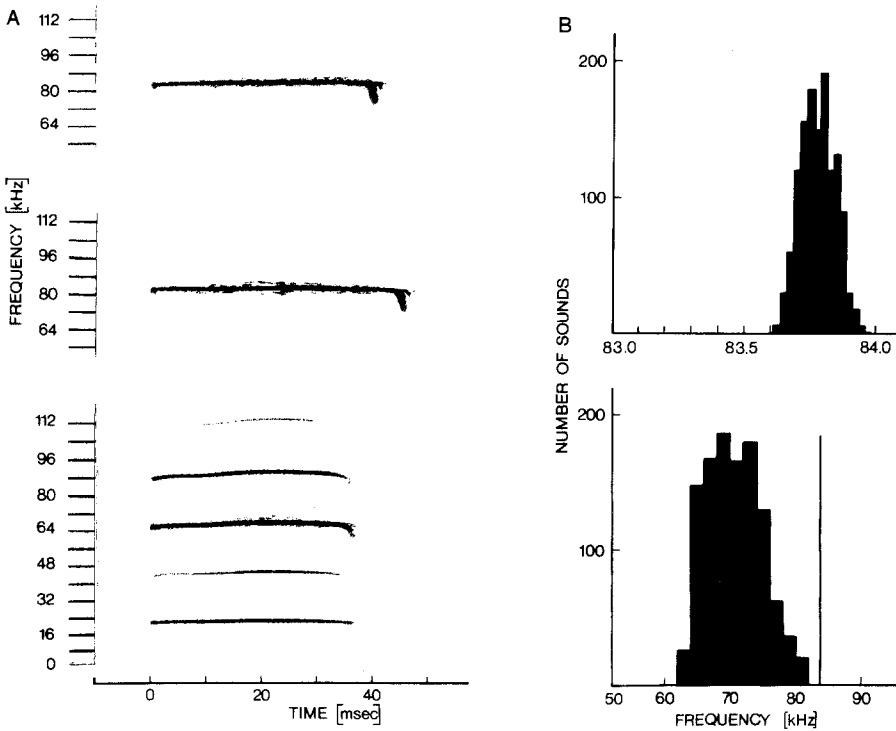
Fig. 1. A Sonagrams of orientation sounds before the transection of the superior laryngeal nerve (top) and after the unilateral (middle) or bilateral transection (bottom). **B** Distribution of frequencies of emitted CFs before (upper graph) and after bilateral section of the superior laryngeal nerve (lower graph). The upper histogram is represented in the lower graph by the vertical line at about 84 kHz

that is, the compensation was not accurate and was not stable and the lowering of the emitted CF could reach 5–6 kHz for Doppler-shifts of only 2–3 kHz.

Cutting both superior laryngeal nerves showed a prominent effect on the frequency spectrum of the emitted sounds, which than contained several harmonics. The frequency pattern within the spectral components was the same as in the usual orientation sounds: a long CF-component followed by a short FM-component (Fig. 1A, bottom sonagram). The frequencies of the spectral components drifted with time. The bottom figure of. Figure 1 A shows one of the typical sonagrams of orientation sounds after cutting the superior laryngeal nerves on both sides. The spectral components were always harmonically related to each other. The fundamental differed among different sounds emitted by the same bat. The frequencies of the fundamental components lay between 12 and 42 kHz for all bats at different postoperational times. Spectral components falling in the frequency range between 35 and 45 kHz were generally much weeker in intensity than the other components. No common fundamental frequency for all bats could be found. Figure 1 B represents a distribution of frequencies of the emitted CFs before and after cutting of both superior laryngeal nerves. The postoperational distribution was established for the strongest com-

ponent near the resting frequency of the bat for a 5 min long recording period. The scatter in frequency of the emitted CF after surgery was much larger (about 4.2 kHz standard deviation) than the preoperational one (less than 0.2 kHz). These distributions give evidence that compensation for frequency shifts covering about a range of 4–6 kHz is not possible in the bats with denervated cricothyroid muscles. The frequency pattern within the sounds was usually slightly disturbed in the first 2 or 3 days after surgery, and the CF component could than show frequency changes of about 2–4 kHz.

The intensity of the harmonic components with the exception of that around 80 kHz decreased with time. In two out of four bats, one month after surgery the spectral components nearly disappeared and only the harmonic at about 80 kHz remained. The frequency of this harmonic was not stable but drifted in a range of 4–6 kHz below the resting frequency. After sacrificing the bats, reinnervation of the superior laryngeal nerves was not found.

Electrical Activity of the Cricothyroid Muscle

The experiments described above gave evidence that the cricothyroid muscles are controlling the frequency of the emitted orientation sounds and are important for Doppler-shift compensation. The electrical activity of these muscles was studied in conjunction with the frequency of the emitted CF during Doppler-shift compensation. From the cricothyroid muscles, two types of activities were recorded at different places. Figure 2 demonstrates the activity as recorded from the caudal part of the muscle in A and the activity from the cranio-lateral part in B. The activity has been passed through a window discriminator and the activity of a few muscle fibers has been filtered out. A sustained activity (graph A) begins about 50 ms prior to the onset of the vocalization and continues during the sound. In some cases the activity stopped 10–20 ms prior to the end of the orientation sound. The activity before the beginning and the end of the orientation sound (graph B) starts about 20 ms before the sound onset, lasts about 10 ms. The activity at the end of the sound coincides with the final frequency-modulated part of the orientation sound.

The relation between the frequency of the emitted CF and the number of spikes per sound was established for the sustained activity during Doppler-shift compensation. Slow transitions in emitted frequency (2–5 kHz in 10–20 s) yielded clear dependence of the number of spikes per sound on the emitted frequency. As the larynx moves considerably at every vocalization the recording situation could change in a long recording period. The measurements of the spike number per sound were therefore made with transitions of frequency lasting not longer than 20 sec rather than with long lasting constant Doppler-shifts.

Figure 2C gives an example of the spike count versus frequency function obtained when the frequency of the emitted CF changed from 5 kHz below the resting frequency to the resting frequency. The measurements did in general not cover such a large frequency span of 5 kHz, but the dependence measured with smaller frequency spans fit in the curve represented in the figure. The maximum muscle activity was recorded when the bat emitted the CF signal
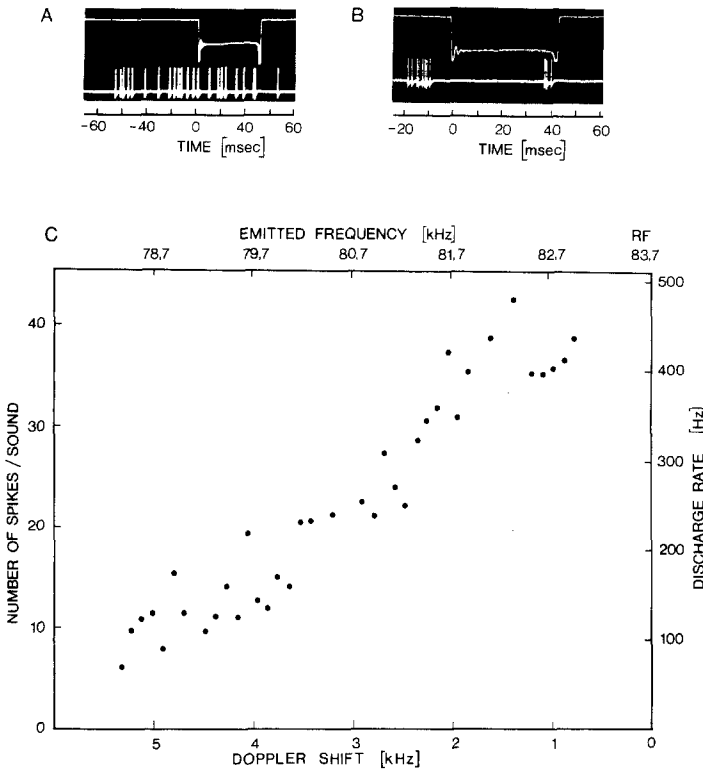
Fig. 2 A–C. Action potentials of the cricothyroid muscle fibres recorded from its caudal part **A** and cranio-lateral part **B**. These action potentials are the largest ones in the gross activity, which were selected with a window discriminator. **C** Relationship between the number of spikes per orientation sound of the cricothyroid muscle fibres and the frequency of the emitted CF. The frequency of the emitted CF varied with the Doppler-shift introduced in artificial echoes. Note that the action potentials were not recorded from a single fibre, but from a few fibres (see text)

at its resting frequency. When the emitted CF was lowered during compensation, the muscle activity decreased linearly with it. In different bats the slope of the curve in Figure 2 C was different what is due to different recording conditions as the number of muscle fibers contributing to the recording can change. The linear dependence of the spike rate on frequency, however, was always found.
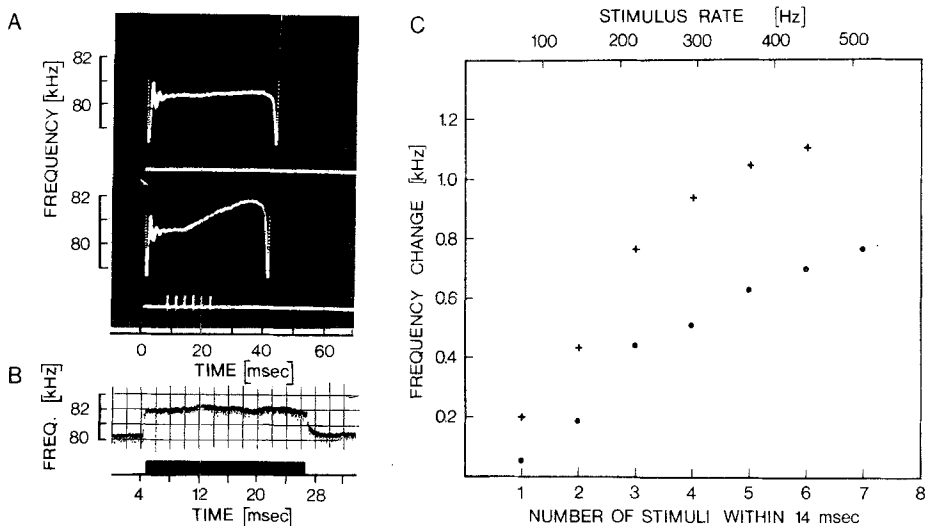
Stimulation of Cricothyroid Muscles

The denervation experiments and the recordings of the electrical activity of the cricothyroid muscles indicated that these muscles controlled the frequency of the emitted CF during Doppler-shift compensation. Electrical stimulation of the cricothyroid muscle should therefore change the frequency of the emitted CF. The electrical stimuli were continuously delivered at certain rates or delivered as a short train of pulses triggered at the beginning of the emitted

orientation sound. The frequency of the emitted CF could be altered either by the acoustical playback of frequency-shifted echoes or by electrical stimuli. When the bat emitted the CF signal at its resting frequency and got no Doppler-shifts in echoes, the electrical stimuli induced a limited rise of the outgoing frequency which was 500 Hz at maximum. This upper limit of the frequency of the emitted CF corresponds well to the behavioural finding that the bat cannot raise its emission frequency in order to compensate for negative Doppler-shifts.

When the bat emitted a sound with a CF lower than its resting frequency, induced by positive Doppler-shifts in its echoes, the electrical stimuli of the cricothyroid muscles increased the frequency of the emitted CF. The rise in frequency by the electrical stimuli depended on the intensity and the rate of the stimuli. Figure 3 A shows how the frequency changed during the CF component of the orientation sound. The frequency started to change 10–20 ms after the beginning of the stimulation and reached the maximum after another 20 ms.

The diagram in Figure 3 C gives the dependence of the maximum frequency change during the CF component on the number of stimuli delivered during 14 msec in the first part of the orientation sound. The maximum frequency change that could be induced by such stimuli was about 1,200 Hz.

Whereas stimuli triggered by the orientation sound changed the frequency within the constant frequency component, the sound frequency as a whole could be shifted when continuous electrical stimuli were applied. This was done while the bat was compensating for a positive 3 kHz-Doppler-shift. Switching

Fig. 3A–C. Effect of the electrical stimulation of the cricothyroid muscles on the frequency of the emitted CF during the Doppler-shift compensation of 3 kHz. A An orientation sound which compensated a Doppler-shift of 3 kHz without (upper graph) and with a train of electrical stimuli (lower graph). B Change in the frequency of the emitted CF due to a long train of electrical stimuli delivered at a rate of 400 Hz during the compensation of a 3 kHz Doppler-shift. C Relationship between the frequency of the emitted CF and the number of electrical stimuli delivered during the first 14 ms of the sound

on the continuous electrical stimulus train (400 Hz stimulus rate) induced a rapid change of the emitted frequency as illustrated in Figure 3 B. It was not possible to establish a clear functional dependence of the frequency rise on the intensity and the rate for continuous stimulation, as small intensities produced frequency rises with relatively big fluctuations and at higher intensities the frequency rise ranged always between 1.6 and 2.5 kHz and gave no reproducible functional dependence on the stimulus rate. Qualitatively the stronger stimulation (intensity or rate) led always to larger frequency rises in the emitted frequency.

In most cases the frequency of the orientation sounds rised immediately upon electrical stimulation (within 100–300 ms), i.e. the first sounds after the onset of stimulation were emitted at higher frequencies. The maximum frequency step between two sounds due to electrical stimulation was 2.2 kHz. After the stimulation ceased, the frequency of the orientation sounds came back to the initial value (3 kHz below the resting frequency) rather slowly, but the time course was not at all stereotypical and ranged between 1 and 10 s.

The tetanus fusion frequency was tested either by trains of electrical stimuli delivered only during the orientation sounds or by giving continuous electrical stimuli. In both situations the tetanus fusion frequency was determined as the lowest rate of the stimuli at which the frequency modulation of the emitted CF due to each stimulus evoked muscle contraction was not detectable. The continuous stimulation yielded a tetanus frequency of about 200 Hz, whereas bursts of stimuli during the orientation sounds gave a tetanus fusion frequency of about 400 Hz.

Discussion

The denervation experiments in the CF-FM bat, *Rhinolophus ferrumequinum*, yielded similar results as Novick and Griffin (1961) found in FM-bats and some CF-FM bats. The inferior laryngeal nerve innervating the intrinsic muscles of the larynx could be cut without effects on the orientation sounds and Doppler-shift compensation. In humans the inferior laryngeal nerve is very important in speech control and section leads to heavy impairment of the speech production. Bilateral section of the inferior laryngeal nerve, however, caused always suffocation probably due to adduction of the vocal cords in horseshoe bats as known also from human surgery (King and Gregg, 1948), so that its functional role in the emission of the orientation sound was not adequately studied. After bilateral section of the superior laryngeal nerve innervating the cricothyroid muscles, orientation sounds emitted showed a high instability in the frequency of the orientation sound and contained several harmonics. However its frequency pattern was maintained the same as in the normal orientation sound. The fundamental frequency differed widely in different sounds and in different bats in a range from 12 to 42 kHz. Doppler-shift compensation was not possible. The cricothyroid muscles are therefore necessary for maintenance of a stable resting frequency and to control the frequency of the orientation sounds for Doppler-shift compensation. In addition they may be involved in the control of sound

transmission, so that only one strong harmonic at about 83 kHz is emitted. The role of the cricothyroid muscles in the Doppler-shift compensation was also demonstrated by the recordings of the activity of these muscles during compensation of frequency shifts in the echoes. A linear dependence of the number of spikes per sound in multiple fiber recordings and the transmitted frequency was found for a range from the resting frequency to 5 kHz below it. The muscle action potentials began about 50 ms before the vocalization. During these 50 ms the tension necessary to emit the CF probably builds up in the muscles and is then maintained throughout the orientation sound. The experiments give no direct answer to the question whether the phasic activities recorded from the cranio-lateral part of the cricothyroid muscle before the onset and at the end of the sounds are functionally related to the emission of FM-components in the orientation sound.

Morphologically the structure of the cricothyroid muscles in *R. ferrumequinum* is only known from gross preparations (Beissmann, 1975). After this work the muscles devide into two muscle bundles: one bundle stretching in cranio-caudal direction on the ventral surface of the larynx (pars recta), which covers the other muscle bundle originating at the medio-ventral portion of the cricoid and radiating in cranio-lateral direction to the thyroid cartilage (pars obliqua). The sustained activity was recorded from the surface of the pars recta, whereas the phasic activity before the onset and the end of orientation sounds came probably from the pars obliqua. A detailed functional description of these muscle bundles in frequency control or Doppler-shift compensation is not possible on the basis of the present experiments.

Electrical stimulation of the cricothyroid muscles resulted in an increase in the frequency of the emitted CF as was expected from the recording experiments. The frequency of emitted CF was never higher than 400–500 Hz above the resting frequency even at strong stimulus intensities or high stimulus rates. This corresponds well to the behavioural results showing that the Doppler-shift compensation system compensates only for positive frequency shifts and that the bat cannot be caused to emit frequencies above the resting frequency when negative frequency-shifts are played back in the echoes (Schuller et al., 1975). The unidirectional compensation mechanism for Doppler-shift is paralleled by the inability of the larynx to produce frequencies higher than the resting frequency as long as the superior laryngeal nerves are intact.

During Doppler-shift compensation the electrical stimulus rate was quantitatively related to the rise of the frequency of the emitted CF. But the resting frequency was never fully reached upon electrical stimulation, which may be due to the small size of the stimulation electrodes. The stimulation did probably not reach all the muscle fibres involved in the frequency control.

The contraction speed of the cricothyroid muscles in terms of the possible speed of frequency change in the orientation sounds is much higher than the speed needed to follow high repetition rates in sound bursts or to track quick frequency-shift changes in the echoes. The control system for Doppler-shift compensation shows an amplitude response with a cut-off frequency of about 0.65 Hz modulation frequency for positive frequency-shifts of 1 kHz (Schuller et al., 1975), which is a much slower response than could actually be followed by

the contraction speed of the cricothyroid muscles. The larynx is definetely not the speed limiting component of the Doppler-shift compensation system.

Suthers and Fattu (1973) report that the mechanical response of the cricothyroid muscles shows partial relaxation between stimuli up to a repetition rate of 240 per s applied to the motor nerve. We did not measure the mechanical response but the frequency change during the CF component of the orientation sounds upon repetitive electrical stimulation. The tetanus fusion frequency was about 200 Hz when the stimuli were applied continuously to the muscle but it was about 400 Hz when the stimuli were present only during the vocalization. Continuous stimulation caused quicker fatigue of the muscles than the interrupted stimulation did. In comparison with other mammalian larynx, the cricothyroid muscle of this bat contracts about 4–5 times faster than for example that of the dog (Hast, 1966).

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