

PERIPHERAL CONTROL OF ACOUSTIC SIGNALS IN THE AUDITORY SYSTEM OF ECHOLOCATING BATS

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SUMMARY

Many species of echolocating bats emit intense orientation sounds. If such intense sounds directly stimulated their ears, detection of faint echoes would be impaired. Therefore, possible mechanisms for the attenuation of self-stimulation were studied with *Myotis lucifugus*. The acoustic middle-ear-muscle reflex could perfectly and transiently regulate the amplitude of an incoming signal only at its beginning. However, its shortest latency in terms of electromyograms and of the attenuation of the cochlear microphonic was 3.4 and 4.8 msec, respectively, so that these muscles failed to attenuate orientation signals by the reflex. The muscles, however, received a message from the vocalization system when the bat vocalized, and contracted synchronously with vocalization. The duration of the contraction-relaxation was so short that the self-stimulation was attenuated, but the echoes were not. The tetanus-fusion frequency of the stapedius muscle ranged between 260 and 320/sec. Unlike the efferent fibres in the lateral-line and vestibular systems, the olivo-cochlear bundle showed no sign of attenuation of self-stimulation.

INTRODUCTION

When we emit a sound which measures about 110 dB SPL at 10 cm in front of the mouth, the sound pressure level at the entrance of the external auditory meatus is about 105 dB. Apparently, we are stimulating our own ears by intense sounds when we vocalize, but we never perceive the self-vocalized sounds to be disturbingly loud. When the same sound is delivered from an external source so that it measures 105 dB SPL at the entrance of our external auditory meatus, we feel it to be loud. This indicates that the amount of self-stimulation is attenuated by some mechanisms operating synchronously with vocalization.

In many species of echolocating bats, orientation sounds are about 110-120 dB SPL when these are monitored at 8 cm in front of the bat's mouth. If such intense sounds directly stimulated their ears, detection of faint echoes from short distances would be impaired. In humans and bats, the self-stimulation is probably important in controlling vocalization. However, it need not be unnecessarily intense and should be considerably attenuated.

Air-borne sound may be attenuated by some mechanical means at the external and middle ears. This attenuation, however, may be insufficient. The inner ear may receive intense self-vocalized sound through middle-ear ossicles and other bones, so that some neural attenuation of self-stimulation may also be necessary. As mechanisms

for attenuation of self-stimulation, at least the following four are conceivable. (1) The external auditory meatus is closed at its entrance by the pinna and/or protuberance; this is *mechanical shielding*. (2) The middle-ear muscles (MEMs) contract synchronously with vocalization; this is *muscular attenuation*. (3) The olivo-cochlear bundle (OCB) becomes synchronously active with vocalization and attenuates the activity of hair cells and primary auditory neurones; this is *peripheral neural attenuation*. (4) The activity of higher order auditory neurones is inhibited by signals from the vocalization system; this is *central neural attenuation*. Each of the above mechanisms may be divided into two types: (a) a mechanism mediated by sensory cells (reflex) and (b) a mechanism operating without sensory cells (non-reflex). For instance, the muscular attenuation may be performed by the acoustic MEM reflex and/or MEM contraction initiated by signals originating from the vocalization centre. The latter may be called the *vocal MEM activity* or *contraction*, to distinguish it from the acoustic MEM reflex.

In the little brown bat (*Myotis lucifugus*), the pinna and protuberance close the external auditory meatus when an intense sound is delivered (Wever & Vernon, 1961). It is, however, not yet known whether this mechanical shielding occurs synchronously with vocalization. In cats and bats, the MEMs show action potentials prior to and during vocalization. The MEMs apparently contract synchronously with vocalization and attenuate self-stimulation to some extent (Carmel & Starr, 1963; Henson, 1965; Suga, Simmons & Shimozaawa, 1974). Studies on the vocal MEM activity are, however, still very superficial. The frequency characteristic of attenuation by the vocal MEM contraction and differences in vocal MEM activity between the stapedius and tensor tympani muscles remain to be studied.

In the acoustico-lateralis system, sensory cells of the canal organ, vestibular organ and cochlea show common anatomical features. These sensory cells are innervated not only by afferent fibres, but also by efferent fibres. In the lateral-line organ of the African aquatic frog *Xenopus laevis* (Russell, 1971*a*) and the vestibular organ of the goldfish (Klinke & Schmidt, 1970), the efferent fibres are inhibitory and become active prior to and during body movement so that self-stimulation is greatly attenuated. In mammals, the OCB consists of axons of inhibitory neurones. It is divided into two groups, the crossed and uncrossed OCBs, which terminate primarily on the hair cells and the dendrites of primary auditory neurones, respectively, and cause pre- and post-synaptic inhibition of the activity of primary auditory neurones (see Rossi's review, 1967). In cats and guinea pigs, the electrical stimulation of the crossed OCB causes an increase in CM and a decrease in N_1 (Desmedt & Monaco, 1961; Fex, 1962), while the stimulation of the uncrossed OCB evoked no change in CM and a decrease in N_1 (Desmedt & La Grutta, 1963; Fex, 1967). The difference in the effect between the stimulation of the crossed and uncrossed OCBs nicely fit to the difference in the innervation modes between these two groups of efferent fibres. The inhibitory synaptic transmitter appears to be acetylcholine in the lateral-line organ (Russell, 1971*b*) and the crossed OCB (Fex, 1973). In *Myotis*, it has been shown that efferent nerve endings terminate on hair cells and dendrites of primary auditory neurones, as in other mammals (Kimura, 1966). It is thus expected that if the OCB becomes active synchronously with vocalization, an increase in CM and a decrease in N_1 would synchronously occur with vocalization.

In *Myotis*, the presence of a mechanism for the central neural attenuation of self-stimulation has been demonstrated (Suga & Schlegel, 1972, 1973), and it has been determined that this attenuation takes place in the nucleus of the lateral lemniscus (Suga & Shimozawa, 1974). The time course of the neural attenuation and the pathway mediating it remain to be studied.

Bats of the genus *Myotis* emit very short orientation sounds at a high repetition rate of up to 200/sec and listen to echoes returning between the emissions, so that mechanisms for the attenuation of self-stimulation are expected to be specialized in these animals. In the present paper, the physiological properties of the acoustic MEM reflex and vocal MEM activity of *Myotis* are described in Parts I and II, respectively. Part II includes data obtained from the electrical stimulation of the middle-ear muscles. The results of the test of whether the OCB attenuates the self-stimulation are described in Part III. For convenience, the following abbreviations are used: CM (cochlear microphonic) EMG (electromyogram) MEM (middle-ear muscle), N_1 (summated response originating from primary auditory neurones), OCB (olivo-cochlear bundle), SM (stapedius muscle), and TM (tensor tympani muscle).

MATERIALS AND METHODS

Experimental subjects were 22 little brown bats (*Myotis lucifugus*) caught in caves in Missouri. The animals (6–7 g) were anaesthetized with ether only during surgery, unless otherwise stated. A nail, 1.8 cm long, was mounted on the exposed skull of the bat with dental cement. Then the animal was brought into an echo-reduced sound-proofed room and its head was immobilized by fixing the shank of the nail onto a metal rod with a set screw. A tiny hole was made in the skull covering the inferior colliculus. Through this hole, a tungsten-wire electrode was inserted into the modiolus and positioned so as to record N_1 with a large amplitude. Then, the animal was placed ventral side up and surgery was performed to expose the ventral part of the auditory bulla after cutting the omohyoid muscle. The CM and N_1 were recorded with a single tungsten-wire electrode placed at the rim of the round window through a hole made in the auditory bulla. The amplitude of the N_1 was usually larger when recorded in the modiolus than at the round window, so that the former was usually used for measurements. The EMGs of the SM and TM were also recorded with tungsten-wire electrodes placed on the ventral side of these muscles. The EMGs evoked by 8 tone bursts or click trains were often full-wave-rectified and averaged with a computer (Nicolet 1070) to show the response pattern quantitatively. Action potentials of single muscle fibres were recorded with micropipette electrodes filled with 3 M-KCl solution. The responses of these fibres to acoustic stimuli were expressed when necessary in post-stimulus-time histograms. The recording of all the above electrical activities was performed without anaesthetic (i.e. between 2 and 19 h after the termination of ether anaesthesia) in a sound-proofed room, the inner wall of which was covered with fibre glass to reduce echoes. The micromanipulators and amplifiers near the subject were covered with cheese cloth to reduce echoes from them.

The electronic instruments used to generate acoustic stimuli were the same as those in previous experiments (Suga, 1968). For the measurement of threshold curves, pure tone bursts of 10–150 kHz with a 0.5 msec rise-decay time and a 4–80 msec duration

were repeatedly delivered at a rate of 1.5/sec from a condenser loudspeaker placed 60 cm in front of the bat unless otherwise described. The amplitude of the tone bursts was calibrated with a quarter-inch microphone (Brüel & Kjaer, 4135) placed at the bat's ears and was expressed in dB SPL (sound pressure level referred to 0.0002 dyne/cm² r.m.s.).

For electrical stimulation of the SM and TM, a train of 0.1 msec electric pulses was delivered to these muscles through tungsten-wire electrodes (with 1–2 megohm resistance) using an electronic stimulator (Grass Co. S88) and stimulus-isolation unit (Grass Co. SIU). Each tungsten-wire electrode was connected with an a.c. pre-amplifier and was placed on or inserted into each muscle and the EMG was recorded. Then the electrode was connected with the stimulus-isolation unit. This procedure was essential to stimulate the SM because it was covered by the hyoid muscle and was not directly visible, but it was not essential for the stimulation of the TM. The repetition rate of the train was 1.0–1.5/s. The duration of the train was about 80 msec. The rate of the electric pulses within the train varied from 10 to 400/sec, and their amplitude ranged between 1 and 10 V.

The time course and the amount of the MEM contraction were measured in terms of the attenuation of the CM evoked by a test tone. The tension of the MEM was not monitored at all. The amount of attenuation of the CM by the MEM contraction was expressed in decibels. In order to do so, the input-output function of the CM without MEM contraction was first plotted. Then, the difference in amplitude between the CMs evoked by a given pressure level with and without the MEM contraction was measured. The difference was expressed in dB by referring to the input-output function of the CM obtained without the MEM contraction (see Fig. 4 for details).

The excitation of the OCB causes an increase in CM amplitude and a decrease in N_1 amplitude, while the MEM contraction attenuates both the CM and N_1 . Therefore, the vocal MEM contraction interferes with the observation of the changes in the CM and N_1 which may be evoked by the OCB when the animal vocalizes. Thus, the MEMs should be destroyed prior to this kind of experiment. The SM was mechanically destroyed with a needle at the outside of the auditory bulla. The TM, on the other hand, was inactivated with an electric cauterizer. It was then confirmed under a dissection microscope that the MEMs did not contract even for intense acoustic stimuli. Both the CM and N_1 evoked by tone pulses delivered at rate of either 200/sec or 500/sec were simultaneously recorded with tungsten-wire electrodes placed on the round window and in the modiolus, respectively. The pressure level of the test stimulus was set at the level where the amplitudes of the CM and N_1 evoked by it were at the middle of the dynamic range of these input-output functions. Thus, the change in their amplitude would be easily noticed if it occurred synchronously with vocalization.

To elicit vocalization, the bat was mechanically stimulated by touching its back or its tail (or both) with a brush or by moving the plastic ball on which the bat rested. Sounds emitted by the bat were monitored with a quarter-inch microphone (Brüel & Kjaer, 4135) placed 10 cm in front of the bat's mouth.

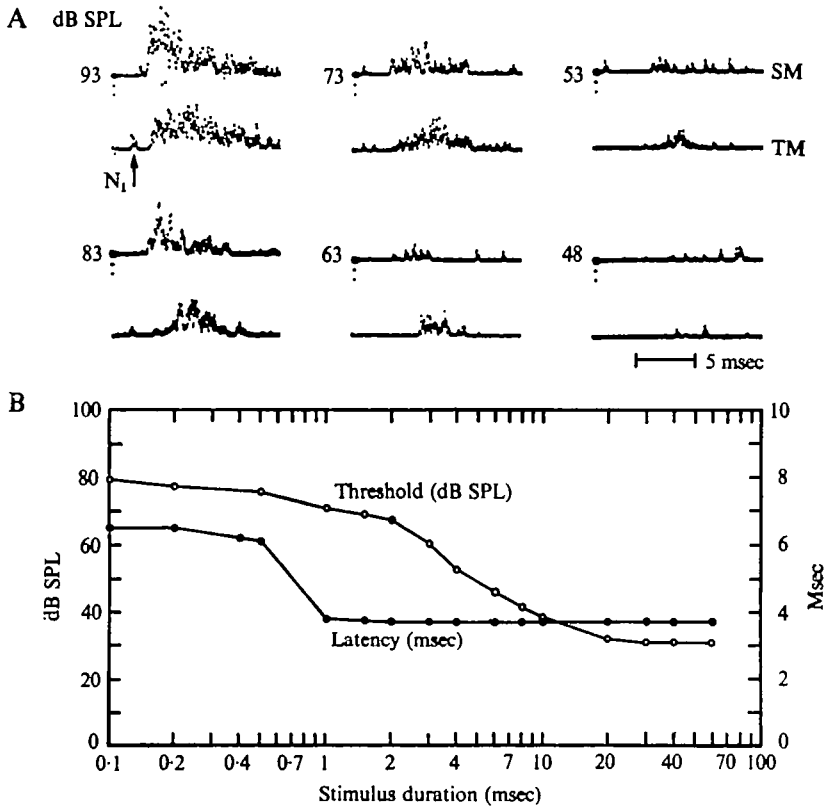


Fig. 1. A, post-stimulus-time (PST) histograms representing the responses of the stapedius muscle (SM, upper trace) and the tensor tympani muscle (TM, lower trace) to a 35.0 kHz tone pulse with a 4.0 msec duration and a 0.5 msec rise-decay time. Responses (EMGs) evoked by the stimulus delivered 8 times at a rate of 1.5/sec were full-wave rectified, added, and expressed by the PST histograms. The bin width of the histograms is 0.04 msec. The figures to the left of the histograms represent stimulus amplitude in dB SPL (decibels referred to 0.0002 dyne/cm² r.m.s.). B, changes in threshold and latency of the acoustic MEM reflex in terms of the EMG of the SM as a function of a duration of a tone burst of 30.0 kHz and 90 dB SPL. The rise-decay time of the tone burst was 0.2 msec at durations longer than 0.5 msec, but it was less than 0.05 msec at durations less than 0.5 msec. The repetition rate of the stimulus was 1/sec.

RESULTS FOR PART I: ACOUSTIC MIDDLE-EAR-MUSCLE REFLEX

Latency of acoustic MEM reflex

When an acoustic stimulus was delivered, MEMs showed action potentials during the stimulus (Fig. 1A) and contracted to attenuate sound energy transmitted to the inner ear through the ossicular chain (Fig. 2). In this acoustic reflex arc, there are at least five intervening synapses. The shortest latency of the MEM reflex in terms of the EMG was 3.4 msec for the stapedius muscle (SM) and 4.4 msec for the tensor tympani muscle (TM). It was usually 3.8–4.4 msec for the SM and 4.8–5.0 msec for the TM. The SM usually showed the EMG at least 0.6 msec prior to the TM. When the stimulus amplitude was attenuated, the latencies of the responses of both muscles lengthened. The lengthening in latency was always more prominent for the TM than for the SM, even in the case where the thresholds of both muscles to an acoustic stimulus were the same (Fig. 1A).

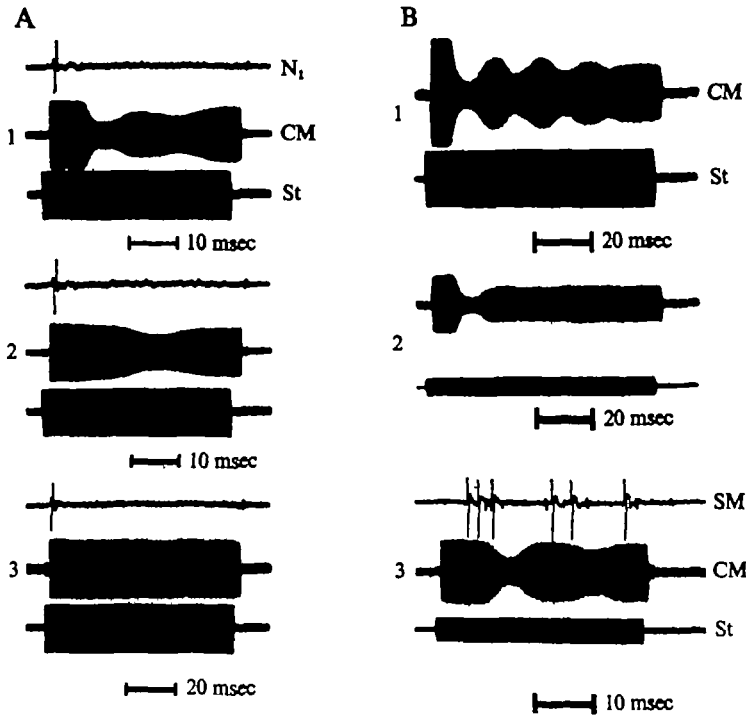


Fig. 2. Amplitude-modulation of the cochlear microphonic (CM) by the acoustic middle-ear-muscle (MEM) reflex. In A, a stimulus (St) is 30.0 kHz, 90 dB SPL and 40 or 80 msec long. 1, the MEMs are intact; 2, the SM is destroyed, but the TM is intact; 3, both the MEMs are destroyed. In B, a stimulus is 30.0 kHz and 40 or 80 msec long, and the MEMs are intact. 1, 90 dB SPL; 2, 80 dB SPL; 3, 90 dB SPL. N_1 , summated action potential originating from primary auditory neurones; SM, action potentials of a single stapedius muscle fibre. No correction of an acoustic delay (1.7 msec) is made in the photographs.

The latency of the MEM reflex became longer when the duration of a stimulus became shorter than a few milliseconds. In Fig. 1 B, the latency of the SM is plotted as a function of duration of a 30 kHz, 90 dB SPL sound. The latency is 3.7 msec for a 2.0 msec duration, but it is 6.5 msec for a 0.2 msec duration.

When the amplitude of a sound between 20 and 40 kHz was more than 90 dB SPL, the summated responses of auditory nerve fibres sometimes became prominent, so that not only N_1 , with a peak latency of 0.6–0.8 msec, but also potential changes following the N_1 were recorded. These neural responses could be discriminated from the EMG because they appeared with a relatively constant latency, while the EMG fluctuated in latency. If these neural responses were considered to be a part of the EMG, the latency of the acoustic reflex would be 1.2–1.8 msec, which is too short to be a latency of a five-synapse reflex arc.

The contraction of the muscles started to occur 1.5–2.5 msec after muscle action potentials. The latency and time course of the contraction were measured in terms of the attenuation of the cochlear microphonic (CM) evoked by a tone burst before and after the destruction of the SM (Fig. 2 A). The shortest latency of the acoustic MEM reflex in terms of attenuation was 4.8 msec for the SM and 6.0 msec for the TM. It was usually between 5.8 and 6.2 msec for the SM and between 6.0 and 8.0 msec for

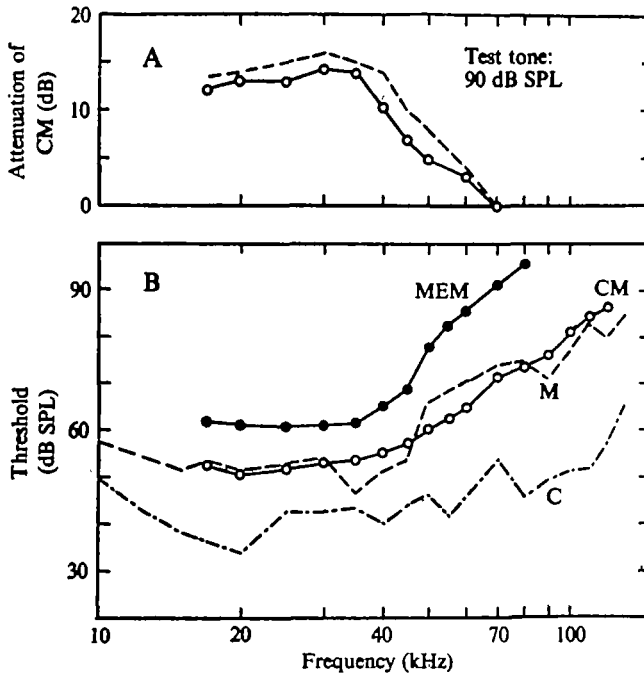


Fig. 3. A, frequency-attenuation curve of the acoustic MEM reflex measured with a tone burst of 90 dB SPL, 100 msec in duration. The amount of attenuation was measured 80 msec after the onset of the tone burst at which the MEM contraction reached a quasi-steady state. The solid curve represents the average of the data obtained from four bats, while the dashed curve shows the largest attenuation obtained from them. B, threshold curves of the CM and the acoustic MEM reflex in terms of the attenuation of the CM. The solid curves represent the average of the data obtained from nine bats, while the dashed curves show the lowest threshold obtained from them. M and C mean MEM and CM, respectively. The ordinate represents the amount of attenuation in decibels in A and stimulus amplitude at threshold in dB SPL in B. The abscissae represent stimulus frequency in kilohertz.

the TM. The maximum attenuation by the SM occurred with a 7.4 msec latency in the shortest case, but it was usually 9–15 msec. The maximum attenuation by the TM occurred with a long latency, 15 msec or longer (Fig. 2A₂). In *Myotis*, orientation sounds are always shorter than 4 msec, so that the MEM reflex fails to attenuate the reception of intense orientation signals emitted by the bat.

When both the muscles were destroyed, the amplitude of the CM became steady during the stimulus (Fig. 2A₃). That is, the attenuation of an intense sound completely disappeared after the destruction of the middle-ear muscles. There was no indication of mechanical attenuation by the pinna and/or protuberance in addition to the muscular attenuation. Stimulus levels higher than 100 dB SPL were not used in the present experiments.

Attenuation of acoustic signals by MEM reflex

The amount of attenuation of incoming acoustic signals by the MEM reflex varied with the frequency and amplitude of the signals. The frequency of a tone burst at 90 dB SPL was changed and the amount of attenuation by the MEM reflex was measured. The amount of attenuation was expressed by the difference between 90 dB and the sound pressure level at which the amplitude of the CM without the MEM

contraction was equal to that with the MEM contraction at 90 dB SPL. In Fig. 3A, the curve indicates that the maximum attenuation takes place at about 30 kHz and the attenuation diminishes with a slope of about 17 dB/octave with the increase in frequency beyond 35 kHz. The attenuation by the muscles was hardly noticeable at frequencies beyond 70 kHz, unless the sound pressure level was higher than 90 dB. The lack of attenuation of sounds higher than 70 kHz at 90 dB SPL was simply due to the fact that the threshold of the MEM reflex was high for sounds above 70 kHz and the muscles did not contract. As described later, the MEMs contract prior to and during vocalization and do attenuate sounds at higher than 70 kHz (Fig. 14B). The frequency-attenuation curve of the acoustic MEM reflex is different from that of the vocal MEM contraction to be presented later.

When the frequency of a tone burst was higher than 40 kHz, the contraction of the MEMs reached a peak at the beginning of the reflex, then monotonically reduced down to a plateau. When the frequency was lower than 40 kHz, however, oscillation of the contraction was usually observed for intense sounds as indicated by the envelope of the CM (Fig. 2B). The oscillation was due to the inert properties of the MEM reflex, which was a negative feedback loop. That is, when an intense low-frequency signal was once greatly attenuated by the MEM reflex, the reflex arc was not activated, i.e. it was self-suppressed. Then, the sound energy going into the inner ear increased, which in turn activated the reflex arc. Thus, the oscillatory contraction occurred. Fig. 2B₃ represents the relationship between action potentials of a single SM fibre and the oscillatory amplitude modulation of the CM by the MEMs. The oscillation could occur several times, decreasing in its amplitude, and finally disappeared (Fig. 2B₁). The amplitude of the CM usually reached a quasi-steady state 80 msec after the onset of a stimulus. When the stimulus amplitude decreased, the number of oscillations became small (Fig. 2B₂). The oscillation thus depended on both the amount of attenuation and the sound-pressure level, as found in humans by Møller (1962).

The relationship between the sound-pressure level and muscular attenuation was studied by measuring the amplitude of the CM evoked by an 80 msec-long tone burst at the moments when the MEMs did not contract, maximally contracted, and contracted in a quasi-steady state. The amplitude of the CM measured at the three locations (see the inset in Fig. 4A) varied with the pressure level of a tone burst as shown in Fig. 4A. Interestingly, the amplitude of the CM at the moment of the maximum contraction of the MEMs stays about the same regardless of the increase in the stimulus level at above 76 dB (curve *b*), while the amplitude at the quasi-steady state monotonically increased with the stimulus level (curve *c*). In order to express the amount of attenuation in dB, the attenuation in dB was calculated as a function of sound pressure level by utilizing curves such as in Fig. 4A. For instance, the amount of attenuation of a 30 kHz, 91 dB tone burst is 14 dB at the quasi-steady state, because the amplitude of the CM for the 91 dB tone burst during a quasi-steady MEM contraction is equal to that for a 77 dB tone burst without the MEM contraction (see the dashed arrows in Fig. 4A).

Fig. 4B represents attenuation-pressure level curves measured with sounds of different frequencies. The curve for the maximum MEM contraction (curve *c'*) indicates that an increase in the sound pressure level beyond 70 dB causes nearly the same amount of attenuation of the CM by the MEMs. In other words, the complete

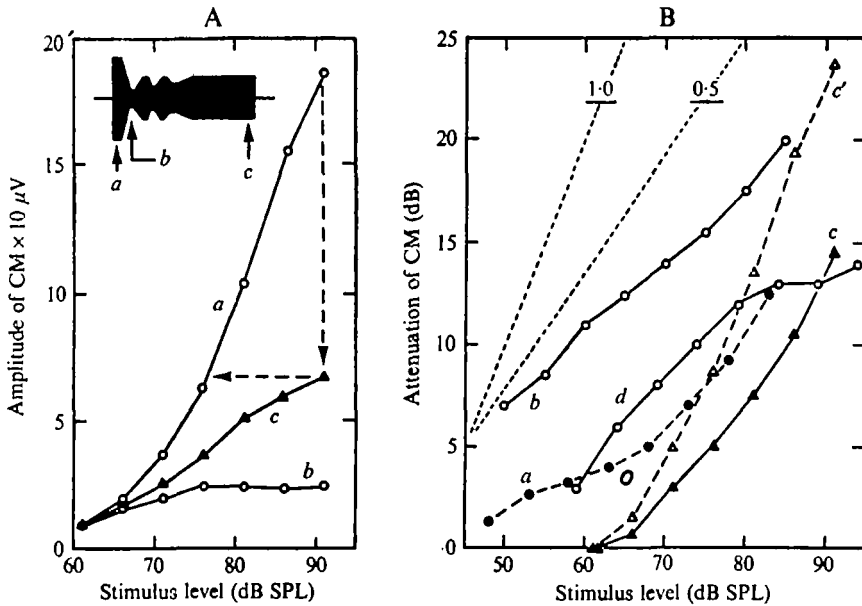


Fig. 4. The relationship between the attenuation of the CM by the acoustic MEM reflex and the stimulus level. In A, the response-amplitude functions of the CM evoked by a 30.0 kHz tone burst are shown. These were measured before the MEM contraction (*a*), at the peak of the MEM contraction (*b*) and at the quasi-steady state of the MEM contraction (*c*). See the inset. In B, the amount of attenuation of the CM is plotted against a stimulus amplitude. Attenuation-amplitude functions *a*, *b*, *c* and *d* were obtained during the quasi-steady state of the MEM contraction, while function *c'* was obtained at the peak of the contraction. The dashed straight lines indicate the slope of the function when a feedback gain is either 1.0 or 0.5. *c* and *c'* were obtained from the data shown in A. The test tone burst was 17.0 kHz for *a*, 20.1 kHz for *b*, 30.0 kHz for *c* and *c'*, and 40.0 kHz for *d*.

regulation of the sound pressure level is transiently performed by the MEMs. Thus, a feedback gain is about 1.0. The dynamic range of the complete regulation was at least 20 dB. The feedback gain was, however, not 1.0 for the quasi-steady state of the contraction, but it was about 0.5 when calculated on the CM amplitude measured at about 80 msec after the onset of the tone burst (curve *c*). In curves *a*, *b*, and *d*, which were obtained with 3 sec long tone bursts between 17 and 40 kHz, the feedback gain ranged between 0.3 and 0.4.

Threshold of acoustic MEM reflex

In nine bats, threshold curves of the CM and the acoustic MEM reflex in terms of the attenuation of the CM were measured with an 80 msec tone burst (Fig. 3B). The threshold curve of the CM showed a minimum threshold of 51 dB SPL at 20–25 kHz on the average. On the other hand, the threshold curve of the MEM reflex showed a minimum threshold of 61 dB SPL at 20–35 kHz on the average. The curve of the MEM reflex was 8–20 dB higher than the CM threshold curve. These high thresholds were greatly dependent on the CM-to-noise ratio. When the recording electrode was placed on the round window, the CM-to-noise ratio was large. The CM evoked by sounds at high frequencies was particularly prominent. When the lowest thresholds of the CM and MEM reflex obtained at each frequency were used to plot their

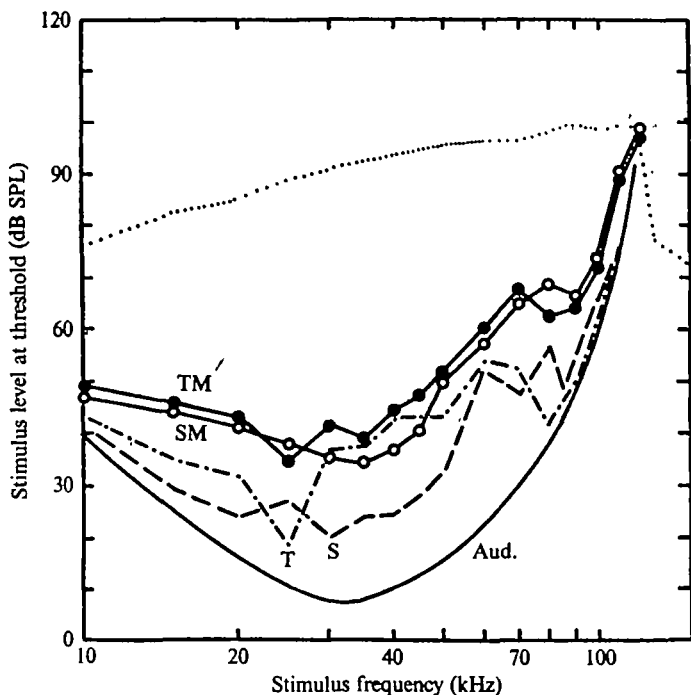


Fig. 5. Threshold curves of the acoustic MEM reflex in terms of the EMGs of the SM and TM and the audiogram in terms of activity of auditory neurones. Curves SM and TM are the average of the data obtained from five bats. Curves S and T are the lowest threshold curves of the SM and TM, respectively. Curve 'Aud.' is the audiogram in terms of neural activity. The dotted curve is the frequency-response curve of the loudspeaker.

threshold curves, both curves were 10–15 dB lower than the averaged ones. The minimum threshold of the MEM reflex was 47 dB SPL (Fig. 3*B*).

The measurement of the threshold of the MEM reflex was more easily performed with the EMG than the CM because the EMG consisted of action potentials with all-or-none properties. The lowest threshold obtained for the MEM reflex in terms of the EMG was 20 dB SPL for both the SM and TM. The lowest threshold obtained for the summated auditory nerve response (N_1) was 8 dB SPL, so that the difference in the minimum threshold between the MEM reflex and the summated auditory nerve response was 12 dB (Fig. 5). In order to examine whether there was a difference in threshold curve between the SM and TM, EMGs were simultaneously recorded from both muscles, and their threshold curves were measured and compared. There was a tendency for the threshold of the SM to be lower than that of the TM. The minimum threshold was, however, 34 dB SPL for both muscles, obtained by averaging data from five bats (Fig. 5).

Tuning curves of single MEM fibres

The broad threshold curve of N_1 was composed of synchronized responses of many single auditory neurones, each of which had a relatively narrow excitatory response area tuned at a certain frequency called the best frequency (Fig. 7*A*). The threshold curves of the MEMs were also as broad as the N_1 threshold curve (Fig. 5). It is not yet known whether these broad threshold curves are composed of responses of single

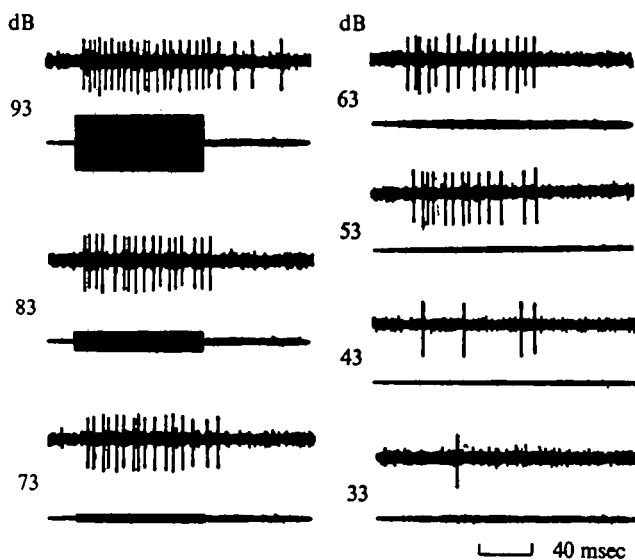


Fig. 6. Responses of a single SM fibre to 35.0 kHz tone bursts delivered at different amplitudes. the tone bursts had a 100 msec duration and a 0.5 msec rise-decay time. The figures to the left represent stimulus amplitudes in dB SPL. The time scale is 40 msec. No correction of the acoustic delay (1.7 msec) is made in the photographs.

muscle fibres with narrow tuning curves similar to those of primary auditory neurones. Therefore, tuning curves of single SM fibres were measured.

About two-thirds of 29 single muscle fibres studied with two bats showed tonic on-responses to acoustic stimuli. The discharge rate in response to a 100 msec tone burst was usually less than 200 impulses per second (Fig. 6). About one-third of the fibres, however, discharged only a few impulses, even for the most effective stimulus. Such phasic muscle fibres showed a very high threshold. Fig. 7B represents the tuning curves of five single muscle fibres selected from the samples obtained from a single stapedius muscle. Unlike primary auditory neurones and cochlear nuclear neurones, single stapedius muscle fibres showed a broad threshold curve. The best frequency varied from fibre to fibre, mainly within a range from 25 to 50 kHz. The minimum threshold also showed some variation. When the best frequency was between 30 and 60 kHz, the slope of a tuning curve was 10–20 dB/octave toward low frequencies and 10–30 dB/octave toward high frequencies in the muscle fibres (curves *a*, *b*, and *c* in Fig. 7B), while it was 100 dB/octave toward low frequencies and 300 dB/octave toward high frequencies in the primary auditory neurones (curves *c*, *d*, *e*, and *f* in Fig. 7A).

Differences between the stapedius and tensor tympani muscles in terms of acoustic reflex

As already described, the latency of the acoustic reflex was shorter in the SM than in the TM, whenever there was a difference (Fig. 1A). The threshold of the acoustic reflex was often lower in the SM than in the TM (Fig. 5). The PST histogram of the EMG often showed quite a difference in its envelope between the SM and the TM. The SM showed more prominent discharges at the onset of the stimulus than the

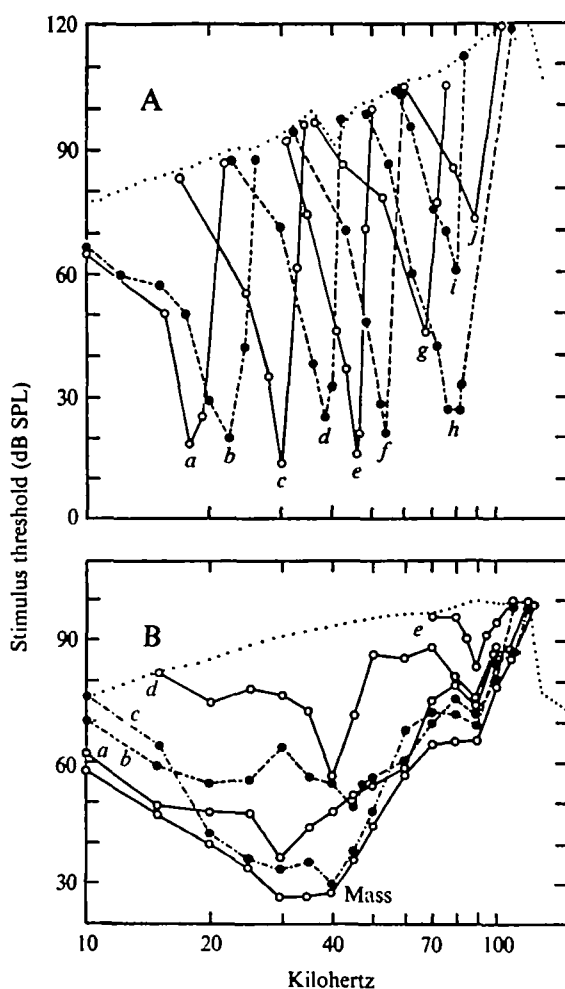


Fig. 7. Tuning curves of single auditory neurones at the periphery (A) and single SM fibres (B). In A, *a* to *j* represent tuning curves of 10 single neurones recorded from the auditory nerve and cochlear nucleus of four bats. In B, *a* to *e* represent tuning curves of five single SM fibers obtained from a single bat. Curve 'Mass' represents the threshold curve of the SM reflex in terms of the EMG of the bat.

TM (Fig. 1A). The SM reacted to acoustic stimuli faster and attenuated them more than did the TM (Fig 2A). Furthermore, these two muscles showed different responses to repetitive stimuli.

When a tone pulse with a 0.5 msec duration was delivered repetitively at different rates, the EMG of the SM nicely followed the stimuli up to a rate of 330/sec, while that of the TM fused each other at a rate of about 220/sec and the response to each stimulus became hardly discriminable (Fig. 8). The SM obviously reacted more quickly and discretely to acoustic stimuli than the TM. Fig. 8 indicates that the EMG of the SM shows facilitation and that the latency of the TM becomes shorter with an increase in the repetition rate.

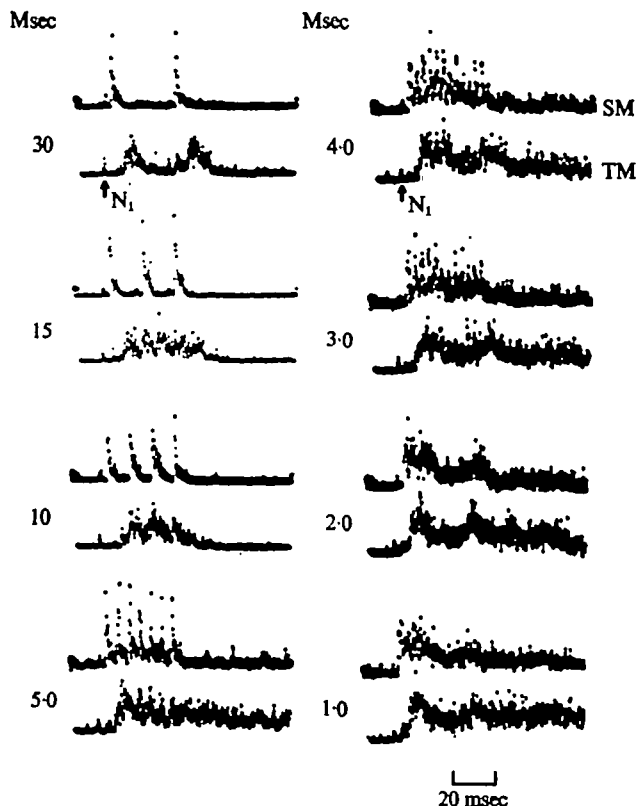


Fig. 8. PST histograms representing the responses of the SM (upper trace) and TM (lower trace) to a train of 40.0 kHz, 94 dB SPL tone pulses with a 0.5 msec duration and a 0.2 msec rise-decay time. The same train was delivered 8 times at a rate of 1/sec, and responses to these were averaged by a computer. The tone pulse in the train was delivered with different inter-pulse intervals as indicated by the figures to the left of the histograms. The bin width is 0.10 msec.

RESULTS FOR PART II: VOCAL MIDDLE-EAR-MUSCLE ACTIVITY

EMGs of MEMs and vocalization

When the bat emitted sounds, EMGs of its SM and TM were simultaneously recorded. It was then noticed that both the muscles always discharged action potentials synchronized with vocalization regardless of the types of sounds emitted (Fig. 9A). The SM and TM became active at least 6.1 and 4.2 msec prior to vocalization, respectively. The EMG of the SM always appeared more than 2 msec earlier than that of the TM. When the animal emitted more intense sounds, the EMG became larger. In Fig. 9, the orientation sound is about 100 dB SPL in A1 and about 108 dB SPL in A2. It is about 110 dB SPL for the second and third vocalizations in A3. The rate of the emission of orientation sounds in our experiments often reached 100/sec. The EMGs of both the muscle always appeared prior to each vocalization even at such a high repetition rate (Fig. 9A3). The SM always discharged more action potentials than the TM did. The shortest duration of the vocal MEM activity in terms of EMG was 3.0 msec for the SM and 1.9 msec for the TM. It is thus clear that the MEMs always start to contract prior to vocalization and attenuate the amount of self-

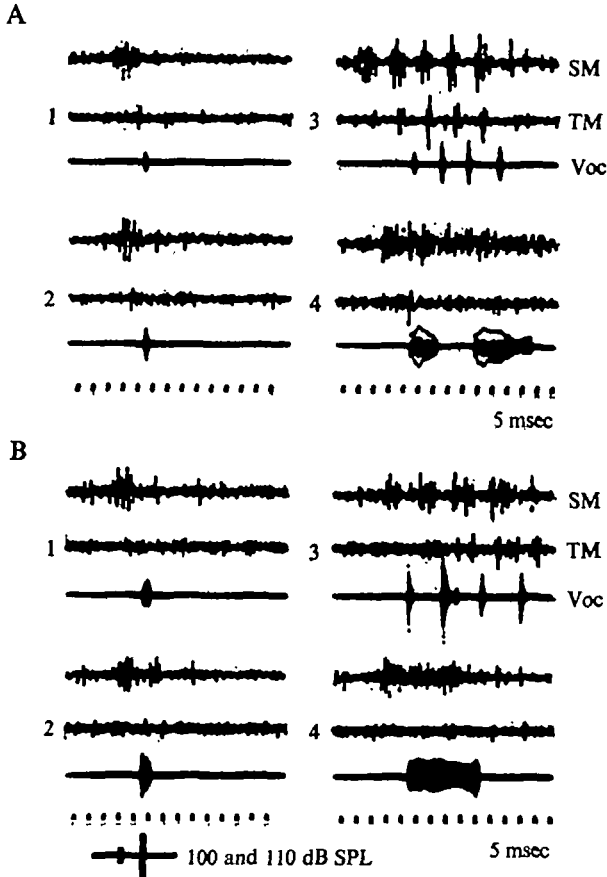


Fig. 9. The vocal MEM activity. The EMGs of the SM (upper trace) and TM (middle trace) and vocalized sounds (lower trace) were simultaneously recorded. *A* and *B* represent, respectively, recordings made without and with a continuous test tone of 16.8 kHz and 78 dB SPL. 1 and 2, single emission of orientation sounds; 3, multiple emission of orientation sounds; 4, emission of a squeak. At the bottom of *B*, 5 msec time signals and 100 and 110 dB acoustic signals are shown.

stimulation and that the mode of contraction is similar to a single twitch, when the repetition rate of sound emission is high.

When the animal emitted non-orientation sounds such as squeaks with a duration longer than 10 msec, the EMGs of both the muscles appeared prior to and during the vocalization. The EMG of the TM was, however, not prominent when compared with that of the SM (Fig. 9A4).

The easiest way to measure the amount and time course of the muscular attenuation synchronized with vocalization is to record the CM evoked by a continuous test tone and to measure the amount and time course of the change in the CM which occurs with vocalization, as Henson (1965) did. The delivery of such a long test tone obviously activates the MEM reflex arc, so that the amount and time course of the muscular attenuation measured with this method are modified by the MEM reflex. The effect of the continuous tone on the EMGs of both the MEMs synchronized with vocalization was thus studied.

Fig. 9 represents the EMGs of the SM and TM synchronized with vocalization without (A) and with (B) a continuous tone of 16.8 kHz. The acoustic MEM reflex evoked by this test tone had reached a steady state before vocalization. With the continuous tone, the amplitude of the EMG of the SM becomes small and its duration becomes long. These differences are particularly clear between A₃ and B₃. Apparently, the synchronization of action potentials of single SM fibres was disturbed by the continuous tone. The effect of the continuous tone on the EMG of the TM was so prominent, that the EMG synchronized with vocalization often became hardly recognizable. These studies on the EMG indicate that, with the continuous test tone, the time course of the vocal MEM contraction becomes long and the amount of attenuation by it becomes small. In particular, the attenuation by the vocal TM contraction virtually disappears with the continuous tone.

Time course and amount of attenuation by vocal MEM contraction

Since the vocal MEM activity was influenced by the acoustic MEM reflex, a test tone pulse could not be delivered prior to the vocal MEM activity for accurate measurements of the amount and time course of the attenuation by the vocal MEM contraction. The best method for the measurement would be to deliver a tone pulse less than 8 msec in duration at, or after, the beginning of the EMG of the SM. The test tone pulse would be so short that the CM evoked by it would not be modified at all by the acoustic MEM reflex. In this method, however, extensive tape recording and data processing would be required, so that a continuous pure tone was used as a test tone. As already described, the vocal MEM activity was desynchronized by the continuous tone, and its duration became long (Fig. 9), so that the muscular attenuation with a long duration was ignored, and only that with a short duration was selected for the analysis of the time course and amount of attenuation by vocal MEM contraction.

As a test tone, a continuous pure tone was delivered at 16.8 kHz, because it caused a large CM and was away from the best frequency for the MEM reflex and also from the frequency of orientation sounds. The CM response to this test tone was recorded from the round window. This response was, however, attenuated by about 9 dB by the acoustic MEM reflex which had reached a steady state before vocalization. When the animal vocalized, the MEMs transiently further contracted and attenuated the amplitude of the CM response (Fig. 10).

When the bat emitted orientation sounds 1–3 msec long, the MEMs started to contract 3–8 msec prior to vocalization and usually reached the maximum contraction during the vocalization (Fig. 10A₁). However, the maximum contraction sometimes occurred within one millisecond after vocalization when the animal vocalized at a high repetition rate (Fig. 10A₂–A₄). The muscles then relaxed within 2–6 msec after vocalization. The time course of the muscle relaxation was quite variable as shown in Fig. 10A. For instance, in A₂, the relaxation time is respectively 5.6 and 7.3 msec for the 2nd and 3rd vocalizations, but it is only 2.3 and 3.4 msec for the 1st and 4th vocalizations. In A₄, on the other hand, the relaxation time is short for the 1st and 2nd vocalizations, but it is long for the 3rd, 4th and 5th vocalizations. This variation may be due to inert jitter in the system or to intentional control.

Since the shortest duration of the contraction-relaxation cycle of the MEMs in terms of the CM attenuation was 6.8 msec as shown by the last vocalization on

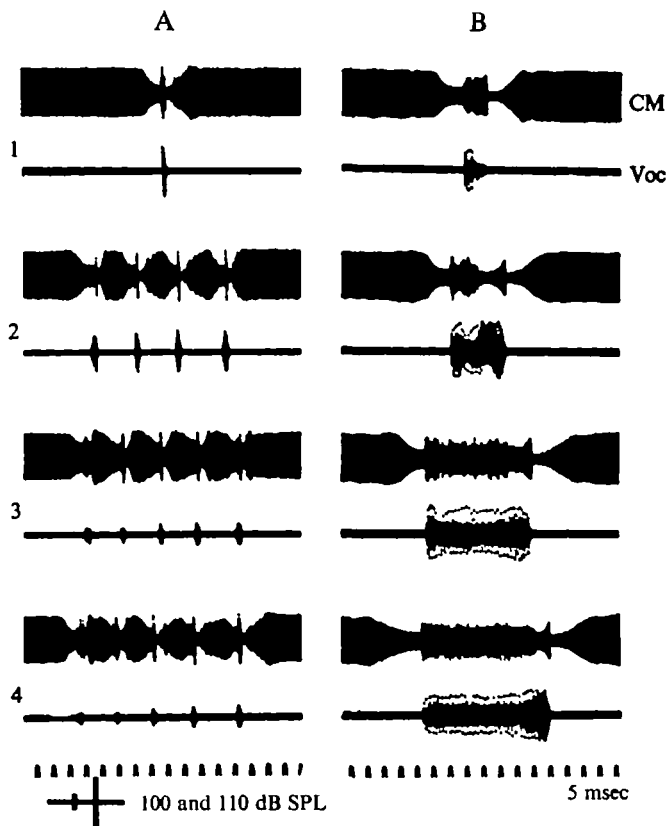


Fig. 10. The time course of the vocal MEM contraction. The CM evoked by a continuous test tone of 16.8 kHz and 78 dB SPL (upper trace) is transiently attenuated by the MEMs when the animal emits sounds (lower trace). In the upper trace, the CM evoked by self-vocalized sounds is also clearly recognizable, although it was passed through an electronic filter set at 15 kHz low-cutoff and 19 kHz high-cutoff. In *A*, the bat emitted orientation signals at different rates and in *B*, squeaks of different durations. At the bottom, 5 msec time signals and 100 and 110 dB SPL acoustic signals are shown. It should be noted that the CM was attenuated by about 9 dB by the acoustic MEM reflex.

Fig. 10A₃ the MEMs could theoretically contract at a rate of 150/sec without overlap. The highest rate of vocalization observed in this experiment was 100/sec. At this rate, the MEM obviously contracted synchronously with each vocalization without overlap. The tetanus fusion frequency of the MEM contraction was studied with electric stimuli applied to them, as described later (Figs. 13 and 14).

In order to express the magnitude of the muscular attenuation in dB, the relationship between the amplitude of the CM and the stimulus level was studied with a 4 msec tone pulse of 16.8 kHz. Then, the amplitude of the CM as shown in Fig. 10A was converted into dB (see Methods and Fig. 4). In Fig. 11, curve *a* shows that the attenuation by the vocal MEM contraction starts to occur 8 msec prior to the emission of a sound 1.7 msec long and increases at a rate of 3.1 dB/msec. The maximum attenuation occurs at the end of vocalization and is 23 dB. The attenuation decreases at an average rate of 3.4 dB/msec. Curve *d*, on the other hand, represents the attenuation occurring synchronously with the 4th sound of a 2.2 msec duration emitted at a rate of 100/sec. In *d*, the attenuation starts to occur 7 msec prior to vocalization at

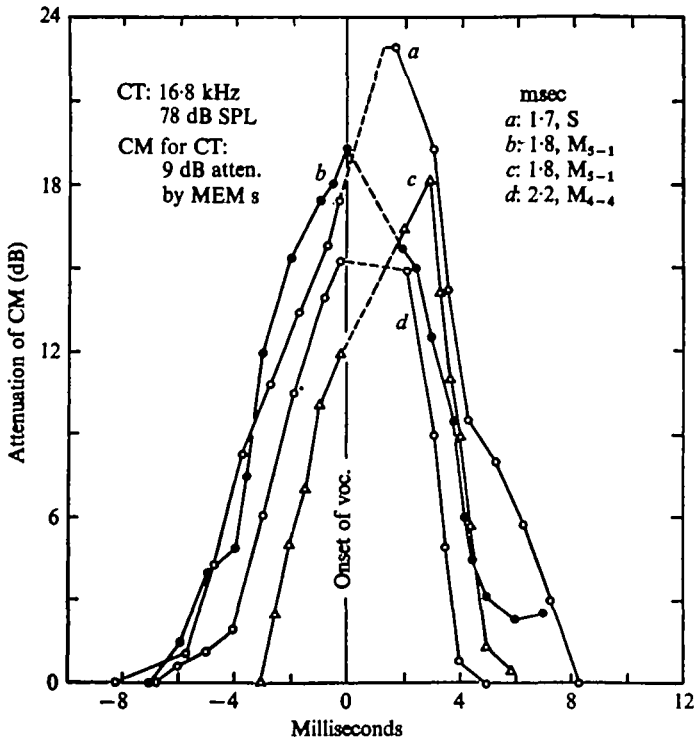


Fig. 11. The time course of attenuation of the CM by the vocal MEM contraction. The CM was evoked by a continuous test tone of 16.8 kHz and 78 dB SPL. The amount of attenuation of the CM in decibels was calculated from the response-amplitude function of the CM obtained without MEM contraction. The abscissa represents the time before and after the onset of vocalization in milliseconds. The emitted sounds for curves *a-d* are as follows: single emission of an FM orientation sound 1.7 msec long for *a*, and multiple emissions of FM signals 1.8–2.2 msec long for *b*, *c*, and *d*. It should be noted that the CM was attenuated by about 9 dB by the acoustic MEM reflex.

a rate of 3.8 dB/msec. The maximum attenuation occurs during vocalization and is at least 15 dB. The attenuation diminishes at a rate of 8.0 dB/msec. The rate of decrease is very large. As already described, the MEMs were tonically contracted and attenuated the CM response to the continuous test tone by 9 dB, so that the amount of attenuation by the vocal MEM contraction without the reflex activity is expected to be larger than that shown in Fig. 11.

When the bat produced non-orientation sounds such as squeaks, the MEMs also contracted synchronously. The time course of the attenuation of the CM amplitude before and after the vocalization was often comparable to that for the emission of a single orientation sound. In Fig. 10B1, for instance, the attenuation of the CM started 8.3 msec prior to vocalization and ended 11 msec after it. The amount of attenuation was also similar to that when orientation sounds were emitted. The maximum attenuation appeared to occur during the vocalization.

In order to examine to what extent such attenuation was due to the SM or TM, the SM was mechanically destroyed. It was then found that there was no apparent attenuation. This was due to the fact that the EMG of the TM synchronized with vocalization

was greatly suppressed by the continuous test tone (Fig. 9B) and that the TM was less effective in attenuation than was the SM (Fig. 2A). Wever & Vernon (1961) found that the pinna and protuberance mechanically shielded the external auditory meatus to protect the ear from intense sounds delivered from an external source. When the bat emitted sounds, its pinnae moved slightly forward, but never shielded the external auditory meatus. The pinnae were not folded to reduce the 90 dB continuous test tone at the external ear. Thus, the absence of attenuation synchronized with vocalization after the destruction of the SM indicates that the pinna and protuberance take no role in attenuating self-stimulation.

Vocal MEM activity and acoustic MEM reflex

When the bat emitted orientation sounds, the amount of self-stimulation in terms of CM and N_1 was about 30 dB weaker than the sound pressure level (about 110 dB SPL) monitored at 5–10 cm in front of the bat's mouth (see also Henson, 1970; Suga & Schlegel, 1973). Since the threshold of the acoustic MEM reflex was as low as 20 dB SPL, the MEMs might be activated by self-vocalized sounds. If this reflex activity were prominent, it would interfere with the detection of echoes, because the MEM reflex started to occur with a 4.8 msec or longer latency in terms of attenuation of CM, as shown in Fig. 2. In the experiments to explore the amount and time course of attenuation by the vocal MEM contraction, no clear sign of the contraction by the MEM reflex was found during and after the relaxation phase of the vocal MEM contraction, although the relaxation phase sometimes consisted of two components, one of which might be due to the reflex activity (e.g. Fig. 10A1). The EMGs of both the SM and TM, on the other hand, showed some sign of the reflex activity evoked by vocalization. That is, the SM and TM sometimes showed action potentials with a latency of about 5 msec from the beginning of vocalization (e.g. Fig. 9). This very poor reflex activity is ideal for echolocation.

As mechanisms for this very poor reflex activity, the following two are conceivable.

- (1) The durations of orientation sounds are so short that the reflex arc is not activated.
- (2) The message from the vocalization system activates the motoneurons of the MEMs, but presynaptically inhibits the reflex activity at the motoneurons. The first possibility was examined by measuring the threshold of the acoustic MEM reflex in terms of the EMG of the SM as a function of stimulus duration (Fig. 1B). The threshold was 31 dB SPL for a 30 kHz sound longer than 30 msec, but it was 79 dB SPL for the sound with a 0.1 msec duration. The change in threshold was thus very large, 48 dB, although the N_1 threshold stayed nearly the same regardless of such a change in duration. The threshold of the reflex was about 70 dB SPL for a sound 1–2 msec long. Thus, the self-stimulation by orientation sounds was approximately 10 dB above the threshold for the reflex and only slightly excited the reflex arc.

Tetanus-fusion frequency of MEMs

As already described, *Myotis lucifugus* emits orientation sounds at a rate of up to 200/sec in the terminal phase of insect hunting (Griffin, Webster & Michael, 1960). It is important to know whether the MEMs can contract at such a high rate without

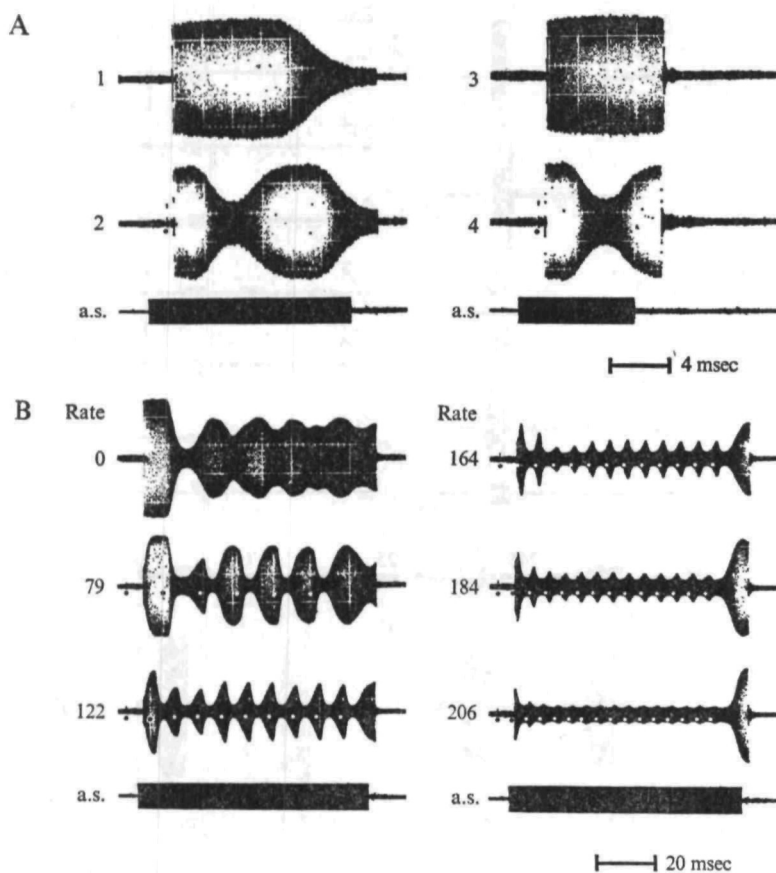


Fig. 12. Attenuation of CM by single (A) and repetitive twitches (B) of the SM elicited by electric pulses. The test tone burst used (a.s.) is 31.0 kHz, 90 dB SPL, with a 0.2 msec rise-decay time. Its duration is 14, 8, and 80 msec in A₁-A₂, A₃-A₄, and B, respectively. The electric pulse (dot) is 0.1 msec in duration and 4.6 V in amplitude. In A₁, the CM is attenuated by the acoustic MEM reflex with a 6.7 msec latency. In A₂, the CM is attenuated by the SM contraction evoked by a single electric stimulus delivered to the SM 0.3 msec prior to the acoustic stimulus. The latency of the acoustic MEM reflex is delayed by the above attenuation. In A₃, the CM is not attenuated by the acoustic MEM reflex because the test tone pulse was short. In A₄, the CM is attenuated only by a single twitch of the SM elicited by an electric stimulus. In B, the SM was stimulated with a train of electric pulses which started prior to the onset of the acoustic stimulus and ended prior to its cessation. The rate of electric pulses in the train was changed from 0 to 206/sec as indicated by the figures to the left of the CM. At 0, the CM is attenuated, in oscillating fashion, only by the acoustic MEM reflex. No correction of the acoustic delay (1.7 msec) is made in the photographs.

showing complete tetanus. It was, however, difficult to have vocalizations at such a high rate as 200/sec from the bat whose head was fixed. Thus, the tetanus-fusion frequency of the MEMs was studied by stimulating each of them. Since it was found that the vocal MEM activity was influenced by the delivery of a continuous test tone (Fig. 9) and that the SM was much more influential in controlling signals than was the TM (Fig. 2A), the time course of the SM contraction evoked by a 0.1 msec electric pulse was mainly studied with the CM evoked by a test tone pulse so short that the CM response to it was not affected by the acoustic MEM reflex evoked

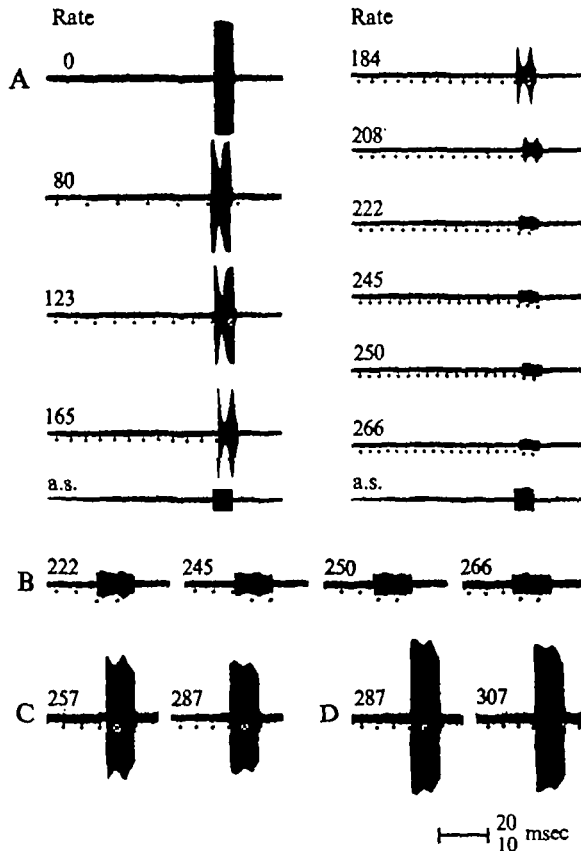


Fig. 13. Attenuation of CM by repetitive twitches of the SM elicited by a train of electric pulses, without the interference of the acoustic MEM reflex. The test tone pulse (a.s.) was 31.0 kHz, 90 dB SPL, and 6–8 msec long. The train of electric pulses was 70–80 msec long and was delivered at a rate of one per second. The electric pulse was 0.1 msec long and was delivered at different rates as indicated by the figure to the left of each record. The amplitude of the electric pulse was 4.6 V for A and B, 3.2 V for C and 2.8 V for D. Since the amplitude modulation of the CM by each SM contraction became less prominent at high rates of repetitive stimuli, the CM in B–D was photographed using magnification two times greater than that in A. Thus, the time scale at the bottom right is 20 msec for A, but it is 10 msec for B–D. '222' to '266' in A are the same as those in B. No correction of the acoustic delay (1.7 msec) is made in the photographs.

by the test tone. Hereafter, the time course and amount of the muscle contraction were expressed by those of the attenuation of the CM response to the test tone pulse.

When a single electric pulse was delivered to the SM, the SM contracted with a 1.8–2.0 msec latency. The durations of the contraction and relaxation were about 2.6 and 3.6 msec, respectively. Thus, the duration of the contraction-relaxation cycle was very short, about 6.2 msec even when the SM strongly contracted and attenuated 31.0 kHz by 25 dB (Fig. 12A2 and A4). When such an electric pulse was repetitively applied to the SM, the SM repetitively contracted. Such a repetitive contraction was easily shown when the electric stimuli were applied to the muscle while the CM response to a long tone burst was recorded.

Fig. 12B shows the CM response to a 31.0 kHz, 90 dB tone burst of an 80 msec duration. The CM was amplitude-modulated by the MEM reflex in B 0, but by both the reflex and the SM contraction evoked by electric stimuli in B 79–B 206. When the electric pulses were applied to the SM at a rate of less than 120/sec in a train, the SM repetitively contracted without fusion. The CM did not immediately return to the original amplitude after the SM contraction evoked by the last electric stimulus (B 122). This indicates that the MEM reflex was activated by the test tone during the electrical stimulation. At rates higher than 160 pulses per second, the SM greatly attenuated the sound energy going into the inner ear, so that the MEM reflex was less activated. Accordingly, the CM returned close to the original amplitude after the SM contraction evoked by the last electric stimulus (B 206). The tetanus-fusion frequency ranged between 200 and 240/sec. In the same experiment described by Fig. 12B, for instance, it was 220/sec. In the same material and the same electric stimulus condition, the tetanus-fusion frequency was 260/sec when the 8 msec test tone was used (Fig. 13A). The tetanus-fusion frequency was clearly lowered by the interference of the acoustic MEM reflex evoked by the long test tone burst.

The 'terminal buzz' lasts 50–100 msec during which the repetition rate of sound emission often goes up to 200/sec (Griffin *et al.* 1960). The muscle contraction evoked by repetitive electric stimuli reached a quasi-steady state in 40 msec after the beginning of the repetitive electric stimuli (Fig. 12B). Thus, a test tone pulse 6–8 msec long was delivered about 70 msec after the beginning of the train of electric stimuli. As shown in Fig. 13, the SM contracts discretely to each stimulus at 123/sec with almost no fusion. At 165/sec, the contractions summate to some extent, so that the peak amplitude of the CM is 6 dB smaller than that without the muscle contraction. The notch amplitude is 21 dB smaller than the CM amplitude without the muscle contraction (Fig. 14Aa). In other words, at such a high rate echoes between the contractions will not be significantly attenuated by the contraction, but the self-stimulation will be greatly attenuated. At 208/sec, the overlap of the contraction increases (Fig. 13A 208). The peak and notch amplitudes are now 18 and 25 dB smaller than the CM amplitude without muscle contraction, respectively (Fig. 14Aa). The muscle still contracts to each stimulus. At repetition rates higher than 208/sec, the peak amplitude of the CM becomes much smaller because of the tetanic contraction of the muscle. The tetanus-fusion frequency was observed at about 260/sec (Fig. 13A and B). The attenuation of the CM by the complete tetanus is 29 dB, which is 16 dB larger than that by a single twitch (Fig. 14Aa). The tetanus-fusion frequency depended on the extent of the contraction. With the decrease in the amplitude of the electric pulse, the tetanus-fusion frequency sometimes increased up to 320/sec, which was the highest rate obtained from four bats (Figs. 13D, and 14Ac).

It was difficult to insert a tungsten-wire electrode for the electric stimulation into the TM without a strong push, so the electrode was placed on the surface of the muscle. Accordingly, a large electric pulse was required to stimulate the TM in order to observe the attenuation of the CM. With a stimulus pulse larger than 7 V, it was observed that not only the TM, but also the SM contracted. At above 10 V, even the pinna started to move. Thus, the SM was mechanically destroyed and no contraction of it was observed under the dissection microscope. Then the attenuation of the CM due to the TM contraction was studied.

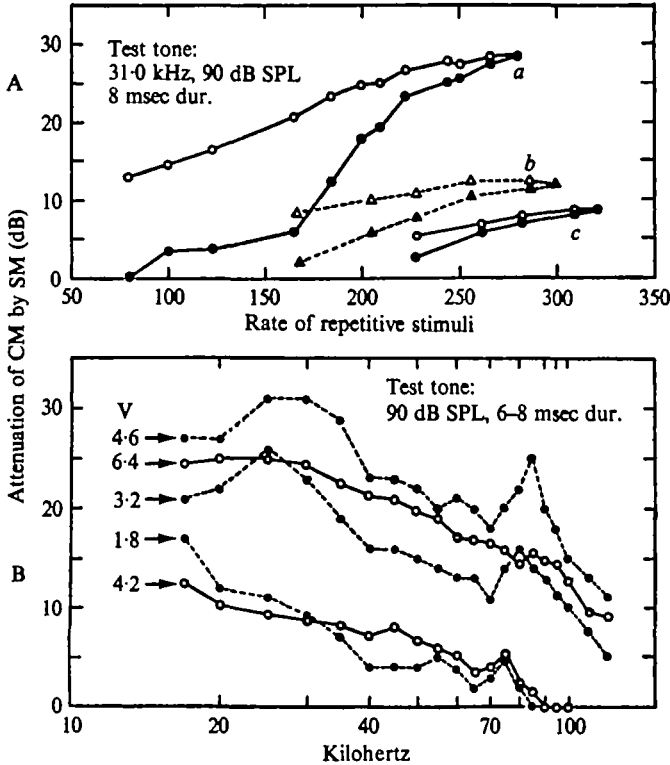


Fig. 14. Attenuation of CM by the SM contraction evoked by electric pulses delivered at different rates (A) and the frequency-attenuation curves obtained at different extents of contractions of the SM (B). In A, both the maximum (open symbols) and minimum (closed symbols) attenuations of the CM at the quasi-steady state of the SM contraction are plotted against the repetition rate of electric stimuli applied to the SM. The test tone pulse was 31.0 kHz, 90 dB SPL, and 6-8 msec long. It was delivered near the end of the train of electric pulses as shown in Fig. 13A. *a* indicates that the SM contraction was strong and the tetanus-fusion frequency was about 280/sec, at which the CM was attenuated by 29 dB. *b* and *c* indicate that the SM contraction was weak and the tetanus-fusion frequency was about 300 and 320/sec, at which the CM was attenuated by 14 and 9 dB, respectively. In B, the attenuation of the CM by a certain extent of contraction of the SM is plotted against the frequency of a test tone pulse. The SM was stimulated by a train of electric pulses and the test tone pulse of 90 dB SPL and 6-8 msec long was delivered nearly at the end of the train, as shown in Fig. 13A. The electric pulses were delivered at 300-350/sec and differed in amplitude as indicated by the figure to the left of each curve. The solid curves represent the average of data obtained from four bats. The average amplitude of the electric pulse was either 4.2 or 6.4 V. The dashed curves were obtained from a single bat. The amplitude of the electric pulse was either 1.8, 3.2 or 4.6 V. The parameters of the train of electric pulses were the same as those described in Fig. 13.

The TM contracted with a 2.2 msec latency after an intense electric stimulus. The duration of the contraction was 2.8 msec, and that of the relaxation was 4.5 msec. Thus, the contraction-relaxation time was 7.3 msec, which was 1.1 msec longer than that of the SM. The tetanus-fusion frequency ranged between 200 and 240/sec, which was significantly lower than that of the SM.

Frequency-attenuation curve for vocal MEM contraction

The frequency characteristic of muscular attenuation due to the acoustic MEM reflex (Fig. 3A) was not applicable to that due to the vocal MEM activity, and the amount of muscular attenuation measured with the CM evoked by a continuous test

tone was undoubtedly affected by the acoustic MEM reflex evoked by the test tone. Furthermore, it was not practical to obtain a constant vocal MEM contraction for each vocalization in order to study the frequency characteristic of the attenuation of self-stimulation. Therefore, the SM was repetitively stimulated with electric pulses delivered at a rate of 300–350/sec, which caused the tetanic contraction, and a CM response to a 90 dB SPL test tone pulse with a 6–8 msec duration was recorded during the tetanic contraction in order to obtain the frequency-attenuation curve of the vocal MEM contraction. The CM response observed was not affected by the acoustic MEM reflex, but only by the SM contraction evoked by the electric pulses. The amplitude of the CM response, which was suppressed by the tetanic contraction of the SM, was measured. Then, the acoustic stimulus alone was delivered and the pressure level was adjusted so that the amplitude of the CM response was the same as that during the tetanus. The difference between this pressure level and 90 dB SPL, i.e. the amount of attenuation expressed in decibels, was measured as a function of frequency.

As shown in Fig. 14B, the frequency-attenuation curves were obtained from four bats. When a sound between 17 and 30 kHz was attenuated by about 25 dB, a 100 kHz tone was attenuated by about 13 dB on the average. The slope of the curve between 30 and 100 kHz was about 6 dB/octave (curve 6.4). With a decrease of the electric pulses in amplitude, the amount of attenuation was reduced. When a sound of 17 kHz was attenuated by about 12 dB, sounds higher than 90 kHz were not attenuated at all, but transmission of sounds about 90 kHz was increased a few decibels in two bats. The slope of the curve was about 3 dB/octave between 20 and 80 kHz (curve 4.2). The averaged frequency-attenuation curves were smooth, without prominent peaks and notches. However, some individual curves showed prominent peaks. Three dashed curves in Fig. 14B were obtained from a single bat. For instance, the curve 4.6 shows peaks at 25–30 and 85 kHz. A 25–30 kHz sound was attenuated 31 dB and an 85 kHz sound was reduced by 25 dB. The frequency-attenuation curve extended beyond 100 kHz, where the amount of attenuation was 15 dB. With the decrease in the amplitude of the electric pulses, both the peaks shift toward lower frequencies (curves 3.2 and 2.8). At 2.8 V electric pulses, the attenuation became zero for sounds higher than 85 kHz.

Fig. 11 shows that a 16.8 kHz tone was synchronously attenuated more than 24 dB with a single emission of orientation sound and more than 15 dB with repetitive emission at a high rate. On the basis of the data presented in Figs. 11 and 14, it becomes clear that when the bat emits an orientation sound sweeping from 100 to 45 kHz, the self-stimulation by it is attenuated more than 13 dB and that when an orientation sound sweeping from 40 to 20 kHz is emitted at a rate of 100/sec, the self-stimulation by it can be attenuated more than 10 dB.

After the destruction of the SM, the frequency-attenuation curve of the TM was measured by the same method described above. The curve for the strong TM contraction was relatively flat between 17 and 70 kHz, being 12 dB. Beyond 70 kHz, the curve descended down to zero at a rate of 20 dB/octave. The attenuation was not observed at frequencies higher than 90 kHz.

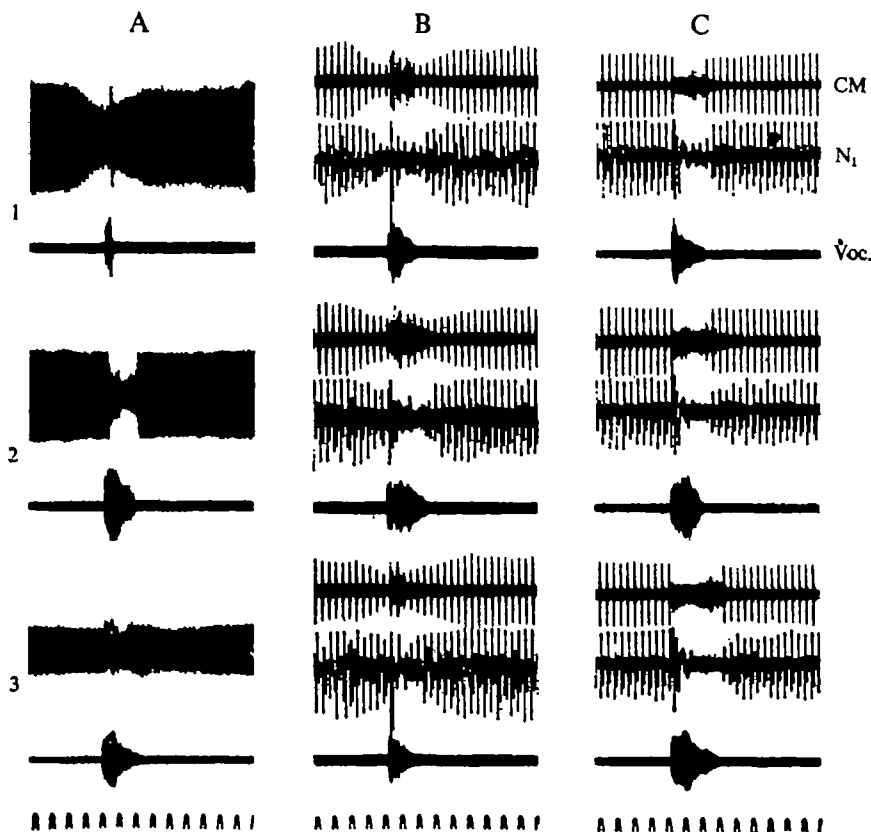


Fig. 15. The change in amplitude of the CM and N_1 with vocalization before (A₁ and B) and after (A₂, A₃ and C) the destruction of the SM. A 43.0 kHz test sound was delivered as either a continuous tone (A) or 0.5 msec-long tone pulses with a 0.2 msec rise-decay time at a rate of 500/sec (B). The amplitude of the tone pulse was 94 dB SPL, except for A₃ in which it was 84 dB SPL. In A, the upper and lower traces represent the CM and emitted sounds, respectively. The emitted sounds were squeaks, except for A₁, in which an orientation sound was vocalized. In B and C, the upper middle, and lower traces represent the CM, N_1 and emitted sound, respectively. At the bottom, 5 msec time signals are shown.

RESULTS FOR PART III: OLIVO-COCHLEAR BUNDLE AND VOCALIZATION

In order to examine the increase in CM and the decrease in N_1 which might be caused by OCB activity synchronized with vocalization, the MEMs had to be destroyed. This was necessary because the vocal MEM contraction caused a decrease in both the CM and N_1 . In four bats of the eleven, the SM and TM were successfully destroyed with almost no bleeding, and vocalization frequently occurred while CM and N_1 were being recorded. Since the MEMs showed a tendency to start to contract earlier for the emission of squeaks rather than orientation signals, it was possible for the OCB to show this same tendency. The change in the amplitudes of the CM and N_1 were studied not only when the bat emitted orientation signals, but also when it produced non-orientation sounds. Fig. 15 shows a set of data obtained from one of these four bats. Before the destruction of the MEMs, both the CM and

N_1 evoked by tone pulses repetitively delivered, became small synchronously with vocalization. The self-evoked N_1 was usually very prominent (Fig. 15B). After the destruction of the SM, however, both the CM and N_1 showed a few dB attenuation or no significant change prior to vocalization (Fig. 15C). The amplitude of the CM never became larger, neither prior to nor after vocalization. The small attenuation which sometimes appeared was apparently due to the vocal TM contraction which was greatly reduced by the test stimuli.

During vocalization, however, both the CMs and N_1 s for the tone pulses and self-vocalized sounds were very small (Fig. 15C). The CM evoked by self-vocalized sounds was even smaller after the SM destruction than before. After vocalization, the CM for the tone pulses immediately returned to the original amplitude, while the N_1 slowly returned. The time course of this suppression of the CM was better studied when the CM evoked by a steady pure tone was recorded during vocalization (Fig. 15A). Unlike the suppression of the CM occurring synchronously with vocalization before the destruction of the SM (Figs. 10 and 15A1), the suppression after the SM destruction occurred and terminated with vocalization (Fig. 15A2). Its time course was quite different from that of the muscular attenuation and also from that of neural inhibition, such as that caused by the OCB. In Figs. 10 and 15A1, there is no sign of this suppression in addition to the muscular attenuation. Thus, the suppression in Fig. 15A2 is related to the result of the destruction of the SM.

The above data indicate that the suppression is probably due to the non-linearity of the mechanical system in the ear. If so, the attenuation of the continuous test tone should reduce the suppression. If not, one cannot explain this by the non-linearity. When the amplitude of the steady test tone was attenuated by 10 dB, the suppression nearly disappeared (Fig. 15A3). With a 20 dB attenuation the CM for the steady tone was not suppressed and that for self-vocalized sound was prominent.

In cats and guinea pigs, the density of efferent endings is not uniform along the cochlea, but it is high in the basal turn (Smith & Sjöstrand, 1961; Spoendlin, 1962). The effect of the OCB is thus more prominent on high-frequency sensitive neurones than on low-frequency sensitive ones (Wiederhold, 1970). In bats, the efferent fibres have been poorly studied, and information about the density of efferent endings is not yet available, but the density may not be uniform along the cochlea. Consequently, a steady test tone was delivered at different frequencies (20, 30, 40, 60, and 80 kHz) and the above experiments were repeated. All the data were similar to those shown in Fig. 15. That is, both the CM and N_1 showed no change prior to vocalization. No sign of activity of the OCB synchronized with vocalization was thus obtained.

Since the time course of the inhibition caused by the electrical stimulation of the OCB is slow (Fex, 1972; Wiederhold, 1970), the CM amplitude may become larger with a considerable delay from vocalization. The CM, however, did not show any such change. If this did occur, responses of auditory neurones to echoes would be reduced, but those to emitted sounds would not.

DISCUSSION FOR PART I

Latency of acoustic MEM reflex

The shortest pathway for the acoustic MEM reflex consists of hair cells, primary auditory neurones, cochlear nuclear neurones, superior olivary neurones, motoneurones in the trigeminal and facial nuclei, and MEMs (Gacek, 1970). Thus, the reflex arc contains at least five synapses. In *Myotis lucifugus*, the shortest latency of the MEM reflex in terms of the EMG is 3.4 msec for the SM and 4.4 msec for the TM. In awake cats, it is 3.7 msec for the SM and 4.2 msec for the TM (Simmons, 1962). Our data obtained from *Myotis* are very similar to Simmons' data obtained from cats. Eliasson & Gisselsson (1955) reported that the latency in terms of EMG was 6 ± 0.5 msec for the SM and 7 ± 0.7 msec for the TM in anaesthetized cats. There are, however, a few papers reporting latencies much shorter than the above. In *Tadarida*, Henson (1967) found that the latency of the MEM contraction in terms of attenuation of the CM was 1.4 msec for an intense sound. Since a muscle usually starts to contract with a 1–2 msec delay from a muscle action potential, the latency of the SM in terms of EMG would be less than 0.4 msec in *Tadarida*, if the observation was correct. In cats, Kirikae (1960) found that the shortest latency of the MEM reflex in terms of EMG was 1.3 msec for the SM and 2.1 msec for the TM. In *Myotis*, the summated auditory nerve response (N_1) shows a 0.6–0.8 msec peak latency when recorded from the round window, and the shortest latency of single auditory neurones measured at the modiolus is 0.6 msec (Suga, 1964). In deeply anaesthetized cats, the peak latency is 1.3 msec for N_1 and 2.3 msec for N_2 (Kiang, 1965; Peak, Goldstein & Kiang, 1962). Thus, the very short latencies reported by Henson (1967) and Kirikae (1960) are difficult to explain with the reflex arc containing five synapses. Even if one surmises that the animals have a shorter reflex arc than the above, it should contain at least hair cells, primary auditory neurones, motoneurones, and MEMs, so that there would be three synapses: and in this case, the shortest latency should be longer than 1.8 msec because of synaptic delays and conduction times of action potentials, unless one further assumes the presence of electrical synapses.

In *Myotis*, the shortest latency of the MEM reflex in terms of the attenuation of the CM was 4.8 msec. Orientation sounds used by *Myotis* are usually shorter than 4 msec, so that the acoustic MEM reflex cannot attenuate stimulation by intense orientation sounds produced by the bat itself and other bats. If the MEM reflex were evoked by self-vocalized orientation signals, it would selectively attenuate echoes. For echo-location, the MEM reflex should not be evoked. Communication sounds with components lower than 20 kHz are, however, longer than 10 msec, so that these may be effectively attenuated by the MEM reflex.

Feedback gain

Attenuation of acoustic signals by the MEM reflex has been studied in bats and cats. Wever & Vernon (1961) found that the feedback gain is nearly 1.0 between 72 and 97 dB SPL in an anaesthetized *Myotis*. Henson (1967) also obtained data similar to the above with unanaesthetized *Tadarida*. The dynamic range for the perfect regulation is 28–30 dB. This was true in our data only when the measurement was performed at the moment when the MEMs maximally contracted. When the measure-

ment was performed during a quasi-steady state, the feedback gain was less than 0.5 (Fig. 4B). It has been known that an anaesthetic reduces the activity of the MEM reflex. Henson (1970) described that the normal MEM reflex was not apparent until 4–7 days post-operatively, and stressed how drastically the reflex activity was affected for a long period of time after barbiturate anaesthesia. It is expected that the latency, strength, and threshold of the MEM reflex are affected by an anaesthetic. There have been, however, no systematic studies on the relationship between the depth of anaesthesia and the above parameters of the MEM reflex, although some experiments with regard to this aspect have been performed (Okamoto, Sato & Kirikae, 1954). It is interesting to note that Wever & Vernon (1961), Henson (1967), and we, have all obtained a feedback gain of about 1.0, although the technique and anaesthesia used in each case were quite different. In anaesthetized or decerebrated cats, a feedback gain of about 1.0 and a dynamic range of 20 dB for perfect regulation have also been obtained (Wever & Vernon, 1955).

Threshold of acoustic MEM reflex

The threshold curve of the acoustic MEM reflex is greatly affected by methods of measurement. In our data, the minimum threshold of the reflex in terms of the attenuation of the CM was 61 dB SPL on the average (Fig. 5), which is nearly the same as the 62 dB SPL obtained by Wever & Vernon (1961). Their threshold curve is similar to ours for sounds below 40 kHz. Above 40 kHz, the threshold is lower in their data than ours. In Wever & Vernon's data, the difference in threshold between the CM and MEM reflex is 10–20 dB and is similar to ours for sounds below 40 kHz. But there is a large difference between the two sets of data. In their data, the threshold is lower for the reflex than for the CM at sounds higher than 70 kHz. We never observed such a phenomenon. In *Tadarida*, Henson (1967) found that the threshold curves of the CM and MEM reflex were nearly the same, regardless of frequencies. The minimum threshold was 45 dB SPL, which was the same as the lowest thresholds obtained from *Myotis*, but 16 dB lower than the averaged minimum threshold of *Myotis*. In three out of the nine *Myotis* studied, the thresholds of the CM and MEM reflex were similar only when a sound was between 30 and 40 kHz. The lowest threshold of the reflex in terms of EMG was 20 dB SPL for the MEMs of *Myotis*.

Tuning curves of single SM fibres

Unlike tuning curves of primary auditory neurones, those of single SM fibres are very broad. Where does this change take place in the reflex arc? The innervation ratios (motor fibres to muscle fibres) are 1–2.4 and 1–3.5 for the SM and TM of a cat, respectively (Blevins, 1963, 1964). According to Erulkar *et al.* (1964) and Fernand & Hess (1969), the MEMs of a cat consist of twitch and slow muscle fibres. The twitch fibres have a few end plates in a restricted area, while the slow fibres have several small synapses originating from a single motoneurone. It is thus unlikely that many single motoneurones with a narrow tuning curve converge upon a single muscle fibre to produce a broad tuning curve.

Single neurones in the cochlear nucleus of a cat have tuning curves which are only slightly broader than those of primary auditory neurones (Pfeiffer, 1964). However, some neurones in the posterior part of the posterior ventral cochlear nucleus and the

dorsomedial preolivary nucleus of a cat have a broad tuning curve and show phasic on-responses to tone bursts (Kiang *et al.* 1973). Since the MEMs tonically respond to acoustic stimuli, presynaptic neurones of their motoneurones should be tonic on-responding. In *Myotis*, some neurones in the superior olivary complex have a broad tuning curve and show tonic on-responses to tone bursts (Jen, 1974). These tuning curves were broader than those of primary auditory neurones, but were still much narrower than those of the SM fibres. Thus, there are possibilities that these neurones with broad tuning curves are involved in the MEM reflex and also that motoneurones of the MEMs integrate signals from many superior olivary neurones with different best frequencies.

DISCUSSION FOR PART II

Functions of the MEMs and vocal MEM activity

Five possible functions of the MEMs have been considered. (1) Protection of the inner ear from an intense sound delivered from an external source (protection theory, Politzer, 1864). (2) Expansion of the dynamic range of response (dynamic-range theory). (3) Attenuation of self-stimulation (self-stimulation-attenuation theory, Carmel & Starr, 1963). (4) Accommodation of the ear for the effective transmission of sounds at particular frequencies (accommodation or frequency-selection theory, Mach, 1863). (5) Stabilization of the ossicular chain (fixation theory, Magnus, 1961).

Protection theory

Since an intense sound is attenuated by the MEMs, the protection theory has been accepted, with reservations which result from: the weakness of the attenuation, its high threshold, and absence of attenuation of high-frequency sounds and brief vocalization. However, the protection theory has not been actually tested because it has not yet been studied whether the elimination of the MEMs (i.e. the elimination of 'protection' for externally originating intense sounds), causes degeneration of cochlear hair cells of animals in their normal habitat. The kinds of intense sounds which actually exist in the normal habitat should also be considered. *Tadarida* and *Myotis* are very colonial and frequently emit orientation and communication sounds. In *Myotis*, the threshold of the acoustic MEM reflex is low. The feedback gain is 1.0 at the very beginning of the reflex, but it is 0.3–0.5 in a quasi-steady state. The muscular attenuation, however, starts to occur with a 5.8–6.2 msec latency, so that the MEMs play a role in attenuating only sounds longer than 6 msec. Orientation sounds of *Myotis* are usually shorter than 4 msec. These sounds are apparently not attenuated. Their communication sounds are, however, long in duration and low in frequency and subject to an effective attenuation due to the MEM reflex. Obviously, one of the functions of an acoustic MEM reflex is to attenuate incoming signals which are unnecessarily intense, but it remains to be studied whether its function is to protect the inner ear from damage by intense sounds originating from external sources or whether it serves another function.

Dynamic-range theory

Wever & Bray (1937, 1942) systematically increased the tension of the MEMs of a cat by pulling them and found that the response-amplitude function, indicating the relationship between the CM amplitude and sound pressure level, shifted in parallel

toward higher pressure levels. On the basis of their data, it is conceivable that with an increase in sound pressure beyond a certain level, the CM amplitude increases, and then deviates from the power function, reaches a peak and then decreases. At the pressure level where the deviation starts to occur, the MEM reflex starts to operate and retains the CM response within the dynamic range of the response-amplitude function. The MEMs play a role in preventing the response from reaching saturation and setting it within a dynamic range. In other words, the MEMs would increase the dynamic range of the ear. This effect of the MEM reflex would have a greater influence on lower frequency sounds than on higher ones. In rabbits, the attenuation of the CM by the MEM reflex does not saturate below 125 dB SPL, so that the CM amplitude increases with a sound pressure level up to 125 dB. Without the MEM reflex, the CM saturates at about 110 dB SPL (Borg, 1972). In our experiments, sounds with larger amplitudes than 100 dB SPL were not delivered because of harmonic distortion so that no measurements were made of CM amplitude either with or without the MEM reflex at the saturation point. However, we believe that one of the functions of the MEM reflex is to expand the dynamic range. The CM originates from many sensory hair cells. It is essential to study to what extent the dynamic range of a single hair cell or a single primary auditory neurone is expanded by the MEM reflex.

Self-stimulus-attenuation theory

In nature, intense sounds which most frequently stimulate the ears are self-vocalized ones, so that the self-stimulation should be attenuated. The vocal MEM activity has been demonstrated in humans (Starr, 1969), cats (Carmel & Starr, 1963), and bats (Henson, 1965). In *Myotis*, the MEMs also synchronously contracted with vocalization and attenuated the self-stimulation. The contraction-relaxation time was very short, 6.8 msec for the vocal MEM contraction and 6.2 msec for the electrically elicited strong contraction in the shortest case. The tetanus-fusion frequency went up to 320/sec in the SM and 240/sec in the TM. These high tetanus-fusion frequencies appear to be related to the unique acoustic behaviour of the bat which emits short orientation sounds at a rate of up to 200/sec and listens to echoes. Our data indicate that the MEMs synchronously contract with the emission of sounds even at a rate of 200/sec, and partially relax between the emissions. In the searching phase of echolocation, the bat emits intense FM orientation sounds at a rate of 10–15/sec. Within each sound, the frequency sweeps from about 100 to 45 kHz. The vocal MEM contraction in this phase was very strong and attenuated a continuous test tone of 17 kHz by 23 dB or more (Figs. 10 and 11). The frequency-attenuation curves obtained at different amounts of MEM contraction (Fig. 14B) indicate that the self-stimulation by the orientation sound sweeping from 100 to 45 kHz is attenuated more than 13 dB at 100 kHz and more than 20 dB at 45 kHz. When orientation sounds sweeping from about 40 to 20 kHz are emitted at a rate of about 100/sec, the vocal MEM contraction is not necessarily strong, but still attenuates the continuous test tone by 15 dB or more (Figs. 10 and 11). The frequency-attenuation curves (Fig. 14B) indicate that the self-stimulation by the orientation sounds sweeping from 40 to 20 kHz is attenuated more than 10 dB at 40 kHz and more than 13 dB at 20 kHz. Since the CM response to the test tone is attenuated by 9 dB by the acoustic MEM reflex, the amount of attenuation may be 9 dB more than the above values.

The MEMs quickly relax at a rate of 3–8 dB/msec for a 17 kHz sound after each emission of orientation sounds. Fig. 14 indicates that the rate of relaxation is 2–4 dB/msec for a 100 kHz sound, so that echoes received during the search phase and the early approach phase may not be attenuated at all by the vocal MEM contraction. However, echoes received during the late approach phase and the terminal phase may fall into the relaxation period, so that these echoes may be attenuated by the vocal MEM contraction to some extent. Studies on recovery cycles of single auditory neurones and summated activity indicate that the response to a tone pulse following another depends on the difference in amplitude as well as interval between them, and that the more intense the second tone pulse than the first, the larger is the response to the second (Friend, Suga & Suthers, 1966; Grinnell, 1963*b*). Thus, the vocal MEM contraction improves echo-detection, in so far as it attenuates the self-stimulation more than echo-stimulation.

As reported in humans (Simmons, 1960), cats (Carmel & Starr, 1963) and *Tadarida* (Henson, 1965), the MEMs of *Myotis* contract synchronously with various activities of the animal, such as movements of the head, mouth and body which produce noises which are conducted to the ears. The self-generated noise which stimulates the inner ear through tissues probably consists mainly of low frequency components, because of smaller conduction loss in tissues for lower frequencies. The MEM contraction more effectively attenuates lower sounds than higher sounds (Fig. 14). Thus, the MEMs can effectively reduce such noise. One of the functions of the MEMs is undoubtedly to attenuate the self-stimulation due to vocalization and similar activities. If it is demonstrated that cochlear hair cells are damaged by self-stimulation after the elimination of the MEMs, it may be concluded that the MEMs serve to protect the inner ear from damage by self-stimulation.

Accommodation theory

In a cat, the MEMs take a role in reducing an antiresonance notch at about 2 kHz and producing the smooth audiogram in the awake cat (Simmons, 1964). When the MEMs weakly contract, the sensitivity of the ear to 0.7–2 kHz sounds increases a few decibels (Møller, 1965; Starr, 1969; Wever & Bray, 1942; Wever & Vernon, 1955). These findings appear favourable to the accommodation theory. Wever & Bray (1937, 1942) systematically changed the tension of the MEMs within a wide range and studied a change in the CM threshold curve of the cat. They found that thresholds for sounds lower than 2 kHz increased more than those for sounds higher than that, but the best frequency at about 3 kHz did not significantly change in spite of a big change in MEM tension. Thus, the MEMs do change the frequency characteristic of sound transmission across the ossicles to some extent, but the function of the MEMs in frequency selection appears to be very limited.

In *Rhinolophus ferrumequinum* (Ajrapetianz & Vasilyev, 1971; Neuweiler, 1970) and *Pteronotus parnellii*, previously called *Chilonycteris rubiginosa* (Pollak, Henson & Novich, 1972; Suga *et al.* 1974), the threshold curve of the auditory system shows a very sharp notch and peak at certain frequencies. For instance, in *Pteronotus*, the frequency of the notch is related to the frequency of a constant-frequency component of about 61 kHz which is predominant in the orientation signals. These notches and peaks probably correspond to resonance and antiresonance peaks of the mechanical system for sound transmission to the sensory hair cells. According to Pollak *et al.* (1972), these notches

and peaks in terms of a CM threshold curve become less prominent when the animal is anaesthetized and the MEMs presumably somewhat relax. The MEMs appear to take a role in enhancing them. If this is the case, the MEMs of *Pteronotus* may be a unique example fitting the accommodation theory. We have, therefore, tested whether the MEMs can shift the sharp tuning at about 61 kHz to other frequencies and/or can sharpen the threshold curve. We have found no significant shift and sharpening (Suga, Simmons & Jen, 1975). In *Myotis*, it is not yet known whether there is a significant difference between the audiograms obtained from awake and anaesthetized subjects. A behavioural audiogram of an awake *Myotis lucifugus* (Dalland, 1965) and a neurophysiological threshold curve of an anaesthetized *M. lucifugus* made without opening the auditory bulla (Grinnell, 1963a) are smooth and are similar to each other. The smooth threshold curve of the CM was also obtained from anaesthetized (Vernon, Dalland & Wever, 1966; Wever & Vernon, 1961) and unanaesthetized *M. lucifugus* after making a tiny hole in the auditory bulla (Fig. 3B). The frequency-attenuation curves obtained as a function of MEM contraction (Fig. 14; Wever & Vernon, 1961) suggest that the MEMs do not play a significant role in frequency selection. The application of the accommodation theory to the MEMs of *M. lucifugus* appears to be very limited.

The accommodation theory remains to be tested by systematic studies on how the audiogram changes with the amount of the MEM contraction and whether the amount of the MEM contraction is pre-adjusted according to frequencies of acoustic signals by an animal.

Tetanus-fusion frequency and muscular specialization for echolocation

In mammalian muscles, the tetanus-fusion frequency is usually less than 100/sec. The internal rectus of the cat's eye is known to be an unusually fast muscle. Its tetanus-fusion frequency is between 240 and 300/sec for the electrical stimulation of the horizontal canal nerve (Suzuki & Cohen, 1966). Recently, Suthers & Fattu (1973) reported that the cricothyroid muscle of *Eptesicus fuscus* showed a tetanus-fusion frequency of 220–240/sec. Its contraction time was 6.5 msec and the contraction-relaxation time was 12–16 msec. In our data, the tetanus-fusion frequency was 260–320/sec for the SM and 200–240/sec for the TM, and the contraction time was 2.6 msec for the SM and 2.8 msec for the TM. The contraction-relaxation time was 6.2 msec for the SM and 7.6 msec for the TM. In *Tadarida*, the contraction time is 8–10 msec and the contraction-relaxation time is 16–20 msec when the MEMs are activated by an intense sound (Henson, 1970). Electrical stimulation of the MEMs of *Tadarida* is not yet performed. The bats of family Vespertilionidae, which produce short frequency-modulated orientation sounds at high repetition rates, are thus equipped with unusually fast muscles for echolocation.

It is interesting to note that some teleosts also have very fast muscles for sound production. The tetanus-fusion frequency of the sonic muscle attached to the swim-bladder is 290/sec in the teleost, *Therapon* (Schneider, 1961), 300/sec in the sea catfish, *Geleichthys* (Tavolga, 1962), 340/sec in the slender sea robin, *Prionotus*, and 340–380/sec in the toadfish, *Opsanus* (Tavolga, 1964). In avians, it has been shown that the pectoral muscle of hummingbirds shows a very high tetanus-fusion frequency 250–300/sec. The contraction time is about 8 msec (Hagiwara, Chichibu & Simpson, 1968).

Vocal MEM activity and acoustic MEM reflex

In non-echolocating mammals, vocalized signals are usually much longer than 10 msec and detection of acoustic signals following within several milliseconds after vocalization is not essential in their acoustic communication. Therefore, it is not an important problem whether the MEM reflex is wholly activated by self-vocalized sounds. In echolocating bats, however, this problem is very important. *Myotis* emits very brief orientation sounds at a rate ranging between 10 and 200/sec (Griffin, 1958). The duration of the sound decreases with the shortening of the distance between the bat and a target so that the emitted signal and primary echo never overlap each other (Cahlandar, McCue & Webster, 1964). When the MEM reflex is repetitively activated, it shows facilitation or summation (Fig. 8). If the MEM reflex was facilitated by the vocal MEM activity and was effectively activated by self-vocalized orientation sounds, the detection of echoes would be reduced. The bat may possess a special mechanism to solve this problem. When the bat vocalizes, the vocalization centre sends messages to excite motoneurons for the vocal MEM contraction, but it may send messages to suppress the MEM reflex, for instance, by causing presynaptic inhibition of the motoneurons. Actually, attenuation by the MEM reflex, following the vocal MEM contraction, is absent or unrecognizably weak as shown in Fig. 10A. It remains to be tested whether the suppression of the MEM reflex takes place synchronously with vocalization. It was, however, found that the poor reflex activity evoked by the emission of orientation signals was partially due to the very short duration of the signals (Fig. 1).

DISCUSSION FOR PART III

As to the function of the OCB, several possibilities have been considered. (1) Increase of signal-to-noise ratio (Dewson, 1968). (2) Gating to focus on particular signals (no supporting data were obtained by Picton, Hillyard & Schiff, 1971). (3) Improvement of frequency analysis (no supporting data were obtained by Igarashi *et al.* 1972). (4) Expansion of the dynamic range of intensity coding. (5) Attenuation of self-stimulation. The fifth possibility was tested in our experiments.

Unlike the efferent fibres of the lateral-line system of the African aquatic frog (Russell, 1971a) and the vestibular system of the goldfish (Klinke & Schmidt, 1970), the olivo-cochlear bundle of the bat showed no sign of attenuation of self-stimulation due to vocalization. We are very confident about our result that the OCB did not become synchronously active with vocalization when it was tested with tone pulses delivered at the high rates. However, a possibility remains that the activity of the OCB synchronized with vocalization is interfered with by the long-lasting train of test tone pulses. Therefore, the activity of the OCB remains to be studied with a single test tone pulse. Although it is very difficult to record action potentials from the OCB during vocalization, this technique may be used to obtain convincing data as to whether the OCB plays a role in attenuation of self-stimulation.

When two sounds, one of which should be intense, are simultaneously delivered, the phenomenon called two-tone suppression is commonly evoked in the cochlea (Engelbreton & Eldredge, 1968; Nomoto, Suga & Katsuki, 1964; Sachs & Kiang,

1968; Arthur, Pfeiffer & Suga, 1971). This two-tone suppression can be explained with a non-linear distortion and filter (Pfeiffer & Kim, 1973). The suppression of the response to the test tone by a self-vocalized sound, observed after the destruction of the SM, or SM and TM, is probably due to the non-linearity of the mechanical system of the ear. It is probably the same as the two-tone suppression, i.e. the suppression caused by the test tone and self-vocalized sound. Then, the question arises as to why this suppression did not occur when the MEMs were intact.

When the MEMs were intact, a 17–25 kHz sound of 90 dB SPL, for instance, was attenuated by about 12 dB by the acoustic MEM reflex (Fig. 3A). When the bat emitted sounds of 100–110 dB SPL, the sounds were further attenuated by 14–23 dB by the vocal MEM contraction (Fig. 11). In total, the pure tone going into the inner ear was transiently attenuated by 26–35 dB when the animal vocalized. On the other hand, a 17–25 kHz component in the self-vocalized sound was also attenuated by 14–23 dB by the vocal MEM contraction. Of course, the amount of attenuation varied with the frequency (Figs. 3A and 14B). For simplicity, when the test tone is 90 dB SPL and the self-vocalized sound is 110 dB SPL, the actual stimulation at the ear is 90 dB by the test tone and 105 dB by the emitted sound without muscular attenuation, while it is 60 dB by the test tone and 85 dB by the emitted sound with muscular attenuation. Thus, the ear is strongly stimulated without the MEMs, so that non-linear distortion becomes prominent and the two-tone suppression increases.

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