NEUROPHYSIOLOGICAL AND BIOPHYSICAL STUDIES ON COUPLING OF AUDITORY AND VOCALIZATION SYSTEMS IN ECHOLOCATING BATS

BY

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INTRODUCTION

Living organisms have been interacting over for billions of years with the sea of variety of energy after chaos parted into voice and silence, light and dark, being and naught. Mechano-sensitive membrane changes its electrical or chemical properties as a result of distortion on the membrane itself which can be represented in terms of kinetic energy in the medium, vibration, sound or pressure. Contractile element with or without elastic structure makes the organism possible move, or give a mechanical energy to the adjacent material by a result of conversion of chemical energy stored in various types of the carbon compounds.

There are tremendous varieties of energy absorption and emission through the living organisms or organella; photic to chemical, mechanical to chemical, chemical to electrical energy and vice versa.

In the field so called sensory- or neuro-physiology, these energy conversions are termed stimulus and response if one can assume a causality between the phenomena. The response to the stimulus causes the change in the state of the animal, the new environment including the state of the animal then may cause the change in susceptibility to the stimulus, or in the capability of continuance of the stimulus itself. If this stimulus-response interaction through the

animal makes a new steady or quasi-static state of equilibrium compared with the duration of the response and stimulus, the animal may continue to be existent; adaptive to environment and evolutionable when the interaction acts on the animal itself.

Nature gives several receptive system to living organism; visual, auditory, tactile, taste and olfactory organs corresponding to the physical potential of light, mechanical, and chemical activity known as Aristotelian five sense.

In contrast to the passive reception of the incoming physical energy in variety of sensations as above mentioned by so called sensory or afferent system, the animals or lower organisms are able to emit the physical energy to the surroundings. The types of energy are heat, sound, locomotion, chemical substance and light. Emitter organ of these are called the effector and the control system of the effector in animal is called the efferent system.

The animals must utilize the afferent and the efferent systems in order to survive in the changing surroundings. The animals recognize the predator by visual perception, or by sound hearing and smelling, and the final recognition would be manifested as behavioral responses such as escaping, threatening, or akinesis. Those are passive recognition of the outside world.

Animals can perform the intra- or inter-species communication by coupling the efferent system with the afferent

one by using of the variety sets of information carrier energy.

Indeed, for communication between organisms and their environment as well as between individuals, light; electromagnetic wave is the most usefull of all physical vehicles, throughout the animal kingdom. But another physical avenue for the transfer of information consists of mechanical vibration rather than electromagnetic one. In fact certain advantages are involved in the use of sound waves and in the physiological mechanisms evolved in animals to produce and detect them. While visual organ in any organism has a sensitivity range for not more than an octave of wavelength or frequency (400 nm to 800 nm), the sense of hearing in birds and mammals including human, extends over seven to ten octaves. As a result there is a far wider range for possible variation in the quality of an auditory stimulus, and delicate meaning may be conveyed by a single complex sound made up of a large number of component frequencies and its time course.

Another advantage of sound waves over those of the electromagnetic spectrum is that animals have succeeded in generating sound waves for their own purposes to a far greater degree than in the other, and that they, except for a few species equipped with luminous organ as in the deep sea fish, must rely for light on what is obtained from the sun or other outside sources.

Of cause there is physical limitation of advantages as well as disadvantages in both the waves, electromagnetic and mechanical. Main reason of limitation is the difference in wavelength in comparison with the size of object or source to be detected. The ratio between the shortest wavelengths of visible light and those of sound used or audible by any animal is of the order of 10^4, so that the angular resolution by sound wave has far little power compared with that by light. It is also true that physiological specialization of eyes seems to have in the way of improving the spatial acuity whereas the evolution of hearing has been in the direction of an increased ability to resolve frequency components in complex sounds.

There is another way to recognize the outside world that would be termed "active perception" or "probing". Active perception involves the emission of the information carrier energy by the animal itself through an effector and the efferent system, and the world would be recognized by the extraction of the information born by the reflected energy. This may be metaphorized to the way that one must aim a flash light to prevent tripping against a chair in his office when the electric power was shut down in an evening. Similarly the blind people can recognize the ambient properly by hearing the reflected sounds made with snapping his fingers or tapping his heel or knocking his white cane against a floor.

Nature has developed a conspicuously sophisticated animal, bats of the order Chiroptera. The bats in suborder Microchiroptera and one species in suborder Megachiroptera emit sound, in most case the sound is far beyond the human audible range in frequency, and hear the returning echoes from the objects. Bat also is strange in the sense of flying mammal with membranous wings. The animal can discriminate by difference in echo feature whether the echo comes back from solid wall of the cave, or a branch of tree, or from a mosquito on which the animal must concentrate his attentions to pursue as a prey. This mechanism utilizing the echo to locate the object is known as "echolocation". In order to perform the echolocation, bat must emit the sound strong enough to obtain the echo from the mosquito so as to overwhelm the naturally existent noise such as rustling of leaves or water stream, and then animal must listen to the faint echo which may return within a few thousandth of a second, in short, very soon after the strong sound emission.

The sound used for echolocation in bats is called orientation sound or sonar cry. The emission of orientation sound also should be controlled by the results of recognition of the echo to ensure the acuity of echolocation.

Usually, one can not hear the sound following a relatively strong sound in a short time, i.e., refractory or recovery phase, and if the ears are plugged with sound

proofing material, one can not also speak properly. Therefore, in the bats there must be an interaction between auditory and vocalization systems, in order to keep the sensitivity to weak echo even after strong out-going sonar sound and to control the sound emission appropriate to the situation recognized by afferent system.

Discovery of echolocation.

In the last decade of eighteenth century, the Italian scientist Lazzaro Spallanzani was much impressed by the ability of bats to pass through objects during their fly even under complete darkness as in the cave. Spallanzani found that even blinding a bat did not affect in the slightest its ability to avoid obstacles. And two years later, in 1793, the Swiss entomologist Louis Jurine as his co-worker found that a plugging the ear of bats with waxy material did prevent the bat from avoidance of obstacles. Spallanzani concluded that hearing was the essential sensory window for location of obstacles in flying bats.

At that time this conclusion seemed to be unreasonable and it was forgotten by the naturalists through the nineteenth century (Galambos, 1942; Dijkgraaf, 1960).

More than one hundred years after the Spallanzani's conclusive observation, Griffin and Pierce demonstrated that a bat actually emits the ultrasonic sound during its

flight (Griffin, 1958). In 1938 they attempted to probe the world of bat by using a developed modern apparatus which can detect sound above the frequency range of human hearing that is above approximately 20 kHz (kilohertz or hertz abbreviated in kHz or Hz is the measure of frequency in terms of cycles repeated in one second of time called after German physicist Heinrich Hertz). The apparatus showed that the bats emitted intense sound which were inaudible to the human ear and that the sound had frequencies ranging from 30 to 70 kHz. Intensity of the sounds were more than 100 dB SPL, it is so strong sound that if one is exposed in the sound field of 100 dB SPL in audible frequency range, one may feel a pain in his ear rather than loudness or that such sound field is equivalent to an engine room of the battle ship (dB SPL represents the intensity of the sound pressure level in decibels relative to 0.0002 dyne/cm^2, threshold in human hearing at 1 kHz is approximately zero dB SPL).

During the next two or three years Galambos and Griffin demonstrated that the ear of bats are actually sensitive to those high frequency sound waves (Griffin and Galambos, 1941). According to their work it is conclusive that the bats can emit the high frequency sounds and hear the echoes reflected back from obstacles ahead. If either the ears or the mouth of the bats were covered they became helpless and collided with even conspicuous obstacles.

Griffin proposed in 1944 the term "echo-location" to the mechanisms probing the surroundings by the sound emission and the echo listening.

It is theoretically plausible that the echolocating bat utilize the ultrasonic orientation sound to locate a small insect in order to ensure the size relationship between object and wavelength. According to the physical principle, the intensity of the wave reflected or diffracted by an object sharply decreases when the wavelength becomes shorter than or comparable with the size of object. Therefore it is advantageous for bats to emit and hear sounds with as high a frequency or as short a wavelength as possible.

It might be supposed that as bat evolved in the early Cenozoic era from an Insectivoran ancester they developed larynxes and cochleas tuned to higher and higher frequencies by a very reason of above mentioned principle of reflection, until another physical limitation began to outweight this benefit. The chief disadvantageous limitation is the fact that the absorption of sound energy propagating in air increases in proportion to a square of the frequency. In the range of human hearing it is not a problem, even at 20 kHz a planer sound wave lose one half of its initial energy after traveling 6 meters, but this loss of energy will occur for only 25 cm traveling at 100 kHz, and at 1000 kHz it will occur extremely soon; 3 mm!

Thus the lower frequencies give the better advantages,

and with the object of between only a few millimeters (mosquitoes and fruit flies) and a few centimeters diameter (moths), frequency range from 40 to 100 kHz (8 to 3 mm in wavelength) would have been suitable to the advantage in wavelength as well as disadvantage in absorption (Griffin, 1953).

Echolocation.

Nature has given other opportunities to employ the principle of echolocation to the other members of animals.

A weak electric fish, Gymnotidae, living in the turbid water of South American river in which eye could not act as an effective detector of objects, generates a high frequency (several hundreds Hz) electric field in the surrounding water by activating an electric organ specialized for this purpose, and they can locate the object whose electrical conductivity is much more or less than that of the surrounding water with electro-static (quasi-static) field instead of electromagnetic wave, reflection of the electric field by an object causes an unevenness of the distribution of current flow into the body surface (Lissman, 1958). The electroreceptors distributed on the body surface respond to the electric current density at the place and the central system analyzes the informations collected at each receptor organ. The electric organ which generates the electric field is also controlled by central nervous system, they change the frequency when

there is the possibility of jamming with other electric fishes. Thus they survey their surroundings with the electric field generated by each individual (Bullock and Hamstra, 1972).

A deep sea fish of Salmoniformes, Aristostonias scintillans, has two visual pigments with sensitivity maxima in green and red regions of the spectrum. This fish also has two bioluminescent organs, a suborbital and post-orbital organs which emit red and green light respectively. In contrast to many other deep dwelling marine fishes whose visual pigments are especially sensitive in the blue wavelengths, and to the fact that the spectrum of the light after filtering through hundreds of meters of sea water consists only of blue wavelength, this fish seems to be specialized to emit the red light and to look at object illuminated with the own emitted red light (O'Day and Hector, 1974). This specialization coincidence between light emitting organ and visual organ would be assumed as an evolutional realization of the echolocation principle or more properly, active probing mechanism. Other possible explanation may take place to this coincidence that the fish can perform effectively an intra-species communication through their specialized channel with red light.

In these two cases, the types of emitted energy are electro-static or electromagnetic field whose velocity of propagation is 300 million meters per second, i.e., almost

meaninglessly infinitive in biological sense, so that there is no chance to utilize the time delay from the emission to reception of the energy due to the traveling time.

In contrast to electricity, mechanical vibration has a relatively slow propagation velocity in the order of hundred meters per second at most. This velocity is in the range being able to give a meaning in nervous system in animal where the time scale of event is of millisecond. For examples, sound pulse reflected back from a surface at 1 meter in air gives about 6 msec delay time after the emission, the rippling wave propagating on the surface of water gives about 60 msec when reflected from 1 cm distant object.

There is a trickily echolocating insect, water beetles, Gyrinidae, these beetles are darting and whirling incessantly over the surface of small streams and ponds. These beetles can continue to avoid the walls of a glass even when their eyes are in dark (Egger, 1923). They have rather elaborate club-shaped antennae instead of whiplike appendages as in most insects, and the second segment with stiff hairs that float on the surface film of the water seems to be essential for the bug.

If the second segment of the floating antennae is injured or the sensory nerves to it is damaged, the bugs would no longer be able to detect the glass wall and collide with the wall. The insects interrupt their swimming movements briefly from time to time. Apparently the surface

wave generated by the beetle's swimming movements propagating to the surroundings are reflected from solid object and detected through the antenal organ during the interrupted periods. The surface wave is a transverse vibration instead of the compression wave as sound is, but the principle is quite compatible with the echolocation using sound wave.

It is known that the oilbird, Steatornis carpensis in South America and the swiftlet, Collocalia in South Asia can perform the echolocation. These birds emit the click sounds whose power spectrum lied around 7kHz, when the visual information is not available. If the ears of the oilbird is plugged, the bird would not avoid the wall and collide with it in the dark(Griffin, 1958).

Cetaceans use sounds for orientation and for communication. The porpoise use the click sound which duration is less than 2 msec for echolocation (Norris, 1964). The click sound contains acoustic energy from 0.1 to 100 kHz which main spectrum is about at 20-30 kHz. The rate of sound emission is controlled upto several hundreds pulses per second for difficult discrimination task.

In addition to those animals cited above, certain species of mice, shrews, even fish and penguins are proposed to be able to echolocate in certain condition (Poulter, 1963).

Among those animals that echolocate, the Microchiropteran bat should be placed at one of the most developed and elaborate one in terms of task performance; object dis-

crimination, directionality, distance ranging. They recognize and utilize those informations in accordance with their maneuveral high speed flight.

Microchoropteran bats show a marvellous performance for echolocation. Little brown bats, Myotis lucifugus flying at speed of 3-4 meters per second, easily can detect and pass through an array of metal wires of 0.18 mm diameter. Since the orientation sounds of Myotis are frequency modulated (FM) sounds sweeping down from 100 to 40 kHz or from 3 mm to 10 mm in wavelength, the bat thus can detect the object even whose diameters are sixteen times smaller than the wavelength of the orientation sound. In natural Myotis can locate and catch a flying small insect such as fruit flies within a half second at a rate of 10-14 insects per minute whose size is as small as one half of the shortest wavelength involved. Horse-shoe bats, Rhinolophus can successfully avoid a wire of 0.08 mm in diameter. Big brown bat, Eptesicus shows a discrimination performance for distance difference by 12 mm between two target at about 60 cm, this difference gives only 0.04 millisecond in echo-arrival time difference. They also discriminate angular difference by 6 degrees in horizontal plane. Fish-catching bat, Noctilio leporinus can detect a ripple on water surface due to a wire of 0.2 mm diameter and 1 mm out of water surface.

Millions of years later, since Nature has developed

those built-in-sonar system in many animals, human did apply the principle of echolocation in engineering devices during the twentieth century.

Sonar and radar were developed to detect underwater submarines and flying airplanes, various devices are now available as an artificial aid for blind people, as a powerful instrument for cancer or tumor detection and as an absolute altitude meter for airplane landing.

Orientation sound

To emit orientation sounds, bats of Microchiropterans contract the laryngeal muscles with coordinations of pharyngeal and respiratory ones which supply an air flow through the larynx as a power supply for sound energy. The orientation sounds are radiated through the mouth or nostril appendage (Novick and Griffin, 1961). The properties of orientation sounds are species specifically different. Each individual utilizes different sounds depending on the situation of biological tasks, corresponding to the searching, approaching or terminal phase of insect hunting or landing.

Little brown bats emit orientation sound with a duration of 3-5 msec at a repetition rate of 10-20 sounds per second during the searching phase, i.e. flight in open space or roosting. In this phase, the frequency in individual

sound changes downwards from 100 to 45 kHz with a certain amounts of second harmonics in the ending portion of the sound. During the approaching phase that the animal pays attention to an object and is tracking it, the duration of the sounds becomes 1 to 2 msec and the repetition rate increases from 25 per second upto 70 per second, and the range of the frequency sweep shifts downwards. At the terminal phase, the orientation sounds are emitted at a high repetition rate of upto 250/sec and the duration of each sound becomes as short as 0.5 to 0.3 msec. This high rate emission of the orientation sound at the terminal phase is called "Buzz", since the audible clicks converted by an electronic ultrasonic detector (Bat detector) sound like a buzz sound. The frequency sweep in such a short sound shifts downwards to the range of 30-15 kHz.

Those changes in properties of orientation sound are considered to be important to prevent the overlapping of the emitted sound and echoes. While animal must increase the rate of sound emission in order to increase the aquisition rate of information when the distance between animal and object becomes short and/or the relative velocity between them becomes high during attack, the time echo comes back from object becomes shorter the shorter the distance is; only 2 msec after the sound emission for 30 cm distant object.

However, the lowering the frequency range in approaching

and terminal phase is theoretically unreasonable because that lower the frequency or longer the wavelength, the resolution of both distance ranging and target size becomes poorer, so that the sweep range during those attacking phases may be due to an inevitable mechanical reason of vocal cord as a result of high emission rate. In addition to an example of little brown bat, gray bat Myotis grisescens, big brown bat Eptesicus fuscus and many other members of Microchiropterans; Vesperliotionids, Molossids, and Natalids emit the downward sweeping frequency-modulated orientation sound in any situation of echolocation. These bats therefore are called "FM bat".

The orientation sound has theoretical advantages in echo ranging and size or feature extraction of the object. Sonar theory implies that the wider the spectrum band of the sonar signal, sonar system offers the better ranging, quality, and acuity.

On the other hand, horse-shoe bats (Rhinolophidae), mustache bats (Chilonycterinae), spear-nosed bats (Phyllostmatinae), for examples, emit the orientation sound of constant frequency (CF) followed by brief frequency modulated part (FM), so that these bats are called "CF-FM bat". Frequencies of CF part of the orientation sound in these bats are species specific and may be individually different. CF part is to utilize for measurement of relative velocity between bat and object with regard to the Doppler shift effect in

frequency.

Rhinolophus ferrumequinum changes the frequency of CF part of emitted sound so as to obtain a Doppler shifted echo at a certain preferred frequency just above the resting one (Schnitzler, 1973). They can compensate the Doppler shifted echo by lowering the frequency of the emitted sound as small as 300 Hz and upto 4 kHz, which correspond to the flight speed of 1.0 m/sec and 12 m/sec, while the resting frequency emitted is about 83 kHz. A similar change in the frequency of CF component of emitted sound is also observed in Pteronotus parnellii (Schnitzler, 1970). The sensitivity of the auditory system in P. parnellii has its maximum at the frequency (e.g. 63 kHz) slightly higher than that of CF part of orientation sound (62 kHz), and this fact may be responsible to the possibility for Doppler measurement in this animal.

CF-FM bats also change the rate of emission of orientation sound and its pulse duration during the approaching and terminal phase as in FM bats, but unlike in FM bats the frequency does not change except by an amount due to the Doppler compensation.

Nycteridae and Megadermatidae emit the ultrashort sound pulse in duration of 0.1-1.0 msec consisted of multiple harmonics of a constant frequency, they are called as "pure tone bats", though the orientation sound is physically not a pure tone. A CF-FM bat Noctilio emits

the orientation sound consisting of only CF part or FM part during approaching phase intervening in the usual CF-FM sound (Suthers, 1965).

The intensity of orientation sound emitted is as high in bats such as Myotis, Eptesicus, Rhinolophus and Chilonycteris, as 100-140 dB SPL at even 30 cm in front of the mouth of bat, this sound is rather loud in the sense of sound intensity level so that they are called "loud bat". On the other hand, some CF-FM bats such as Phyllostomus and Hipposideros or FM bat Plecotus emit a relatively weak orientation sound with the intensity of about 70 dB SPL. Those bats are called "whispering bats". Usually loud bats are fast flyer and they hunt for flying insect, while whispering bats fly relatively slowly and hover to hunt insects resting on flowers or on fruits, even the bats eat fruits, pollen and nector.

Bats of the genus Rousettus, Megachiroptera, have evolved a sonar system independently on the Microchiropteran. Rousettus emits the audible click sound made by tongue movement, while Microchiropterans so far investigated generate the ultrasonic orientation sound through their larynxes. Rousettus uses this click sound and seems to be echolocating when visual cue is not available i.e., in dawn or in dark cave. The click sound of this bat is the short noise burst less than 1 msec in duration and the acoustic energy scatters over the wide range of from

6 to 100 kHz.

Though Rousettus is an echolocator in fact, the terms "echolocation" or "echolocating bats" will imply those in Microchiropteran bats hereafter unless otherwise noted, because of differences in the methods of emission of orientation sound and its properties, not only in genus.

Specializations in auditory system of bat.

The structure of peripheral and central auditory system of echolocating bats are basically the same as those of other mammals, although some specializations for echolocation are seen. The Microchiropteran external ear consists of two basic parts, the pinnae and the external auditory meatus. The most remarkable features of the external ear in echolocating bats are its variability in size, shape and elaboration of accessory appendages; tragus and anti-tragus. Some Phyllostomatidae and insectivorous Vespertilionidae such as Myotis or Eptesicus have relatively small simple pinnae. Small-eared species are usually loud bats, and whispering bats have enormously large pinnae, upto three times longer than its head size in such as Plecotus, a bat commonly called big-eared bat. The tragus or the anti-tragus is large and prominent in many Microchiroptera, no other mammal has those appendages in such remarkable dimensions relative to body size.

The pinnae in many echolocating bats have high mobility, it can be bent down to cover the external auditory meatus in response to an intense sound, so that an attenuation of 35-40 dB is achieved (Wever and Vernon, 1961). In horse-shoe bat, the pinnae moves synchronously with sound emission during echolocation, it seems to be scanning the surroundings. An artificial displacement of pinnae or tragus disturbs the ability of echolocation in many big-eared bats, though such operation has little effect in small eared bats. It is plausible that the complexly elaborate shape of pinnae and tragus or anti-tragus and the mobilities of those are responsible for increase in sensitivity to sound incoming from certain directions; directionality.

The directionality as an echolocating system involves a directional spread of the emitted sound, too. The irradiation of ultrasonic sound wave greatly depends on the shape of emitting apparatus; mouth or nostril in the case of bats. Mouth as emitter has relatively simple conical horn, but the nostril in many Phyllostomatidae and Rhinolophidae are complex. Those animal move their nostril in connection with sound emission.

The sound wave collected and matched in acoustic impedance by external ear propagates into a middle ear. Middle ear consists of tympanic membrane, three auditory ossicles and two middle ear muscles. As in all mammals, the middle ear consists in a air-filled cavity communicated

with the pharynx through the Eustachian tube which makes the pressure difference between both side of tympanic membrane equilibrated. The tympanic membrane lies at the junction of the external and middle ears. Three auditory ossicles; malleus, incus and stapes conduct the sound wave into cochlea (scala vestibuli) through the oval window (fenestra vestibuli). These three bones are coupled with lever action, and they act as an impedance matching apparatus between air born sound and the pressure wave in liquid, perilymph in cochlea. The acoustic impedance of water is approximately 10^3 higher than that of air, so that if there is mismatching in acoustic impedance along these transmission pathways, the sound stimulus would be greatly attenuated before entering to inner ear.

Two middle ear muscles provide the control of this attenuation mechanism mechanically or acoustically. In Microchiroptera one of the two middle ear muscles, the tensor tympani arises from the sphenoid bone and insert on the muscular process of malleus. The tensor tympani receives the innervation from the motor root of trigeminal nerve, a branch of the Vth cranial nerve.

The other muscle, the stapedius leis between the walls of the stapedial fossa and the head of the stapes, this is innervated by the facial nerve (the VIIth cranial nerve) and glossopharyngeal nerve (IX). The stapedius muscle is well developed on Microchiroptera, especially in loud bats and

it is innervated richly. The stapedius muscle shows phasic or rapid contraction and the tensor tympani is tonic or slow in most mammals, although little is known in bats. The middle ear muscles in bat seem to take an important place for attenuation since the volume of the muscles relative to that of auditory ossicles are 5-10 times larger in echolocating bat than in other mammals (Henson, 1965).

In echolocating bat as well as other mammals, a protecting reflex to contract the middle ear muscles and attenuate incoming stimulus by 20 dB in response to intense sound stimulu is seen. The activity of middle ear muscles in relation to the vocalization would be important for echolocating system.

The sound wave is converted into the hydrostatic (quasi-static) pressure in perilymph of the cochlea. The pressure wave conducts in scala vestibuli along the turns of cochlea and returns to the other side of basilar membrane, scala tympani which is terminated with low acoustic impedance; membrane opened to the middle ear cavity called round window (fenestra cochleae). The hydrolic pressure in perilymph generates the travelling wave in the basilar membrane characterized in frequencies of the input vibration. The hair cells, one row of the inner hair cells and three rows of outer hair cells, composed on the basilar membrane receive a shearing force on its hair attached onto the tectorial membrane as a result of relative displacement between basilar membrane

and the tectorial one. The structure of this sensory mechanism consisted of hair cells and the other supporting apparatus is known as the organ of Corti. Distortion on the hair elicits the electrical responses of the sensory hair cell and a kind of chemical transmitter substance is released to the dendritic processes of the primary auditory neurons. Thus the mechanical vibrations on the basilar membrane are converted to an electrical nerve impulse of the primary auditory nerve. The primary auditory neurons with cell body in spiral ganglion at canalis spiralis modioli forms the auditory nerve which runs from internal auditory meatus to the brain (cochlear nucleus), the nerve is regarded as the VIIIth cranial nerve.

The size of the cochlea in echolocating bats is much greater than in non-echolocating Megachiroptera and other mammals relative to body weight or size (Pye, 1966). The length of the cochlear duct is approximately 20 mm in Rhinolophus, while it is about 30 mm in man which body size is enormously larger. It would be noted that the cochlea in CF-FM bats is best developed in size and number of turns. The cochlea in Microchiroptera is articulated with the skull only at the anterior part with sphenoid bone, there is a wide basiocochlear fissure and no contact between the cochlea and basioccipital bone. This may improve an isolation of cochlea from a bone conducted self-emitted sound, although some species such as Chilonycteris have a large opening

for perilymphatic space which has direct communication with cerebrospinal fluid and thus any vibration of skull bones could be transmitted to the cochlear fluid.

The basilar membrane in mammals is narrow in basal turn of the cochlea and becom progressively wider in more apical turns. The width of the membrame varies from 105 μ in the basal turn to 500 μ in the apical turn in man. Similar change in width is found in Noctilidae, Molossidae and most Phyllostomatidae, although the width is smaller in bat than man of cause. The width in those echolocating bats increases from 80-90 μ in basal turn to 140-150 μ in apical turn. It is noticiable that the basilar membrane in Rhinolophus has a width of 100 μ in the basal turn, it decreases to 80 μ in the second turn in contrast to other bats, then increases to 120 μ in apical turn.

In many CF-FM bats, the basilar membrane has two thickenings, while in some FM bats they are small or absent. These thickenings in basilar membrane may correspond to the sharp sensitivity curve at a certain frequency in those CF-FM bats, regarded as the mechanical tuning theory as proposed to the constricted basilar membrane in Rhinolophus. On the other hand, histological investigations showed the dense innervations to the hair cells at a certain level of organ of Corti (say second turn in Chilonycteris, Engström et al., 1966). This proposed the neural tuning theory for the sharply tuned curve in audiogram of the animal, at the

frequency corresponded to that of CF part of orientation sound. The sensory hair cells of the bats are very similar to those of other mammals, though they are arranged in higher density than in man. The density of the hair cells are 440 hair cells/mm in man, 550 in Rhinolophus, 580 and 570 in Myotis and Eptesicus respectively. High density arrangement of hair cells may provide the high sensitivity in high frequency sound.

The hair cells receive an inhibitory efferent nerve regarded as olivo-cochlear bundle (OCB) though little is known about its function in bats.

Ascending auditory system in the brain of the echolocating bats shows also specialization for echolocation. Figures 2 and 3 represent schematic diagrams of the ascending and the descending auditory pathways respectively. Most auditory fibers terminate in the ventral cochlear nucleus (VCN). The fibers arising from the VCN ascend to both the contra- and ipsi-lateral superior olivary complex (SOC). The SOC fiber forms major part of the lateral lemniscus and changes synapses in the dorsal or ventral nucleus of the lateral lemniscus (DNLL and VNLL). Some of them do not terminate in NLL and ascend directly to the inferior colliculus (IC). Fibers from NLL terminte in IC. Inferior colliculus is an essential part for echolocation in Microchiropteran bats. The ablation of both side of IC causes severe inability in echolocation in the animal without

vision, while the ablation of auditory cortex has little effect (Suga, 1969). The CN in echolocating bats comprises 14-23 % of the brain stem and only 5-8 % of that in non-echolocating bat. SOC and medial geniculate body (MGB) are about three to four times larger in Microchiroptera than in Megachiroptera. Thus the auditory system of echolocating bats are hypertrophied as emphasized with the under-line in Figure 2, relative to the body size, especially SOC and IC are.

SOC consists basically of five nuclei; lateral preolive, medial preolive, lateral superior olive (LSO, S-segment after its shape), medial superior olive (MSO, accessory nucleus) and medial trapezoid body (MTB) in mammals. All those nuclei receive the input from both side of VCN, therefore SOC is the first place where ipsi- and contra-lateral informations encounter. Thus SOC is an important nuclei for the directionality of echolocating system as a result of binaural interaction. SOC in echolocating bats interestingly has no MSO, accessory nucleus or rudimentary one and does developed LSO and MTB, while other acoustically sensuous animals such as cat have all those three nuclei.

In general, echolocating bats have well developed external, middle and inner ear equipped with mobile sound collector and powerful attenuator, and also does the hypertrophied ascending auditory nuclei except for MSO. IC seems to be an essential center for echolocation while auditory

cortex is not specialized compared with other mammals.

Research aim

Echolocating system in Microchiropteran bats is well developed as one of the effective means to survey the environment. The other mean is of cause the vision, in bats the vision is also effective to survey the outside world, in fact they can locate large insects without any help of sonar system when the visual cue is sufficient.

The sonar system can be contrasted with the system analysis in engineering field, by which a characteristics of an unkown system (black box) can be estimated and described equivalently in terms of transfer function between driving input and response output. In analogy with bat's sonar system (Figure 1), the driving input is orientation sound, the output response and the transfer function are echoes and the acoustic impedances distributing in surroundings.

In order to perform the biological tasks with the echolocating system, the bats should control the coordination between auditory nervous system (input channel for bat pickingup an output response from the outside world) and vocalization system (output channel for bat) in an organized way more severely than the other animals do.

The bat must emit intense orientation sound by activating

a set of motor neurons. This activation would occur in cranial nerves IX (Glosso-pharyngeal), X (Vagus), XII (Hypoglossal) innervating to pharynx, larynx and tracheal muscles, and some spinal motor roots to thorax may be involved to air flow supply. An activation pattern in IXth, Xth, XIIth cranial nerves is important to produce whether CF-FM or FM orientation sound. The electrical stimulation in the brain was performed in order to clarify whether a certain part of the brain is concerned with the emission of species-specific orientation sound (CF-FM or FM) or a certain part is concerned with the emission of certain type of sound (CF or FM), in latter case the animal emitting CF-FM orientation sound would activate one part of the brain then the next. The part concerned with the emission of orientation sound was searched by means of electrical stimulation in the brain. The part interested in must have strong connection with IC, analyzing center for echolocation, or other auditory nuclei, because the emission of orientation sound is affected strongly by acoustic environment such as echo-returning time or jamming noise. Several species of CF-FM and FM bats were used in this experiment, and that a part of midbrain is concerned with the species-specific orientation sound production was found. This work is comprised in Chapter I.

In Chapter II, the directional sensitivity of echo-locating system measured will be presented. The directional spread of the orientation sound could hardly be measured

for the flying animal echolocating voluntarily (Möhre, 1966), because of the movement of sound source during measurement. In order to measure the sound field of the orientation sound, the head of animal should be held at a direction, in such experimental condition the animal would never attempt to echolocate. By using a electrical stimulation of the certain part of the midbrain, one can obtain the sufficient number of orientation sound emitted by the animal with a fixed head for accurate sound field measurement. Thus it was possible to measure the directional sensitivity as an echolocating system by comparison with the directionality of orientation sound and that of auditory system in relation to the frequency dependency.

When the animal is emitting an orientation sound, the animal should control the reactivity of auditory channel downward, otherwise the input channel would be stimulated by the strong self-emitted sound and as a result the channel could not recover its sensitivity until the faint echo comes back. This attenuation mechanism to self-stimulation involves both mechanical one occurred in middle ear by means of middle ear muscle contraction and neural one in the ascending auditory pathway by suppression or inhibition in neural activity. Both those are investigated, and the neural one will be described in Chapter III. In this study the electrical stimulation technique was also employed to elicit the vocalization, since by using this experimental

method, the vocalization is predictable in some extent. This predictability was convenient to observe the pre-activity which is an activity occurred prior to the main activity. And it was found that bat's auditory pathway receives an efferent copy-like activities during sonar cry. Mechanical attenuation mechanism will be clarified in Chapter IV.

As mentioned previously, it is behaviorally clear that the vocalization system and the auditory system is coupled strongly in echolocating bats. One side of the view of this coupling from efferent to afferent was investigated and will be described in Chapter III, based on the investigations in Chapter I and II.

The other side of this coupling from auditory to vocalization system will be described in Chapter IV in relation with some neurophysiological studies on auditory neurons. It is found that the electrically activated vocalization in some species of bats could be modified by an acoustic environment. The properties of the acoustic stimuli which is able to affect to the vocalization is closely related with that of auditory system.

This is the present main goal here to shed a light on What kind of interactions take place between auditory and vocalization system in this ingeneous "sonar equipped animal".

Figure 1.

Schematic representation of echolocation system in bats. Bat emits orientation sound (out-going sonar cry) to the outside world (black box). The sound emitted by bat is in turn the driving input for the black box, and the echo is response output. Echoes and jamming noise are received by the ear of bat. Self-emitted sound also stimulates the bat's auditory system (self-stim.). The self-stimulation is attenuated by middle-ear muscles (MEMs) which contract synchronously with vocalization. Nerve impulses converted into by the sensory hair cells (HCs) ascend to higher auditory nuclei (CN; cochlear nucleus, SO; superior olivary complex, NLL; nucleus of lateral lemniscus, IC; inferior colliculus, HON; higher order nervous systems). When the bat emits the orientation sound, a certain part of central gray matter (CGM) or reticular formation (RF) in the midbrain is activated. This activation involves the nerve activity of a set of cranial nerves to larynx and pharynx; IX, X, XII, at least and V, VII, IX to the middle-ear muscles. In addition to those cranial nerve activation there is a neural attenuation mechanism (N.Att.) in synchrony with vocalization at the level of NLL. Olivo-cochlear bundle (OCB) is known as an inhibitory efferent pathway from SO to hair cells but little is known in bats. Echo-perception controls the vocalization activity, this modification is illustrated as vocal response (V. R.).

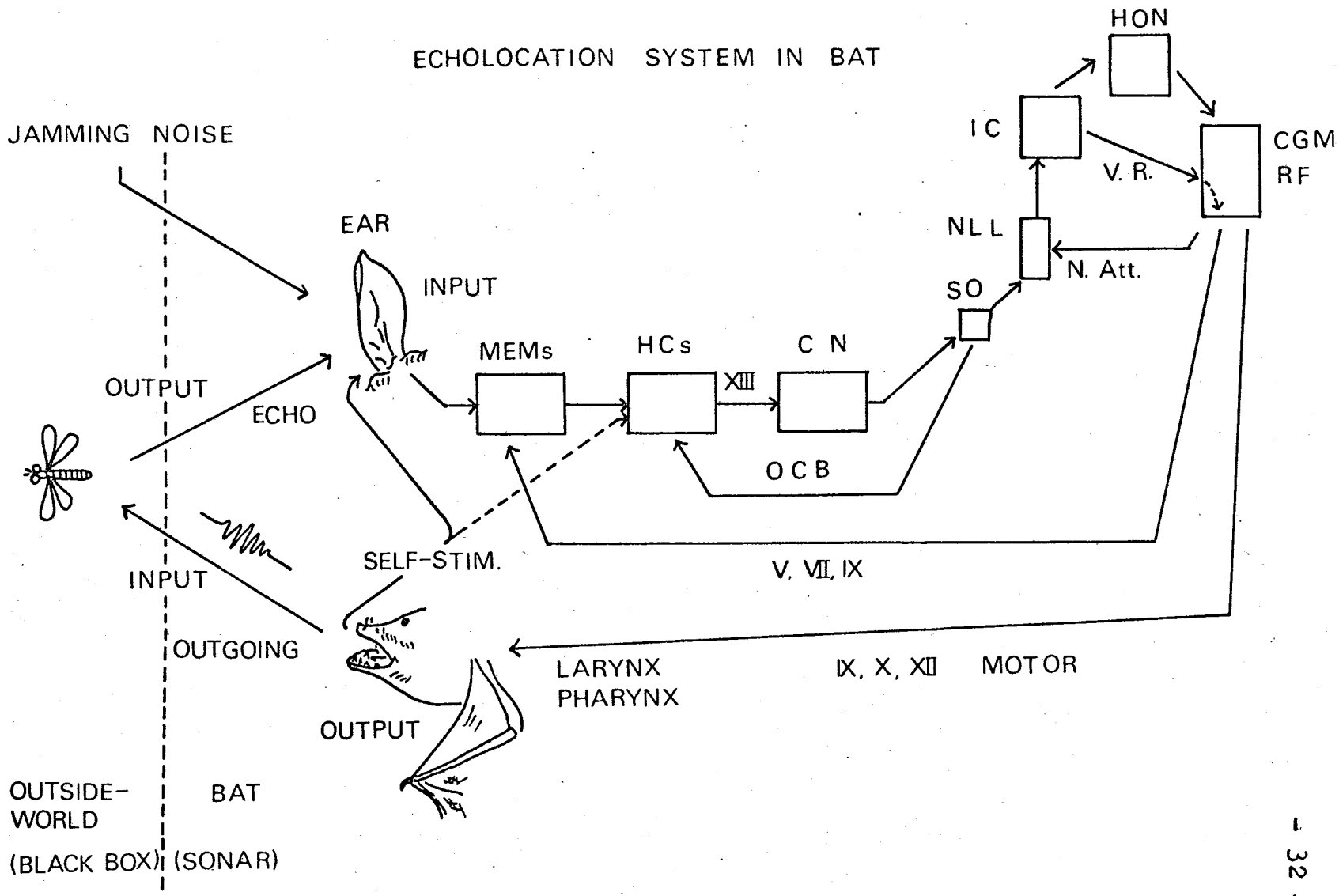

ECHOLOCATION SYSTEM IN BAT
HON
IC
CGM
RF
V. R.
JAMMING NOISE
EAR
INPUT
NLL
N. Att.
SO
MEMs
HCs
C N
XIII
OUTPUT
ECHO
OCB
SELF-STIM.
V, VII, IX
INPUT
OUTGOING
LARYNX
PHARYNX
IX, X, XII
MOTOR
OUTPUT
OUTSIDE-
WORLD
(BLACK BOX)
BAT
(SONAR)

FIGURE 1.

Figure 2.

Simplified schema of the generalized ascending auditory pathways in mammals. Relative location of each nucleus is indicated on left. Sites of the origin of auditory evoked potentials are shown by dotted arrows. Each nucleus may be divided into subnuclei. The underlined nuclei are hypertrophied in echolocating bats. See text for abbreviations.

Ascending Auditory Pathways

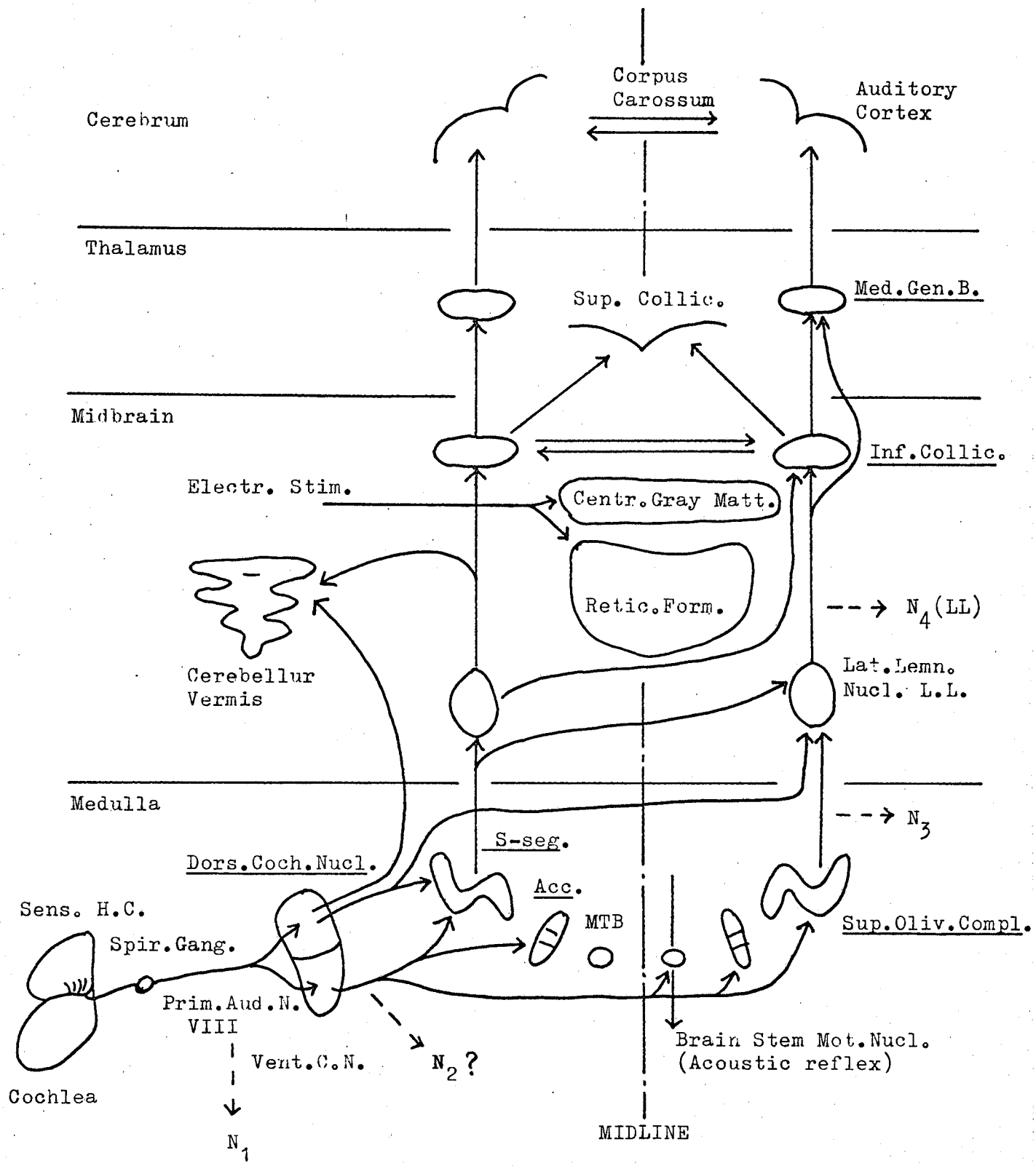


FIGURE 2.

Figure 3.

Simplified schema of the descending auditory pathways seen in mammals. Dashed lines indicate the connections not firmly anatomically established. See Figure 2 for abbreviations.

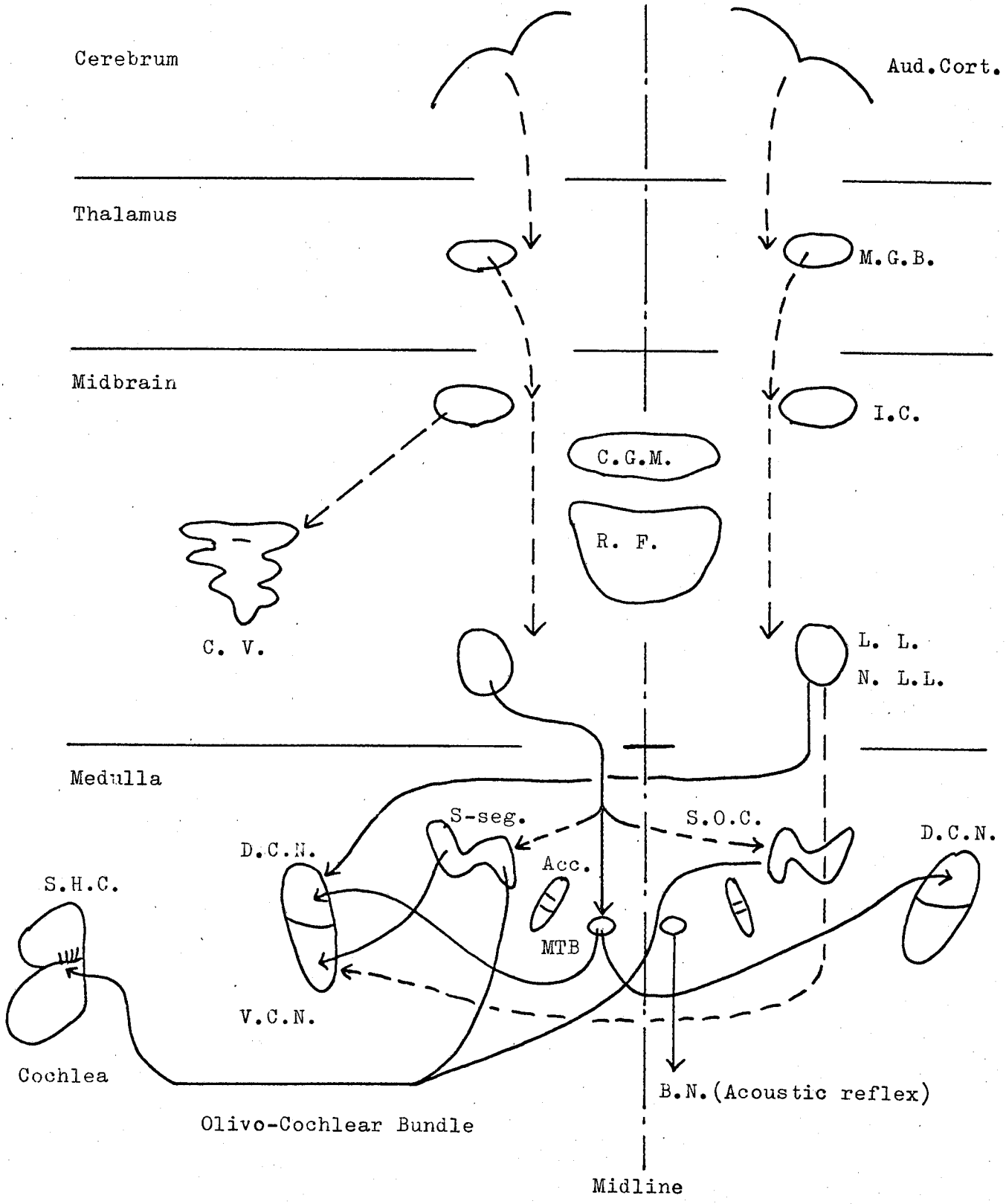

Cerebrum
Aud.Cort.
Thalamus
M.G.B.
Midbrain
I.C.
C.G.M.
R. F.
C. V.
L. L.
N. L.L.
Medulla
S-seg.
S.O.C.
D.C.N.
D.C.N.
Acc.
S.H.C.
MTB
V.C.N.
Cochlea
B.N.(Acoustic reflex)
Olivo-Cochlear Bundle
Midline

FIGURE 3.

MATERIALS AND METHODS IN GENERAL

Experimental materials

Big brown bat Eptesicus fuscus, little brown bat Myotis lucifugus, gray bat Myotis grisescens and Myotis austroriparius, were used as subjects of FM bats all they emit the frequency-modulated (FM) orientation sound for echolocation. Those animals were caught in caves in autumn and winter during their hibernation, or in spring and summer in attics or ceiling of old church or barn. The location of the collection of the animals was southwestern Missouri or southeastern Illinois of the United States of America. The animals with a weight about 5 grams and 12-15 cm of wingspread in Myotis, and about 10 grams and 15-20 cm wingspread in Eptesicus were kept in a flight cage of chicken mesh after training to eat mealworms as a tethered food. When the animals were caught under a hibernating season, the animals were kept in a cold room of a temperature of 14 °C, and the animal was warmed up in a room temperature for a few hours before surgery for experiment. The three species of genus Myotis are all closely related, and there was no noticeable difference in the orientation sounds and audiograms in the experiments among species.

The bats emitting the orientation sounds consisted of a constant-frequency (CF) component with an FM sound (CF-FM)

in the terminal portion were also used for comparison with the FM bats. Mustache bat Pteronotus parnellii (rubiginosus), naked-backed bat Pteronotus suapurensis and fish-catching bat Noctilio leporinus were used. P. parnellii and P. suapurensis were caught in Panama, and N. leporinus was caught in Trinidad. Pteronotus parnellii was previously nomenclatured Chilonycteris rubiginosa (Smith, 1972). Two species of Pteronotus were kept in a humidified room warmed up to 30 °C, after training for mealworms, because the natural cave in which they live in Central America has a high humidity and high temperature. Noctilio was kept in the same conditions as Pteronotus except that Noctilio was not trained to eat mealworms but to take pieces of smelt.

All bats used were loud bats, they emit species-specific orientation sounds from the mouth in the amplitude of 100-115 dB SPL measured at about 10 cm in front of the mouth.

Ether was used to anesthetize the animal only during surgery. For the experimental measurements, awake animal was used at least after 2 hours from the end of the ether administration. Under ether anesthesia, the bat was secured onto a metal plate which was about 4 cm wide and 20 cm in length and was tapered to a point of 5 mm width. The metal plate had holes along its both sides. The bat's wings were folded under the plate and held against the lower surface of the plate with a rubber band. A loop of thread which came through the holes in the plate could hook the bat's

lower jaw and was tightened to hold the jaw firmly in place. The fur on the top of the head was trimmed, and the head skin was incised along the dorsal midline. Temporal muscles on the skull were exposed and were carefully worn out to be removed from the skull bone with a minimum bleeding. After cleaning with a cotton ball soaked with 70 % ethyl alcohol, the dried exposed area of the skull was coated with an acrylic adhesive (Eastman 910), and the flat end of a nail of about 1.8 cm in length was mounted with acrylic dental cement (Fastcure) on the anterior part of the treated skull bone. The incised skin was tightened with sutures to the nail in order to relocate the pinnae to their original position, the skull covering the inferior colliculi, however, was kept visible and accessable to electrode insertion. The animal was then released to a cage untill recovered from the anesthesia. In some case, the animals surgically operated one or two days before experiments were used without any problems. An antimicrobial agent (Sulfathiazole) was applied directly to the incision or to the worn musculature to prevent infections. The same animal can be used repeatedly to the different types of experiments for successive days.

The awake bat was then brought into the sound-proofed room. The nail was locked onto the metal rod with a set screw. The metal rod was mounted on a micromanipulator so that the head of the animal could be positioned properly. The animal was placed on the plastic ball floating on water in

a bowl to absorb the movements of animal, so that the animal sat on the ball with a rigidly fixed head. Noctilio and P. parnellii are large animals whose wingspreads are more than 30 cm, so that the nail and the cementing could not be strong enough to held the animal against its movements. Those animal's heads were supported by three blunt needles pressed against the skull bone after removing the temporal muscles. The needles were supported by three manipulators and cemented on the skull.

All supporting structures and manipulators were at least 10 degrees behind the center of the animal's head. The head of the animal was positioned so that the eye-nostril line became horizontal and that the center of the ears is defined as the center of the perimeter. The surfaces of the supporting structures and experimental equipments except the vibrating (active) membranes of microphone and loudspeaker were covered with absorbent cotton to minimize the possible echoes from these structures to both the sounds emitted by animal and/or by loudspeaker. The inner walls of the sound-proofed room were covered with fiber-glass sheets for the same reason. The sound-proofed room was also electrically shielded.

Stimulating methods

The stimulation and recording system is shown schematically in Figure 4. The continuous sinusoidal waves generated by

a voltage-controlled oscillator (Wavetek model 111) were fed into a tone pulse generator (T. P. G.). The frequency of the oscillator was constant (CF) or modulated (FM) by an output voltage of a sweep function generator (Wavetek model 115) synchronously with a electric pulse from a stimulator (Grass model S-88) providing a master trigger pulse. CF-FM signal could be also generated by triggering the sweep generator with a proper time delay. The frequencies of the CF and/or FM sounds were monitored by a conventional electronic pulse counter with an accuracy of 0.01 kHz. The tone pulse generator formed the envelope of the tone pulse (i.e. 100 % amplitude modulation or electronic switching) in synchrony with the pulse output of the stimulator. The rise-decay time was variable from 0.01 to 10 milliseconds independently each other. In most case the rise-decay time was kept at 0.5 msec in order to reduce transients at the onset and cessation, but changed, otherwise, as stated for the purposes of experiments. When the rise-decay time was reduced, the tone pulses had a scattered frequency components around the vicinity of its fundamental frequency depending on the rise-decay time as a result of acoustic transients caused by the rapid change in amplitude. The duration and the repetition rate were determined by those of the stimulator output. Hereafter, the term "tone burst" means the repetitive tonal pulses whose duration is 2 msec with a 2 msec inter-pulse interval, and the repetition rate is consequently 250 per second,

unless otherwise described. Thus the tone burst is chopped sound wave. The tone burst may consist of a frequency-modulated sound as well as of a pure tone. On the other hand, the term "tone pulse" implies the tonal stimulus whose repetition rate is quite low or the stimulus delivered in order to measure an activity to each stimulus pulse but not to a repetitive stimuli.

On-off ratio of the tone pulse generator was more than 60 decibels. "Decibel" is a technical measure representing conveniently a difference between two physical values, in this case the voltage amplitude of the tonal electric signal when the electronic gate is opened (off) and when closed (on), with a logarithmic scale. Decibel (dB) is a minor scale of Bel (after Alexander G. Bell, the inventor of telephone). For example, 20 dB implies that a potential amplitude is ten times larger or smaller than the other.

The tonal signal from the tone pulse generator were amplified by 40 dB amplifier (Hewlet Packard model 465A). The signal was then attenuated with a decade attenuator (Hewlet Packard model 350D) at a desired level of stimulus intensity by a 1 dB step. There were two systems of the tone pulse generator consisted of sinusoidal wave oscillators and attenuator independently controllable in order to permit a two tone stimulation or masking experiments (dotted box in Figure 4). The two signals were mixed and fed into a power amplifier (Krohn-Hite model DCA-50R). A 40 dB power

attenuator was inserted into line after the power amplifier in order to improve the signal-to-noise ratio when the signal was attenuated more than 40 dB. The signals from the power amplifier were monitored in one channel of an oscilloscope (Tektronix R561) to avoid a distortion in emitted sound due to an overdrive of the amplifier.

The tonal stimulus was delivered from a Myler-filmed condenser loudspeaker (made by Lincoln Laboratory, Massachusetts Institute of Technology) after adding a 200 V bias voltage. The loudspeaker was mounted on an arm of the perimeter at 66 cm distant from the center of the bat's ears. The loudspeaker was placed at the midplane of the animal unless otherwise noted. The distance between the experimental animal and the surface of the loudspeaker caused a 1.9 msec acoustic delay due to the propagation velocity. The amplitude of the emitted sound was calibrated with a calibrated quarter-inch microphone (Brüel and Kjaer 4135) placed at the position substituting the bat's ear. The maximum available amplitude is shown by the dotted curve in each graph for the sensitivity thresholds. All sound amplitude are expressed in decibels in sound pressure level (dB SPL). Zero dB SPL corresponds to the value of 0.0002 dynes/cm^2 (μbar) or 2×10^{-5} N/m^2 in pressure, or 10^{-16} Watts/cm^2 in intensity (energy transmitted through a unit area) level for air born sound. This value was traditionary defined as the reference level of sound because that the psycho-physical threshold of sensation of

human hearing is minimum at about this value around 1 kHz.

The threshold curves were plotted in a way as followed, (1) a criterion of the neural or behavioral response to an acoustic stimulus was defined, (2) at a certain frequency (sweep), the minimum intensity of the stimuli to produce the critical response was determined by changing amount of attenuation with the decade attenuator, (3) the amount of attenuation was plotted by subtracting it from the calibrated frequency-response curve of the loudspeaker, i.e., the maximum available amplitude curve. Because of the frequency-response curve of the loudspeaker, the intensity within the FM sound was not uniform but varied with frequency even at a constant input voltage to the loudspeaker (as discussed in Chapter III). Therefore, the intensity of the FM tone pulse had to be expressed as attenuation below the maximum intesity available from the loudspeaker, i.e., a curve parallel to the frequency-response curve. However, the threshold of the FM tone is given with the linear arrow having the intensity at the center of its frequency sweep for the sake of simplicity of description (see Figures 4 and 5 in Chapter IV).

When the tape recorded orientation sound (see below) had to be emitted through the condenser loudspeaker to stimulate the animal, the playback output of the magnetic tape recorder (Ampex FR-100) was connected to the input of a tone pulse generator instead of the oscillator, after adjusting the amplitude at the peak to that of the oscillator output.

The electrical stimulation of the brain was delivered with a paired electrode. The electrode was made with stainless steel musical wire. After sharpening by an electrolytic etching with solution of sodium nitrate ($NaNO_3$), the electrode was coated with silicon lacquer. Two coated electrodes were then stuck together under a microscope. The tip of this bipolar electrode was trimmed to be less than 50 μ in size and 0.2-0.5 mm apart. The bipolar electrode was employed to minimize the effects of current spread in the brain resulting a wide spread excitation. If monopolar electrode is used, the electric current passes through the brain tissue to the indifferent electrode so that the electrically evoked activity can not be attributed to the specific site of electrode tip. Electric pulses were provided by the stimulator and were applied to the electrode through an isolator unit to minimize the electric current flowing to the indifferent electrode. Electrical stimuli were monitored on the oscilloscope screen and stored in the magnetic tape recorder (Ampex FR-100) for help in the later analysis. The steel electrode has an advantage for identification of the location of the electrode tip, since iron ions deposited from the electrode electrophoretically by passing electric current can be stained easily. Iron ions react with ferri- or ferro-potassium cyanide and result Prussian Blue (Berlin Blau). When such a histological examination was necessary, the experimental animal was sacrified at the end of experiments, and the brain

was extracted. The brain was fixed with Buin's solution saturated with ferro-potassium cyanide.

Recording methods

The sounds emitted by bat were recorded with a quarter-inch microphone (Brüel and Kjaer 4135) placed at various positions depending on the purpose of the experiment. The envelope of the emitted sounds were monitored on an oscilloscope screen. A zero-crossing period meter (made by Lincoln Lab.) was used for the immediate examination of frequency change in orientation sounds, if necessary. The output of the zero-crossing period meter was displayed on an oscilloscope (Tektronix R561). The sound emitted by animal voluntarily or in response to a stimulus were stored after a proper amplification (Hewlet Packard model 465A) with a direct channel of a magnetic tape recorder (Ampex FR-100) having a flat frequency-response characteristics of 300-300,000 Hz at a tape speed of 60 inches/sec, and 50-150,000 Hz at 30 inches/sec. All signals being recorded in tape were monitored with an oscilloscope to avoid the distortion due to overdrive which may result a misunderstanding in later analysis. The sounds stored in the tape were later played back with reduced tape speed of 1/16 or 1/32 of the original tape speed for a detailed analysis. A sonagraph (Kay electric Co.) was used for the frequency component analysis of orientation signals and other sounds emitted by the animals. The sonagram

represents a complex sound with an ordinate of frequency components versus time in abcissa. The intensity of the component is represented by the density of the component appeared, so that one can express the complex sound in terms of changes in frequency components and its intensity with time (e.g. see Figures 5 and 8 in Chapter I). The tape recorded sounds were played back in order to stimulate the animal by the own emitted sound but not at the time of sound emission for the investigation of the auditory responses accompanied with vocalization. The recorded sounds were played back with a reduced tape speed so that each sound with the period meter output could be photographed by a long-recording camera (Nihon-Kohden) automatically by utilizing the tape recorded master trigger pulses for shutter control.

Essentially, neural activities recorded and analyzed in the present experiments were evoked potentials. Recording electrodes were tungsten-wire electrode sharpened electrolytically and coated with silicon lacquer. The etching solution was 10 % NaOH. The recording and stimulating electrodes were introduced into the brain through the hole on the skull made by chipping away the skull bone by surgical blade or forceps. Since the skull bone of the experimental animals was thin enough to be transparent, blood vessels and foldings on the brain were quite visible so that the electrodes were easily aimed to the desired position by using micromanipulators (Narishige MM-3). The electrodes were located at a desired

place in the brain by advancing it, through the inferior colliculus, untill the desired response to acoustic or electrical stimulus becomes maximum and undesired ones do minimum. The indifferent electrode was placed in the exposed temporal muscles. The electrical potential picked up by the electrode was fed into a field effect transistor (FET) input amplifier (Grass P-15). After amplification, the summated neural activities were displayed on the screen of the oscilloscope (Tektronix 565) with the stimulus signals. When the recording electrode was placed on the surface of the inferior colliculus, a complex evoked potential with five positive peaks is easily recorded. Those five peaks are called N_1, N_2, N_3, N_4, and N_5 respectively, meaning the negative waves by character N. In fact the potential change is positive, this is erroneous nomenclature due to a traditional confusion of the definition and terminology for the polarity of excitation in nervous system. Furthermore, there has been a contradictional confusion about N_4 for its origin. In the present experiments it is clear that N_4 is originated mainly from the nucleus of the lateral lemniscus (as described in Chapter III), therefore LL is used in the present work instead of N_4. When an electrode was advanced into or nearby the nucleus originating an evoked potential, the recorded potential became as large as a few millivolts and diphasic or triphasic while the potentials recorded out of the nucleus were monophasic. The electrode was fixed on the skull with acrylic

cement to prevent dislocation of the tip when necessary, so that semi-chronic recording were possible. When the condition of the experimental animal was excellent, the animal could survive a few days or more after starting the experiments, in such case mealworms were given as food during the meal time of experimenter. To identify the location of the tip of a tungsten electrode, recording electrode was connected to the stimulator and electric current was applied at the end of the experiments. Since electric current flow is converging at the tip of electrode, the tip becomes so hot by the heat production at ohmic resistance that the brain tissue at the burning tip was destroyed. The branded brain was extracted and fixed in Bouin's solution. The location of the electrode was thus indicated by an evacuated spot in the histological preparation.

A special purpose computer (Nicolet 1070) was used to average the evoked potentials or to make a post-stimulus-time (PST) histogram for the single unit activity. The computer was triggered by stimulus onset, i.e., stimulator output or the onset of the emitted orientation sound. The averaged responses or PST histograms were plotted on paper with an X-Y recorder (Hewlet Packard model 7034A). Glass micropipette electrode filled with 3 M KCl was used for single unit recording from the primary auditory nerve, though only preliminary result was obtained. In order to approach the auditory nerve, a lateral part of cerebellum

(parafloculus) was aspirated with a small pipette taking a special care of avoiding a destruction of cochlea. Cochlear microphonic potentials (CM) were recorded in order to examine the middle-ear muscle activity. CM was recorded with tungsten electrode as those used in evoked potential recording or simply with coated silver with bear tip placed at the round window. The round window was approached through the dorso-posterior portion of auditory bulla, in order to keep the vocalization system intact. The electronic filter was used to separate the CM and evoked potentials or to discriminate CMs having different frequencies from each other.

All signals described above were fed into the magnetic tape recorder (MTR connections in Fig.4) and recorded or played back when necessary.

Figure 4.

Schematic diagram representing the stimulating and the recording system employed for experiments generally. The equipments inside of the broken line were placed in the sound-proofed room. A rectangle drawn by dotted line represents one of the tone pulse generator system as shown at left of it. MTRs on both sides of CRT oscilloscope represent the input and output panel of the magnetic tape recorder seen at upper-left corner. Some of the connections were modified for the purposes of the experiment as described in each chapter. Only the main equipments and connections are illustrated, see text in each chapter for details.

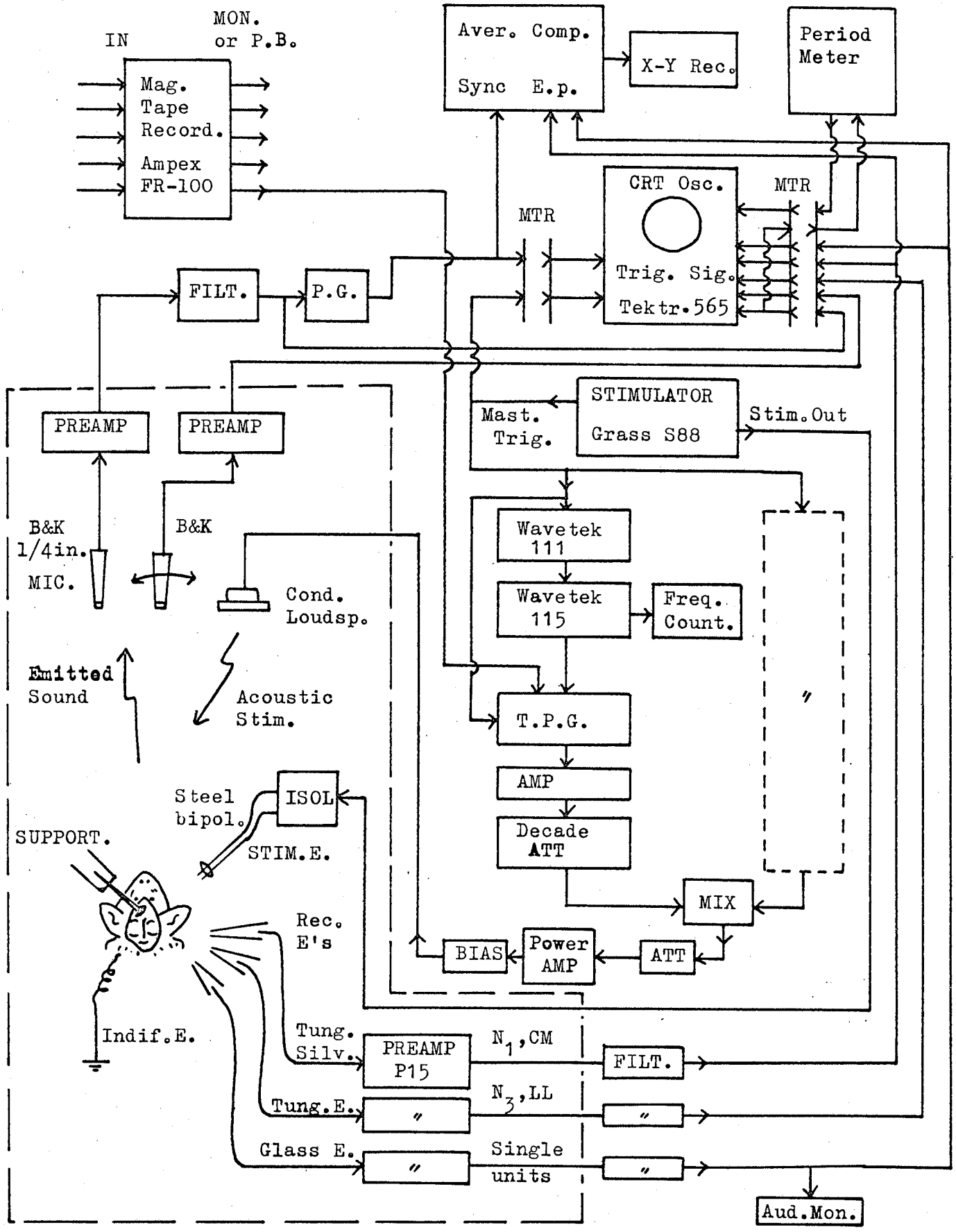

IN
MON.
or P.B.
Mag.
Tape
Record.
Ampex
FR-100
Aver. Comp.
Sync E.p.
X-Y Rec.
Period
Meter
CRT Osc.
MTR
MTR
Trig. Sig.
Tektr.565
FILT.
P.G.
STIMULATOR
Grass S88
Mast.
Trig.
Stim.Out
PREAMP
PREAMP
B&K
1/4in.
MIC.
B&K
Cond.
Loudsp.
Wavetek
111
Wavetek
115
Freq.
Count.
Emitted
Sound
Acoustic
Stim.
T.P.G.
AMP
Decade
ATT
MIX
Steel
bipol.
ISOL
SUPPORT.
STIM.E.
Rec.
E's
BIAS
Power
AMP
ATT
Indif.E.
Tung.
Silv.
PREAMP
P15
N_1,CM
FILT.
Tung.E.
"
N_3,LL
"
Glass E.
"
Single
units
"
Aud.Mon.

FIGURE 4.

CHAPTER I

Orientation Sound Emission by Electrical Stimulation of the Brain

In order to investigate the coupling between auditory and vocalization system, animal must be awake and emit orientation sound. Since neurophysiological research on echolocation has been performed on anesthetized bats passively listening to acoustic stimuli, and the experimental condition under which the animal is held rigidly does not allow the animal to attempt to echolocate, it has been unknown what kind of interaction takes place in auditory and/or vocalization pathways when the animal echolocates.

Since the middle-ear muscles of mammals contract in synchrony with vocalization (Carmel and Starr, 1963; Henson, 1965; Salomon and Starr, 1963), and the responses of auditory neurons in the midbrain to self-vocalized orientation sounds are attenuated by some neural mechanism (Suga and Schlegel, 1972; Chapter III), neural responses to echoes should be studied while the bat is free to vocalize. Furthermore, it is essential to know to what extent the bat hears the self-vocalized sounds during echolocation and whether the hearing of self-vocalized sound plays a role in the detection and processing of echoes. If one could find the regions concerned

with the emission of orientation sounds in the brain, it would greatly facilitate studies on echolocation.

In mammals and birds, certain types of communication sounds can be evoked by electrical stimulation of specific region of the brain which is located near the midbrain auditory nuclei (e.g., Jügens and Ploog, 1970; Potash, 1970). For echolocation, bats emit orientation sound and listen to echoes. The activity of the vocalization system is modified by echo-perception, so that the area concerned with the emission of orientation sound may be located near the auditory nuclei which are important for echolocation. In bats, the inferior colliculus is hypertrophied (Poljak, 1926) and it contains various types of auditory neurons which are clearly associated with the extraction of information from echoes (Suga, 1965, 1968, 1969a). When the inferior colliculi on both sides are severed, the bat emits sound in abnormal temporal patterns and fails to avoid obstacles by means of echolocation (Suga, 1969b). The inferior colliculus thus appears to be an important center for echolocation. Both ascending and descending neural pathways pass through the inferior colliculus, so that it is considered important for acoustic reflexes (Galambos, 1954; Rossi, 1967). The results reported in this chapter indicate that the midbrain regions adjacent to the inferior colliculus are concerned with the emission of species-specific orientation sounds in echo-locating bats.

Materials and Methods

The FM bats, Myotis austroriparius, M. grisescens, M. lucifugus, Eptesicus fuscus, and the CF-FM bats Pteronotus parnellii, P. suapurensis, Noctilio leporinus were used in the experiments. The three species of Myotis are all closely related, and there were no noticeable differences in experimental data among species in this genus.

Under ether anesthesia, nails of 1.8 cm in length were mounted with acrylic dental cement on the skulls of 28 Myotis, seven P. parnellii, two Eptesicus, three P. suapurensis and two Noctilio. Several hours after the operation, the revived animal was placed on a plastic ball floating on water; then the nail was fixed to a metal rod with a set screw. The floating ball absorbed the force of the bat's movements and induced the bat remain inert. Without anesthesia, a small hole was made in the skull, through which a pair of sharpened steel electrodes with about 1 Megohm resistance were inserted for delivering electrical stimuli. The tips of the electrodes were less than 50 μ in diameter and were 0.2 to 0.5 mm apart. The electrical stimuli used to evoke vocalization were short trains of monopolar pulses delivered two times per second. Each train consisted of six electrical pulses, each of which had a 0.1 msec duration and a 1 to 15 volts in amplitude. The inter-pulse interval was 1.7 msec. Sounds emitted by the bat were picked up with a 1/4 inch microphone (Brüel and

Kjaer model 4135) placed 4 to 10 cm anterior to the bat's mouth and were recorded with a magnetic tape recorder having a frequency response of 50-150,000 Hz at a tape speed of 30 inches/sec (Ampex model FR-100). The sounds were later analyzed with a sonagraph (Kay Electric Co.) by reproducing them at 1/16 or 1/32 of the original tape speed. The location of the tip of the stimulating electrodes was determined by histological examination of the Prussian blue spots resultant from the reaction between ferri- and ferro-cyanide and iron ions deposited electrophoretically from the stimulating electrode.

Results

When electrical stimuli were delivered to the dorsal part of the reticular formation in the midbrain of Myotis, an area antero-ventral to the inferior colliculus (Figure 6), the bat moved its mouth and pinnae while emitting an FM sound with an amplitude of 110 to 115 dB SPL (Figure 5, A and B1). The latency of response was 40 to 60 msec after the beginning of each train of electrical pulses. No other body movements were associated with the emission of these sounds. Minor movements of the pinnae, such as those produced by the electrical stimuli, have not been reported to occur synchronously with autonomous echolocation in Myotis. The electrically elicited sounds were about 3-4 msec in duration and they swept downward in frequency from 100 to 40 kHz (Fig. 5, C). The first harmonic sweep always predominated and was the only component consistently recognizable, although at least the second and some times third harmonics were included in the emitted sounds. When an electrical stimulus of a subthreshold voltage was applied repetitively, facilitation and eventual emission of sounds often occurred. For example, at some particular stimulus voltage, too low to immediately evoke vocalization in a one-to-one relationship, no vocalizations would occur for the first 20 pulse trains delivered. After 20 or so stimulations, a weak evoked vocalization with a variable latency would appear, and finally a strong (110

to 115 dB SPL) FM sound would be emitted by the bat with a relatively stable latency after each train of electrical pulse (Fig. 5, B1). The amplitude of the emitted sounds varied somewhat with the stimulus amplitude. Although the FM sounds produced by successive stimuli were variable to some extent in amplitude and duration, the range of the frequency sweeps and the envelopes were relatively constant as long as the stimulus parameters were fixed. The vocalizations always ceased immediately upon termination of the electrical stimulation. Habituation of the evoked vocalizations sometimes occurred. This was manifested in the reduced amplitudes of the evoked sounds or in a rise in the threshold voltage required to produce reliable vocalizations with each stimulation.

Stimulation of the lateral part of the central gray matter, an area antero-ventral to the inferior colliculus (see Fig. 6), always evoked vocalizations which consisted of several short FM sounds of 110 to 115 dB SPL occurring together in a group or in several groups (Fig. 5, B2). The animal only moved its mouth and pinnae, besides emitting sounds. No other obvious body movements were involved. Facilitation of the vocal response was observed for subthreshold repetitive electrical stimuli. When the vocalizations evoked by repetitive stimulation stabilized after a number of stimulus presentations, the latency was 25 to 30 msec. The number of groups of several sounds evoked by

each train of electric pulses increased with increases in stimulus voltage. Each sound had about 1.0 msec duration and the frequency always swept downward from about 40 to 20 kHz (Fig. 5, D). The repetition rate of the sounds in each group was about 140/sec, and the groups occurred about 13 times/sec. The rate of respiration in bats can be as rapid as 10/sec, and a group of orientation sounds is emitted during the exhalation period of respiration (Suthers et al., 1972), so it seems likely that each group of the electrically evoked sounds is related to the exhalation period (Fig. 5, B2). The regions of the brain located close to the inferior colliculus are thus concerned with the emission of orientation sounds.

There are other places in the brain of Myotis where electrical stimulation elicits vocalization. For example, stimulation of the ventro-medial part of the central gray matter or the center of the midbrain reticular formation evoked downward sweeping FM sounds, but in association with various large body movements. Some of these sounds contained human audible components, and these were quite distinct from orientation sounds in duration and frequency sweep. These areas of the brain appear to be unrelated to the emission of orientation sounds. Stimulation of the thalamus and/or the hypothalamus sometimes evoked either a long noise burst (Fig. 5, F), or a complex sound with several harmonics in irregular frequency sweeps or in a nearly constant frequency

(Fig. 5, E). All of these sounds were quite different from orientation sounds. Stimulation of the medulla, cerebellar vermis, or cerebral cortex evoked no vocalization but did elicit twiches, licking and other movements. When the dorsal or posterior part of the inferior colliculus was stimulated electrically, the bat moved its pinnae but did not vocalize. Furthermore no acoustic stimulation evoked vocalizations on a one-to-one basis, whereas vocalization elicited by the electrical stimulation did occur on a one-to-one basis. The vocalizations described above were thus not due to stimulation of the classical auditory pathways by the current spread from the stimulating electrodes.

The orientation sounds of Myotis are short FM signals, while the orientation sound of P. parnellii contains long CF and short FM components. When either the central gray matter or the midbrain reticular formation of P. parnellii was stimulated electrically, the bat always emitted complex sounds containing both the long CF and short FM components (Figure 8, A). The sound pressure level of these electrically evoked sounds ranged between 100 and 115 dB. The boundary between the CF and FM components was always easily identified in the envelope of the sound by the presence of a notch (Figure 7, A and B). Unlike in Myoits, purely FM sounds were not elicited. The latencies of the vocal responses of P. parnellii were 25 to 40 msec. The total duration of the sound was 10 to 30 msec. The second harmonic predominated

in both the CF and FM components. In the second harmonic, the CF component was about 62 kHz and the FM component swept from 62 to 50 kHz. The duration of the FM component was short, always about 3 msec regardless of the duration of the CF component. The first harmonic, at 31 kHz in the CF component was present at a reduced amplitude as was the third harmonic at about 93 kHz (Figure 8, A and B). These vocalizations evoked by electrical stimulation were indistinguishable from orientation sounds emitted autonomously by the bat. Another bat in this genus, P. suapurensis, emits complex sounds that consist of a short CF component followed by a short FM component. Electrical stimulation of the central gray matter or the midbrain reticular formation of P. suapurensis elicited CF-FM sounds highly compatible with species-specific orientation sounds. The CF component was always short in duration and was followed by a short descending FM sweep. The electrically elicited vocalization contained several harmonics (see Figure 21 in Chapter IV) as did the orientation sounds of this species. Unlike the genus Myotis, P. suapurensis did not vocalize purely FM sounds.

These stimulation experiments were repeated in Eptesicus fuscus and Noctilio leporinus. In the case of both of these species, the sounds evoked by the electrical stimuli were similar to the orientation sounds of the species. E. fuscus emitted short FM sounds in response to electrical stimulation. The FM sweeps of these vocalizations were at lower frequencies

than the sounds evoked from Myotis. In N. leporinus electrical stimuli delivered to the midbrain reticular formation or the central gray matter evoked either FM sounds or CF-FM sounds (see Fig. 21 in Chapter IV). In both instances, these sounds were similar to the natural orientation sounds. Sometimes a short and weak CF sound followed by an FM sound with an intervening silent period of 4 msec was evoked (see Figure 22 in Chapter IV). Purely CF sounds alone were not evoked by these electrical stimuli.

Discussion

The orientation sounds used by bats for echolocation are specific to the species of the bat. The orientation sounds of many bats including the genera Eptesicus and Myotis are less than a few milliseconds in duration and are frequency-modulated (FM), sweeping downward by about one octave (Griffin, 1962; Pye, 1967). Other bats emit signals containing constant-frequency (CF) and FM components. The species Rhinolophus ferrumequinum (Schnitzler, 1968) and Pteronotus parnellii (Schnitzler, 1970) use 10-60 msec signals comprised of a long CF component followed by a short FM component. Pteronotus suapurensis emits sounds consisting of short CF components followed by short FM components (Grinnell, 1970). Noctilio leporinus is unusual in that it uses three kinds of orientation sounds; FM, CF, and CF-FM sounds under different conditions (Suthers, 1965). In R. ferrumequinum and P. parnellii, the long CF component is used to detect a target in motion from Doppler-shifted echoes (Schnitzler, 1968, 1970; Schuller, 1974; Simmons, 1973). The significance of short CF components in P. suapurensis is not well understood (Simmons and Howell, 1972). The FM signals are used for target ranging (Simmons, 1971) and are apparently not useful for velocity detection (Altes and Titlebaum, 1970; Cahlander, 1964). The FM signals are, in all likelihood, suited in finding target characteristics and location, for

which most bats incorporate FM signals in their sounds.

The sounds evoked by electrical stimulation of the dorsal part of the reticular formation in the midbrain of Myotis were similar to orientation sounds used by the bats during searching phase of insect pursuit behavior (Griffin, 1958; Griffin et al., 1960; Pye, 1967). When the electrical stimuli were applied to the lateral part of the central gray matter, the evoked sounds had about 1.0 msec duration and the frequency always swept downward from about 40 to 20 kHz and the repetition rate of the sound emission was about 140/sec. These sounds were similar to orientation signals used by Myotis during the terminal phase of insect pursuit or obstacle avoidance (Griffin, 1958; Griffin et al.,1960; Pye, 1967). The regions of the midbrain located close to the inferior colliculus are thus concerned with the emissions of orientation sounds. In squirrel monkeys, vocalizations are effectively elicited by electrical stimulation of the central gray matter, although the latency of the response is considerably longer (over 200 msec) than the bat (Jürgens and Ploog, 1970).

The electrical stimulation of the ventro-medial area of the central gray matter or the center of the midbrain reticular formation could elicit the downward sweeping FM sounds, but in association with various large body movements, and also some of these sound contained human audible component. Stimulation of the thalamus or the hypothalamus

sometimes evoked the sounds quite different from orientation sounds. These places did not seem to be specialized for vocalization, because large body movements were always associated with the vocalization and because the threshold for vocalization was high. The animal appeared to vocalize because of general excitation due to the electrical stimulation.

Electrical stimulation of the several midbrain area in Myotis thus elicited sounds which were very similar to natural orientation sounds. It cannot, however, be strictly concluded that these midbrain area are concerned with the emission of orientation sounds on the basis of data from this one genus, because the midbrain may be involved in the production of FM sounds in all other genera of bats and the orientation sounds of the Myotis genus may coincidentally be FM. Accordingly, experiments were performed on the other bats using different orientation signals.

When either the central gray matter or the midbrain reticular formation of P. parnellii was stimulated electrically, bat always emitted complex sounds indistinguishable from orientation sounds emitted autonomously by the bat. This was also true in another bat in the same genus, P. suapurensis. These two bats emitted CF-FM sounds highly compatible with the species-specific orientation signals, and did not vocalize purely FM signals for the electrical stimulation on the midbrain.

The other species of FM bat, Eptesicus fuscus showed

the vocalization very similar to the species-specific orientation sounds in response to the electrical stimulation to the same areas in the midbrain as in Myotis and Pteronotus. N. leporinus uses three types of sounds in natural condition, the electrical stimulation to the midbrain could elicit two of them, CF-FM and FM. Purely CF sounds alone were not evoked by electrical stimulation, although a short CF sound followed by an FM sound after an intervening silent period of about 4 msec was evoked (see also Chapter IV).

In Chapter IV, it will be described that this electrically activated vocalization can be modified by the acoustic environment.

It appeared as though certain areas in the midbrains of echolocating bats are concerned with the emission of orientation sounds, and that the neural circuitry for the emission of orientation sounds is different from genus to genus. There is a possibility that different electrode sites might be concerned with orientation sounds adapted for various purposes (e.g., the reticular formation may be active during the insect search in Myotis, and the central gray matter during the terminal phase of pursuit), but further evidence will be needed to demonstrate this as a fact. Detailed anatomical studies of the neural pathways involved remain to be performed. It would be most interesting to see whether the electrical stimulation of the midbrain elicits sounds similar to the echolocation signals in the

African fruit bat, Rousettus aegypticus, which emits tongue-clicks for orientation (Kulzer, 1956).

Summary

1. The brains of several representative species of echolocating bats were found to contain regions concerned with the emission of species-specific orientation sounds.

2. When the dorsal part of the reticular formation in the midbrain or the lateral part of the central gray matter is stimulated with a short train of electric pulses, the bat moved its mouth and pinnae and emits sounds identical to orientation sounds, with a latency of 25 to 60 msec. The acoustic parameters of the electrically elicited sounds and the temporal patterns of emissions vary with the location of the stimulating electrodes.

3. Bats of genus Myotis (little brown bat or gray bat) emitted short frequency-modulated (FM) sounds both in nature and when electrically stimulated.

4. Pteronotus parnellii (mustache bat) responded with sounds containing long constant-frequency (CF) and short FM components that were typical of the species. Eptesicus fuscus (big brown bat), Pteronotus suapurensis (naked-backed bat), and Noctilio leporinus (fish-catching bat) also made characteristic orientation sounds, and it was found that a part of the vocalization system is located in the regions juxtaposed to the inferior colliculus which is an important neural center for echolocation.

Figure 5.

Sounds evoked from Myotis austroriparius by electrical stimuli applied to the midbrain. (A) FM sounds evoked by the stimulation of the midbrain reticular formation. The time scale is 1 msec. (B) FM sounds evoked by the stimulation of either the dorsal part of the midbrain reticular formation (1) or the lateral part of the central gray matter (2). The upper and lower traces, respectively, represent electrical stimuli and sounds evoked by these. The time scale is 100 msec. (C) and (D) represent sonagrams of sounds evoked by the stimulation of the midbrain. (C) the sound shown in (B1). (D) The grouped sounds evoked in a stimulus similar to that in (B2). These sonagrams were obtained by reducing the tape speed to 1/32 of the original speed. The ordinates and abscissae, respectively, represent sound frequency in kilohertz and time in milliseconds. (E) and (F) represent sonagrams of sounds evoked by the stimulation of thalamic and/or hypothalamic areas. Myotis can produce sounds quite different from orientation signals. These sonagrams were obtained by reducing the tape speed to 1/16 of the original speed. The abscissae represent time in seconds.

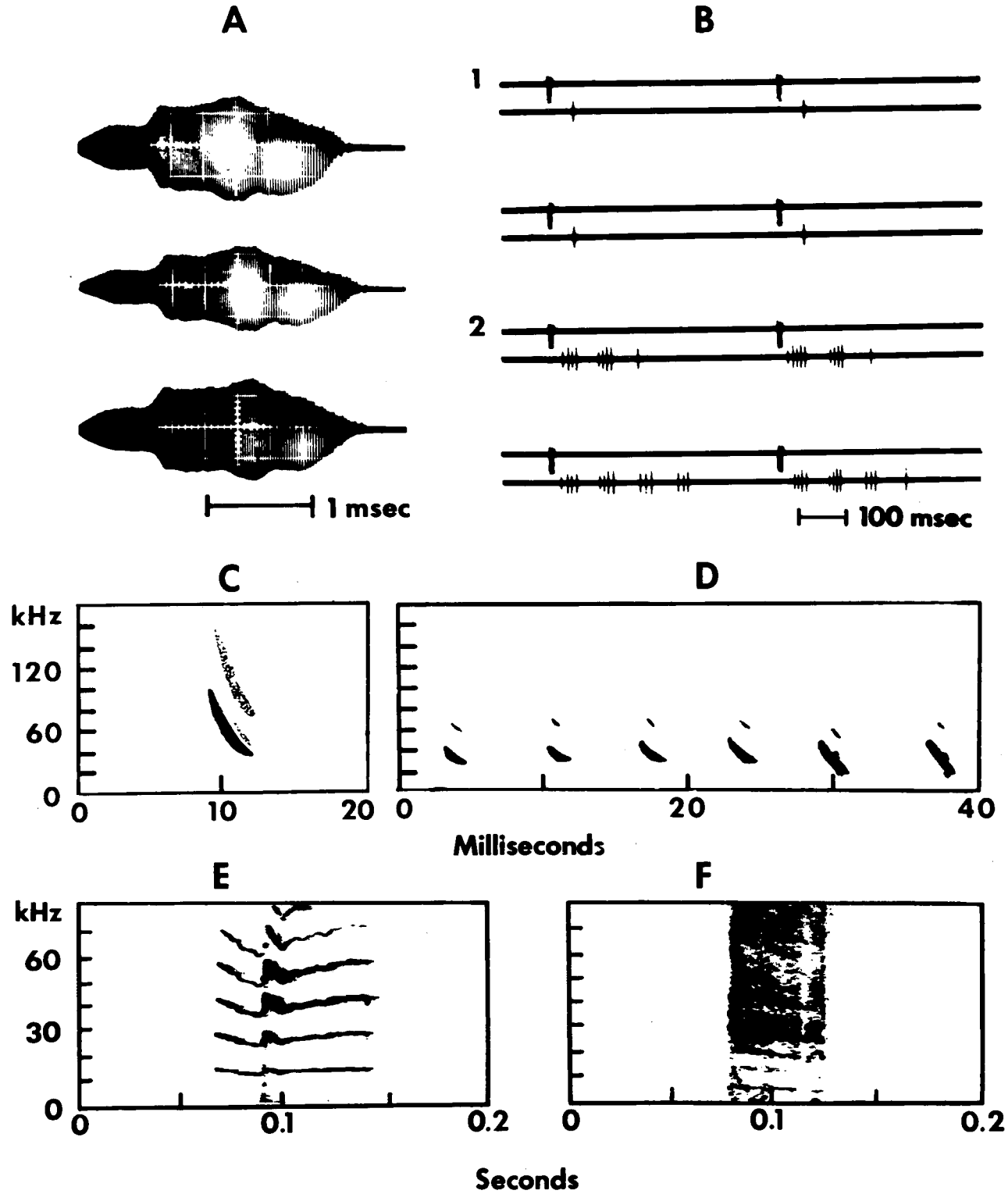

A
B
1
2
1 msec
100 msec
C
D
kHz
120
60
0
0
10
20
0
20
40
Milliseconds
E
F
kHz
60
30
0
0
0.1
0.2
0
0.1
0.2
Seconds

FIGURE 5.

Figure 6.

A frontal section across the midbrain near the boundary between the inferior and superior colliculi of Myotis. The location of the tip of the stimulating electrode is indicated by the shaded areas. (CGM) central gray matter, (CP) cerebellar peduncle, (DBC) decussation of brachium conjunctivum, (DNR) dorsal nucleus of raphe, (IC) inferior colliculus, (ML) medial lemniscus, (MNTN) mesencephalic nucleus of trigeminal nerve, (NLL) nucleus of lateral lemniscus, (NMR) nucleus of medial raphe, (NVT) nucleus of ventral tegmentum, (PF) pyramidal fibers, (RF) reticular formation, (SC) superior colliculus, and (TNP) tegmental nucleus of pons . The horizontal bar is a 1 mm scale.

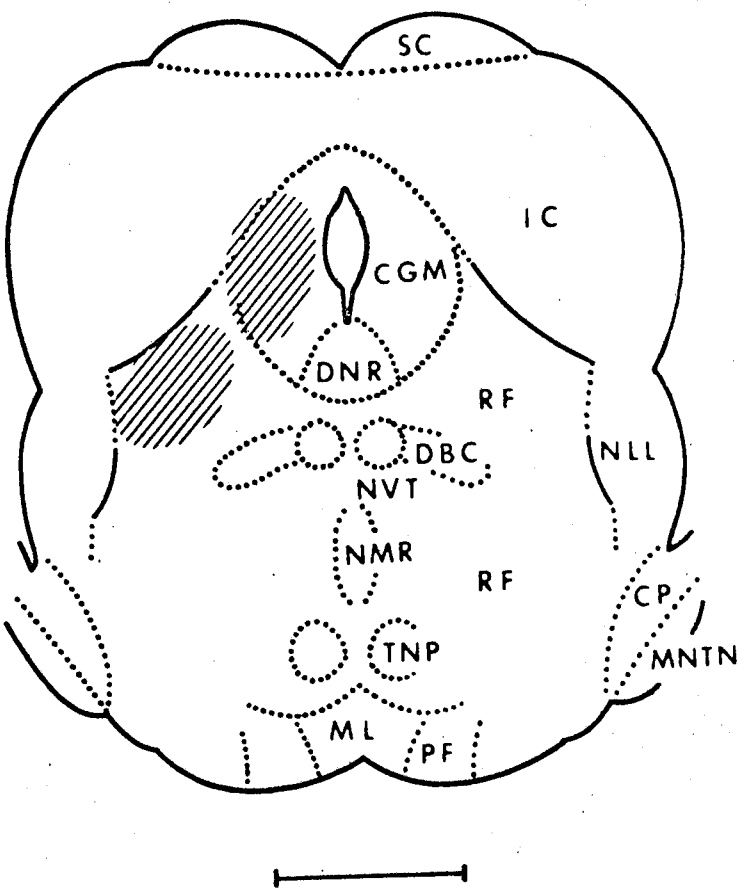


FIGURE 6.

Figure 7.

Sounds evoked from Pteronotus parnellii in response to the electrical stimuli applied to the reticular formation (A) and central gray matter (B).

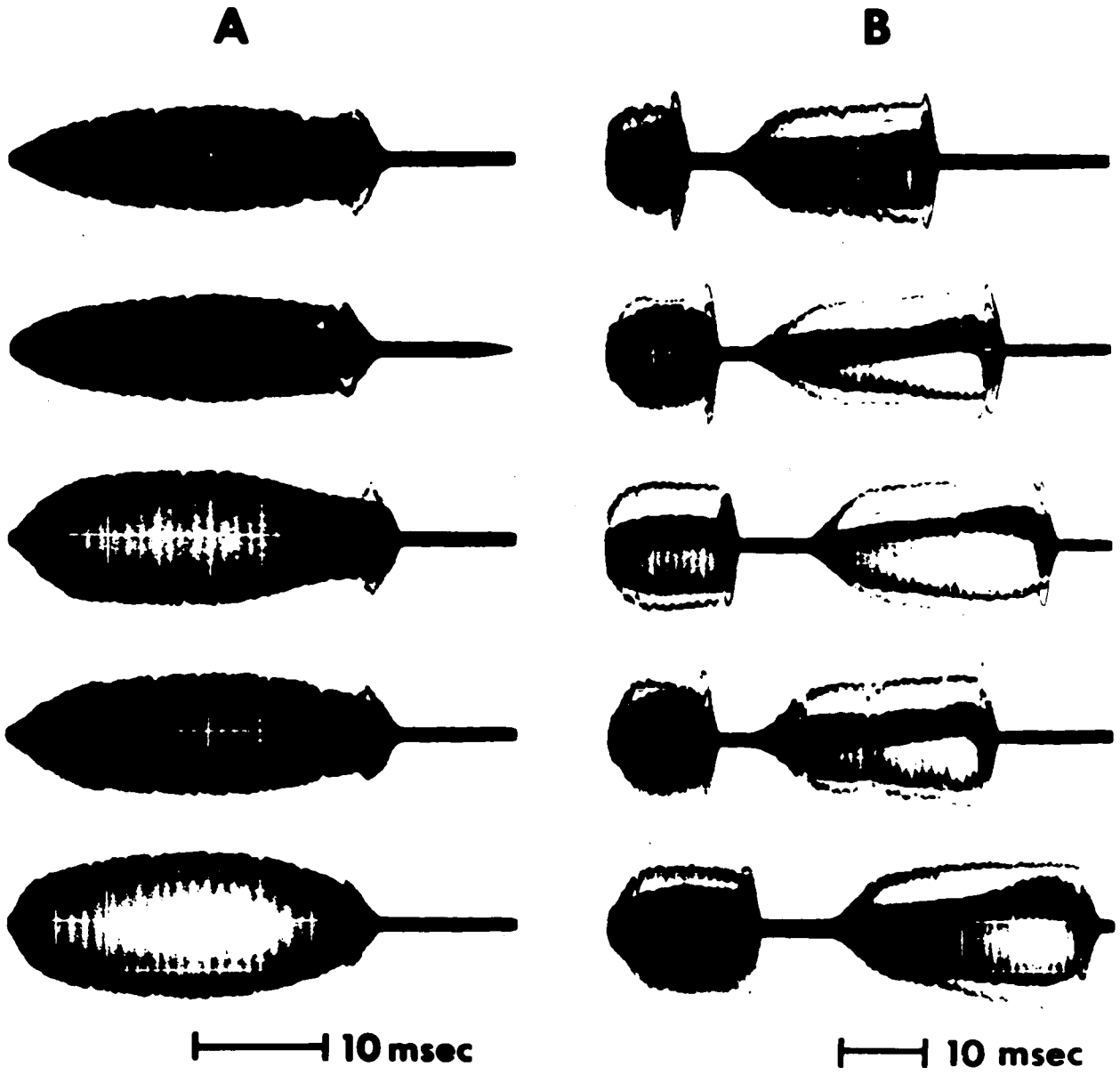


FIGURE 7.

Figure 8.

The sonagrams of some of the sounds in Figure 7 (A) and (B) are shown in (A) and (B,C), respectively. The time scales are 10 msec.

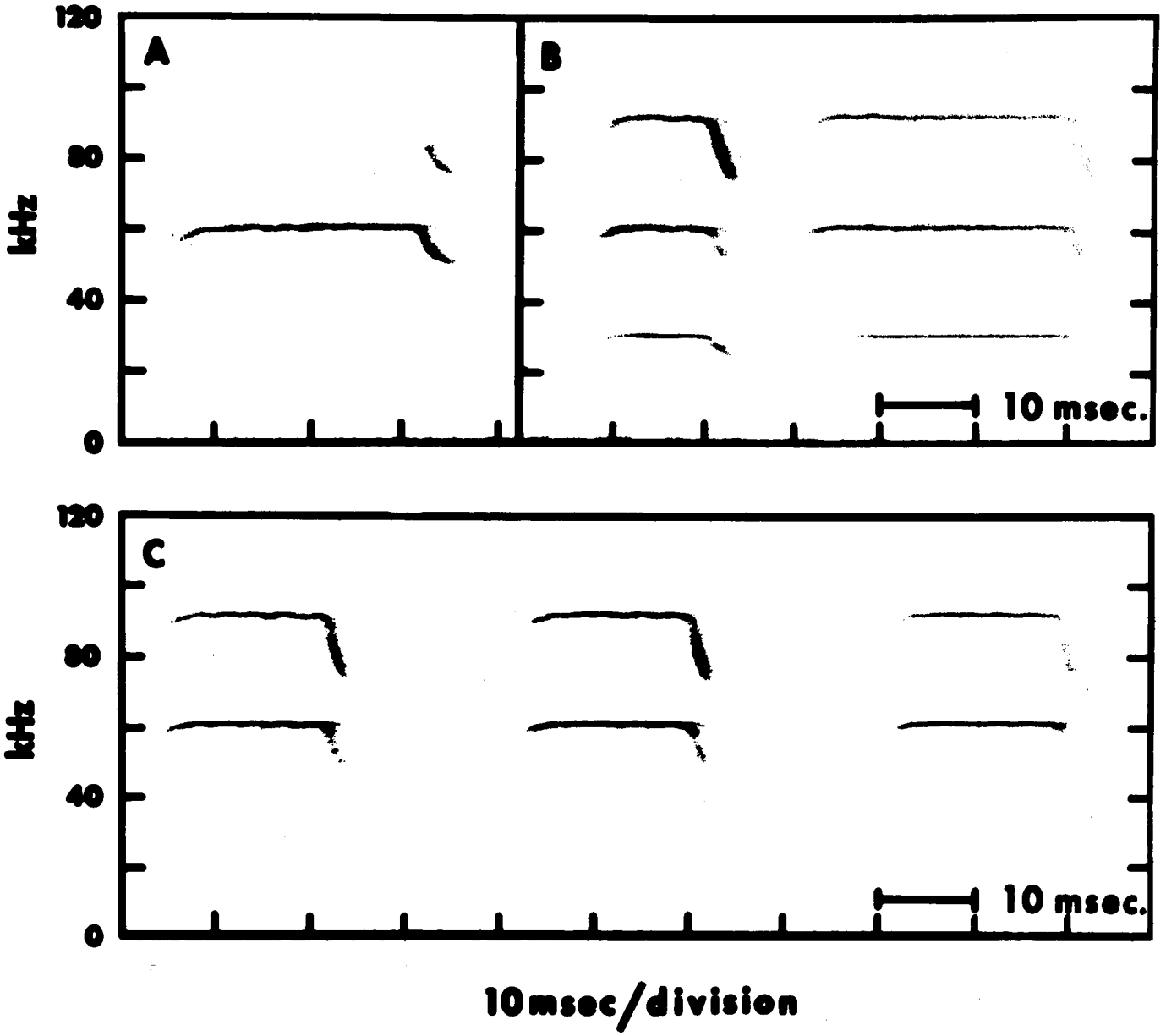


FIGURE 8.

CHAPTER II

Directional Sensitivity of Echolocating System; Orientation Sound and Auditory System.

Bats emit sound and listen to echoes for echolocation. To investigate the directional sensitivity of their echolocation system it is neccessary to measure the directional properties of both the orientation sounds and the ears. The field of the orientation sound around the head has been measured in Eptesicus and Pteronotus (Simmons, 1969), Rhinolophus (Schnitzler, 1968) and Megaderma (Möhres, 1966). In these measurements the animal with its head unrestrained emitted sounds toward a target. Thus these data might include the effect of head movement. Sokolov and Makarov (1971) fixed the head of Rhinolophus and measured the sound field produced by it. As described in Chapter I, sounds very similar to species-specific orientation sounds are elicited by electrical stimulation of the dorsal part of the reticular formation in the midbrain and/or the central gray matter of an unanesthetized bat. In this case the head was immobilized by cementing it to a nail (Suga and Schlegel, 1972; Suga, Simmons and Shimozawa, 1974). This technique makes possible accurate measurements of the sound fields produced by bats.

For sound localization at least two types of cues are

conceivable: interaural pressure and time (or phase) differences. In man the interaural pressure difference (IPD) is used mainly for location of high-frequency sounds, while the interaural time difference (ITD) is used mainly for low-frequency sounds (Mills, 1958, 1960; Stevens and Newman, 1936). Since the bats of the genus Myotis are small and their orientation sounds are higher than 10 kHz, the IPD may be a primary cue for echolocation. The IPD as a function of azimuth is obtained from the directional sensitivity curve of the auditory system, which can easily be measured by recording summated or single neural activity (Grinnell, 1963; Grinnell and Grinnell, 1965; Suga, 1964).

To investigate the directional sensitivity of the echolocation system and the IPD as a function of azimuth, therefore the sound fields produced by bats and the directional sensitivity of the auditory system in terms of summated neural activity, were measured.

Orientation sounds of Myotis are frequency-modulated (FM) so that an ITD can be coded by a group of neurons with different best frequencies on both the left and right sides. Possible roles of the ITD as well as the IPD are also discussed in relation to echolocation.

Materials and Methods

Three gray bats (Myotis grisescens) and five little brown bats (M. lucifugus) were used. These two species are closely related, and there were no noticeable differences in orientation sounds and audiograms. Under ether anesthesia a nail 1.8 cm long was mounted on the skull with acrylic adhesive and dental cement. Then the pinnae were carefully relocated to their original position by tightening the skin with sutures. A few hours to a day later the animal (now recovered from the anesthesia and shock due to surgery) was placed on a vinyl ball of 7.5 cm diameter floating on water. The head of the animal was fixed by fastening the nail to a metal rod with a set screw. The midpoint of the ears was placed at the center of the acoustic perimeter and the eye-nostril line was made horizontal. The nail, the rod supporting it, and the floating ball were at least 20° posterior to the animals mouth.

Without anesthesia, a small hole was made in the skull through which a double steel electrode with a few megohms resistance was inserted for the electrical stimulation of the midbrain to elicit FM sounds similar to species-specific orientation signals (see Chapter I). After fixing the electrode in place with dental cement, the micromanipulator used for positioning it was removed. The electrical stimulus applied to the brain was a short train of pulses delivered

twice per second. Each train consisted of 10 electric pulses, each having a duration of 0.1 msec and an amplitude of 1-15 V. The inter-pulse interval was 1.7 msec.

The sounds elicited by the bat were recorded with two quarter-inch condenser microphones (Brüel and Kjaer 4135). One, "reference microphone" pointed toward the bat's mouth and was positioned 5° down from the eye-nostril line, and 70 cm anterior to the mouth. The other, "the scanning" microphone, mounted on a movable aluminium arm in order to scan the bat's perimeter at a 50 cm radius, was also aimed at the mouth. Since the sounds emitted by the bat were frequency-modulated, the interference between the signal directly incident upon the microphones and the echoes was easily detected, because the overlap of the delayed echoes with the signal produced ripples in the envelope of the recorded signal. To minimize these echoes, objects in the sound-proofed room were covered with cotton. A magnetic tape recorder (Ampex FR-100) with frequency response of 300-300,000 Hz at the feeding speed of 60 inches/sec simultaneously recorded the following signals: electrical stimuli applied to the brain, bat's sounds monitored with the reference and the scanning microphones, and electric signals indicating the position of the scanning microphone. At each position of the scanning microphone about 15 FM sounds emitted by the bat were recorded.

The recorded signals were played back at 1/32 of the

original tape speed. A 2 sec square pulse triggered by the electrical stimulus controlled the shutter of camera, while the pulse synchronized with the onset of the bat's sound triggered the sweep of a cathode-ray oscilloscope. The sound monitored with the reference microphone was fed into the period meter in order to examine the frequency sweep. The output of the period meter and the tape-recorded sounds were simultaneously displayed on the oscilloscope screen and were photographed. A 3,125 Hz signal, which corresponds to 100 kHz at the original tape speed was also fed into the period meter prior to the tape-recorded sound and its output was also displayed for purposes of calibration (Figure 9). In addition, the signal indicating the location of the scanning microphone was displayed on an oscilloscope screen. In Figure 9 the frequency of a bat's sound sweeps from 100-43 kHz. Since there were no noticeable harmonics for the components higher than 50 kHz, the radiation patterns of sounds higher than 50 kHz could be studied by measuring the peak-to-peak amplitudes. In the experiments the amplitude of the 55, 75 and 95 kHz components were measured in FM sounds monitored with the reference and scanning microphones, and the difference in amplitude at the two microphones was expressed in either percentage of, or decibels below maximum. Assuming sagittal symmetry, each point in the data represents the average of six measurements.

To study the relationship between mouth movement and

sound emission a photo-electric displacement transducer was connected to the lower jaw by a rubber band, and a quarter-inch microphone was placed 10 cm anterior to the bat. The outputs of the transducer and microphone were simultaneously displayed on an oscilloscope screen (Figure 10).

For the measurement of the directional sensitivity of the auditory system a tungsten electrode was inserted into the nucleus of the lateral lemniscus to record its activity evoked by acoustic stimuli. This is hereafter called the LL response (equivalent to N_4). The LL response was selected, because it is more directional than the summated neural activity of the primary auditory neurons (N_1) as a result of binaural interaction occurring in medullary auditory nuclei mainly in S-segment (Grinnell, 1963). Instruments for acoustic stimulation and recording were the same as those described by Suga (1971), (see Materials and Methods in General). A condenser loudspeaker was moved around the head in the horizontal plane at 66 cm radius, and thresholds of LL responses to 35, 55, 75 and 95 kHz sounds were measured as a function of azimuth. These acoustic stimuli had a rise-decay time of 0.2 msec and a duration of 4 msec.

Results

Sound field produced by bats. When an electrical stimulus was applied to the dorsal part of the reticular formation in the midbrain, the bat opened its mouth 50-60° and emitted an FM sound with a latency of 30-50 msec. This was very similar to the species-specific orientation sound used in the searching phase of echolocation. The amplitude of the emitted sound ranged from 100-115 dB SPL at 10 cm in front of the mouth. Its duration was between 3 and 5 msec. The frequency always swept downward from 100- ca.45 kHz. If such an FM sound was emitted at different phases of mouth movement, the sound field measured would show large variations; the relationship between the sound emission and mouth movement was thus first studied. As shown in Figure 10, the sound emission was not strictly locked to a certain phase of the mouth movement, but it occurred anywhere between slightly earlier and slightly later than the peak opening of the mouth, so that a given component of the FM sound might be emitted at any aperture of the mouth between 30-60° . Thus, the sound field varied for each sound. This variation was within ± 12 % of the mean amplitude.

Figure 11 shows an example of sound fields produced by M. grisescens (Mg 1). The contour map shows that the sound is strongly radiated in a direction of 5-10° downward from the eye-nostril line and the main lobe is sharper at higher

frequencies. Figures 12-14 show radiation patterns of the 55, 75 and 95 kHz components, respectively, in the horizontal and vertical planes produced by Mg 1. All directional patterns were normalized with respect to the maximum amplitude for each component. The radiation angle at half-amplitude of maximum was 38° lateral, 18° up and 50° down at 55 kHz, 34° lateral, 8° up and 32° down at 75 kHz, and 30° lateral, 5° up and 25° down at 95 kHz (Figs. 12-14). Thus the width of the main lobe becomes narrower with an increase in frequency. At 95 kHz two prominent side lobes were present: one oriented 25° upward and the other 50° downward (Fig. 14). Two other bats showed different values, which were broader in angle at half-amplitude than Mg 1.

Since the measurements of sound pressure was performed in 5° or 10° steps, there was possibility that sharp lobes which might exist for high frequencies were missed. The fields of 55, 75 and 95 kHz sounds were therefore measured around a loudspeaker with an aperture comparable to that of the bat's mouth. This measurement indicated that the presence of such lobes in addition to those in Figure 12 was unlikely. The main lobe calculated by Strother and Mogus (1970) is also compatible with the data shown in Figures 12-14. Furthermore, there was a possibility that the ball under the bat modified the sound field in the hemisphere in front of the bat. The sound field around the loudspeaker was also measured with and without the ball. The result demonstrated that the

sound field in front of the bat was not noticeably different.

Directional sensitivity of the auditory system (DSA).

When a tungsten-wire electrode was inserted into the lateral lemniscus, the LL response (lateral lemniscal evoked potential) to a tonal stimulus was 1-2 mV in amplitude. The loudspeaker was then moved in the horizontal plane including the eye-nostril line, and the threshold of the response to the sound was measured as a function of azimuth angle. The DSA was expressed by the reciprocal of the threshold. Figures 15 and 16 show DSA curves measured with either 35 and 55 kHz or 75 and 95 kHz tones. Each curve is the average of measurements with 5 different bats. The LL response was most sensitive to sounds delivered from the contralateral side, so that impulses for the lateral lemniscus mainly originated from the contralateral ear. The maximum sensitivity appeared at $38 \pm 7.5^\circ$ azimuth to the midline for 35 kHz, $27 \pm 9.0^\circ$ for 55 kHz, $18 \pm 7.5^\circ$ for 75 kHz and $5.0 \pm 8.1^\circ$ for 95 kHz. The slope of the DSA curve toward and beyond the midline was 0.28 ± 0.03, 0.43 ± 0.03, 0.42 ± 0.03 and 0.58 ± 0.03 dB/degree for 35, 55, 75 and 95 kHz, respectively. Since higher-frequency sounds were more directional, it was expected that the slope of the DSA curve would increase with frequency, but it was not anticipated that the maximally sensitive direction would move medially with increasing frequency.

Directional sensitivity of the echolocation system (DSE). The DSE was obtained by adding the two curves for the directionality of the orientation sound (DO) and DSA. As shown in Figures 15 and 16, the slopes of the DSE curve on both side of its peak are about 0.6 dB/degree at 55 kHz, 0.8 dB/degree at 75 kHz, and 1.0 dB/degree at 95 kHz. These results confirm that the DSE increases in sharpness with frequency. The peak for the DSE curve is 15° lateral for 55 kHz, 15° lateral for 75 kHz, and 2.5° lateral for 95 kHz. It was noticed that the peak for a 95 kHz sound was very close to the midline.

Interaural pressure difference (IPD). Since the IPD appeared to be the essential cue for echolocation in bats of genus Myotis, it was calculated as a function of azimuth angle for 35, 55, 75, and 95 kHz. Assuming sagittal symmetry of the auditory system, the DSA or DSE curves in Figs. 15, 16 were inverted at zero degrees. The IPD curves in Figure 17 were then obtained by subtracting the inverted curves from the non-inverted ones. The IPD for a 35 kHz sound changed linearly with azimuth between 0-50° at a rate of 0.4 dB/degree. Beyond 60° it changed at a rate of 0.2 dB/degree. For a 55 kHz sound the slope of the IPD curve was 0.7 dB/degree between 0-30° lateral, and 0.2 dB/degree beyond 30°. For 75 and 95 kHz sounds the slope of the IPD curve was 0.3-0.4 dB/degree between 0° and 30-40° lateral, but it becomes nearly zero beyond 40°. These data indicate that a 55 kHz

sound gives a larger IPD cue than do 35, 75 and 95 kHz sounds and that the IPD cue for sounds higher than 75 kHz is very poor at positions more lateral than 30°.

Discussion

Sound fields. Sound fields produced by different species of bats have been measured by Möhres (1966), Schnitzler (1968), Simmons (1969), and Sokolov and Makarov (1971). Except for Sokolov and Makarov's extensive study, the animals did not have a head-holding device when they emitted orientation sounds toward a target. The azimuth angle at half-amplitude of maximum is 23° at 28-30 kHz for Eptesicus fuscus and Pteronotus parnellii rubiginosus which emit sounds through the mouth (Simmons, 1969), and 21-23° at 83 kHz for Rhinolophus ferrumequinum which emits signals through the nostrils (Möhres, 1966; Schnitzler, 1968), and 35° at 60-80 kHz for Megaderma lyra (Möhres, 1966). According to Sokolov and Makarov (1971) who fixed the head of Rhinolophus, the azimuth angle at a half-amplitude is 28°, but it is 30° after amputating the nose-leaf. Thus, the effect of the nose-leaf on horizontal radiation is very small. This is unexpected data, because the elaborate nose-leaf surrounding the nostril of Rhinolophus gives an impression that it plays role to sharpen the beam of emitted orientation sound, whereas Myotis and Eptesicus have no such an appendage.

In the data on Mg 1, the horizontal angle at a half of maximum amplitude is 34° at 75 kHz and 30° at 95 kHz, which are comparable to the data obtained by Sokolov and Makarov (1971). Conversely, the azimuth angle at half-amplitude

at 55 kHz in Mg 1 (38°) is much wider than that at 28-30 kHz in Eptesicus and Pteronotus (Simmons, 1969).

The vertical distribution of an orientation sound has been measured only with Rhinolophus. The vertical angular width at a half-amplitude of maximum at 83 kHz is 43° according to Schnitzler (1968) and 73°according to Sokolov and Makarov (1971). The large angular width obtained by Sokolov and Makarov is due to the fusion of the main and side lobes. Interestingly, the dissection of the nose-leaf greatly reduces the vertical width down to 46° because of the reduction in the side lobes. Rhinolophus moves the nose-leaf during echolocation, probably to control distribution of orientation sounds. In the data with Mg 1, the vertical angular width of the main lobe at half-amplitude was 68° at 55 kHz, 40°at 75 kHz and 30°at 95 kHz.

Interaural pressure difference (IPD). Since the DSE curves showed sharp slopes (0.6 dB/degree at 55 kHz and 1.0 dB/degree at 95 kHz), the amplitudes of echoes at the ears greatly decrease as echo sources move away from the maximally sensitive direction. Accordingly, clatter from irrelevant sources at the ears is greatly reduced. If the peaks of the DSA curves were more lateral than those in Figures 15, 16, the IPD and the dynamic ranges of the IPD curves would increase, but the peaks of the DSE curves would decrease and move laterally. In other words, the IPD and the DSE are

mutually dependent. For echolocation the peak of the DSA curve should be thus neither too lateral nor too medial. The dynamic ranges of the IPD curves greatly depend on the difference between the slopes of the DSA curves toward the ipsi- and contra-lateral sides. To increase the dynamic range, the slope lateral to the peak of the DSA (or DSE) curve should be smaller than that on the medial side.' This was found true for lower frequencies but not for higher ones.

For comparison with the present data, a DSA curve (Neuweiler, 1970) and a directionality curve of orientation sound (Schnitzler, 1968) of Rhinolophus ferrumequinum are replotted in Figure 18 A. The frequency of sound used for t these measurements was 83.3 kHz. The slope of the DSE curve is 0.5-0.6 dB/degree. The IPD varies linearly with azimuth angle from 0-30 lateral at a rate of 0.5 dB/degree. It does not, however, change as the azimuth angle increases beyond 30° (Fig. 18 B). The IPD curve of Rhinolophus is thus similar to that of Myotis. Rhinolophus moves the pinnae synchronously with vocalization and does not change the frequency of CF-FM sound more than a few kHz during echolocation (Schnitzler, 1968), while Myotis does not move the pinnae during vocalization and greatly drops the frequency of FM sounds when hunting insects and when landing (Griffin, 1958). Thus, the dynamic range of the IPD curve may alternately shift left or right in Rhinolophus, while it broadens in Myotis.

Both the narrow dynamic range of the IPD curve for

higher frequencies and the decrease in frequency of FM orientation sounds during the approaching and terminal phases appear to be disadvantageous for echolocation and target characteristics. However, Myotis may have not only this disadvantage, but also some advantage. During a searching phase Myotis emits FM sounds sweeping from 100-45 kHz. At these frequencies, the slope of the DSE curve toward a lateral side is very sharp, so that any clatter which may be produced by irrelevant objects and/or other bats more lateral than 30° is greatly attenuated. Since the distance to a target is long in the searching phase, a wide dynamic range for echolocation may not be needed by the bats, so that the narrow dynamic range may not neccessarily be disadvantageous for echolocation. The bats can efficiently find small objects in front of them with a sound of such a high-frequency, although higher-frequency sounds are attenuated by the air absorption more than lower-frequency sounds. The target characteristics analysis may be performed during and/or just before the approaching phase. During the terminal phase and the latter part of the approaching phase Myotis emits FM sounds sweeping down about one octave between 50-15 kHz (e.g. from 40-20 kHz) at a high repetition rate. In these phases tracking of the target may be most important. Since the distance to the target is short, the direction of the target may quickly change, so that a wide dynamic range for echolocation and the frequent emission of orientation sounds

may be more important to the bat. Thus lowering the FM sweep at the terminal phase in Myotis may not necessarily be due to the mechanical reason of the vocal cord but may be functional for widening the dynamic range of localization of the echo source.

In man the just-detectable IPD is 0.5 dB at 2-3 kHz and 50 dB above the sensation level which corresponds to a 1.2-2.5° lateral shift of a sound source from the midline (Mills, 1960). In Myotis the slope of the IPD curve around the midline was 0.4, 0.7, 0.3 and 0.4 dB/degree for 35, 55, 75 and 95 kHz sounds, respectively. If one assume that the just-detectable IPD of Myotis is 0.5 dB, they may be able to detect a 1.7-0.7° azimuth difference around the median plane. Harrison and Downey (1970) measured the IPD with Phyllostomus hastatus and obtained the minimum detectable angular difference of 2.0° at 10 kHz and 2.3° at 20 kHz. They assumed the just-detectable IPD to be 0.5 dB. Behavioral experiments with bats indicate that Eptesicus fuscus with an interaural distance of 14 mm can detect an 8-6° azimuth difference and P.hastatus with an interaural distance of 22 mm can detect a 4-6° difference with their orientation sounds (Peff and Simmons, 1971). These behavioral data can be explained by the IPD cue without assuming that the bat can detect a much smaller IPD than man. There is no doubt that the IPD is the essential cue for echolocation. Since Myotis catch two separate Drosophila within a half second (Griffin, Webster

and Micheal, 1960), the minimum detectable azimuth difference in Myotis may be smaller than behavioral values cited above.

Pumphrey (1948) stated that sound localization is accomplished by the binaural comparison of intensity ratio at least at three different frequencies to which the ear shows different polar diagrams of sensitivity. As a matter of fact, the polar diagrams of the ears of owls (Payne, 1961) and bats (Neuweiler, 1970; Grinnell and Grinnell, 1965) change extensively with the frequency of sound and also with the position of the external ear. This is also true in man (Sivian and White, 1933), so that the spectrum density of a complex sound differs at the two ears (Nordlund and Fritzell, 1963). The source of a complex sound is thus more easily located than that of a pure tone. Thus, FM sounds are apparently better signals for echolocation than CF sounds.

Responses of auditory neurons vary not only in discharge pattern, but also in latency with stimulus amplitude. In Myotis the difference in latency between the left and right LL responses to a tonal stimulus due to the IPD appears to be much larger than the ITD and may greatly contribute to the information processing for sound localization. Since this interaural latency difference is due to the IPD, it is not included in the following discussion about ITD.

Interaural time difference (ITD). In man, the just-detectable ITD is about 5 μsec (9 μsec on the average) at

a 75 % correct point for a noise with a band width of 0.15-17 kHz and 60-70 dB above the sensation level (Klumpp and Eady, 1956). This ITD corresponds to placing a sound source 1.1° lateral from the median plane (Fedderson et al., 1957). Fedderson et al. (1957) calculated the ITD as a function of azimuth for a sphere with a diameter of 17.5 cm, which is equal to the interaural distance in man, and found that the calculated values (9 μsec/degree) are very similar to the values measured with microphones placed in the ears of human subjects. In Myotis grisescens and M. lucifugus, the interaural distance is ca. 9 mm. The ITD for Myotis as a function of azimuth angle can be obtained by multiplying the ITD for man by the ratio between the interaural distances, 0.05. The calculated ITD curve showed a slope of about 0.45 μsec/degree from the median plane to 40° lateral. If one assume that the just-detectable ITD in Myotis is 5 μsec as in some human subjects, the theoretical limit for the just-detectable azimuth difference would be 11°. In order to detect a 0.7-1.7 azimuth difference with the ITD cue alone, the just-detectable ITD should be assumed to be 8-17 times smaller than that of man, if the ITD cue is to be considered equally important to the IPD cue for echolocation. No data have been obtained yet to indicate whether the above assumption is reasonable or not. According to the above assumption the minimum time-pressure trading ratio is 0.6-1.6 μsec/dB, which is much smaller than value for man, 9-11 μsec/dB (Hershkowitz and Durlash, 1969;

Gilliom and Sorkin, 1972).

In Eptesicus and Phyllostomus the just-detectable azimuth difference is 6-8° and 4-6°, respectively (Peff and Simmons, 1971). Since the interaural distance is about 14 mm for Eptesicus and 22 mm for Phyllostomus, the ITD at the just-detectable azimuth difference is about 5 μsec. Thus, the ITD cannot be ruled out as a cue for sound localization, although it is apparently inferior as a cue to the IPD.

Primary auditory neurons of mammals commonly show phase-locked responses to sounds lower than 5 kHz (Rose et al., 1967). In bats, orientation sounds are higher than 10 kHz, so that phase-locked responses may not play any role in echolocation. Since the orientation sound of Myotis is always frequency-modulated by about one octave, the ITD is coded by many neurons tuned at different frequencies within the sound. If the ITD plays an important role in echolocation, FM sounds are much better signals than CF sounds or noise bursts.

Summary

1. Radiation patterns of the 55, 75 and 95 kHz components in frequency-modulated sounds emitted by the gray bat (Myotis grisescens) were studied. FM sounds similar to species-specific orientation sounds were elicited by electrical stimuli applied to the midbrain while the head of the animal was immobilized by a nail cemented to its skull. The main beam was emitted 5-10° downward from the eye-nostril line. The radiation angle at one half of maximum amplitude was 38° lateral, 18° up and 50° down at 55 kHz, 34° lateral, 8° up and 32° down at 75 kHz, and 30° lateral, 5° up and 25° down at 95 kHz. At 95 kHz, two prominent side lobes were present.

2. The directional sensitivity of the auditory system (DSA) measured in terms of the potential evoked in the lateral lemniscus was studied with the gray bat (M. grisescens) and the little brown bat (M. lucifugus). The maximally sensitive direction moved toward the median plane with the increase in frequency from 35-95 kHz. The slope of the DSA curve increased from 0.3-0.6 dB/degree with frequency.

3. The directional sensitivity of the echolocation system (DSE) was calculated using both the DSA curve and the radiation pattern of the emitted sound. The maximally sensitive direction of the echolocation system was 15° lateral to the median plane at 55 kHz and 2.5° lateral at 95 kHz. The

slope of the DSE curve increased from 0.6 to 1.0 dB/degree with frequency. Thus, the higher the frequency of sound, the sharper was the directional sensitivity of the echolocation system.

4. The interaural pressure difference (IPD), which appeared to be the essential cue for echolocation in Myotis, changed linearly with the azimuth angle from 0-30° lateral regardless of the frequency of sound, at respective rates of 0.4, 0.7, 0.3 and 0.4 dB/degree for 35, 55, 75 and 95 kHz sounds. Beyond 30°, the change in IPD was quite different depending on frequency. For 75 and 95 kHz sounds, the IPD stayed nearly the same between 30° and 90°. Thus, the 75-95 kHz components in FM orientation sounds were not superior to the 35 and 55 kHz components in terms of the IPD cue for echolocation.

5. Assuming the just-detectable IPD and ITD to be 0.5 dB and 5 μsec respectively, as in man, the just-detectable azimuth difference of Myotis around the median plane would be 0.7-1.7° with the IPD cue and 11° with the ITD cue.

Figure 9.

A photograph used for amplitude measurement of an FM sound emitted by M. grisescens. The sound waves monitored with the scanning and reference microphones are shown in the upper and lower traces, respectively. The period meter output is simultaneously displayed by dots with a short calibration signal of 100 kHz on the left. The arrows indicate the corresponding amplitude of either the 50 or 75 kHz component. The frequency scale for the period meter output is given to the left in kilohertz, and the time scale at the bottom in milliseconds.

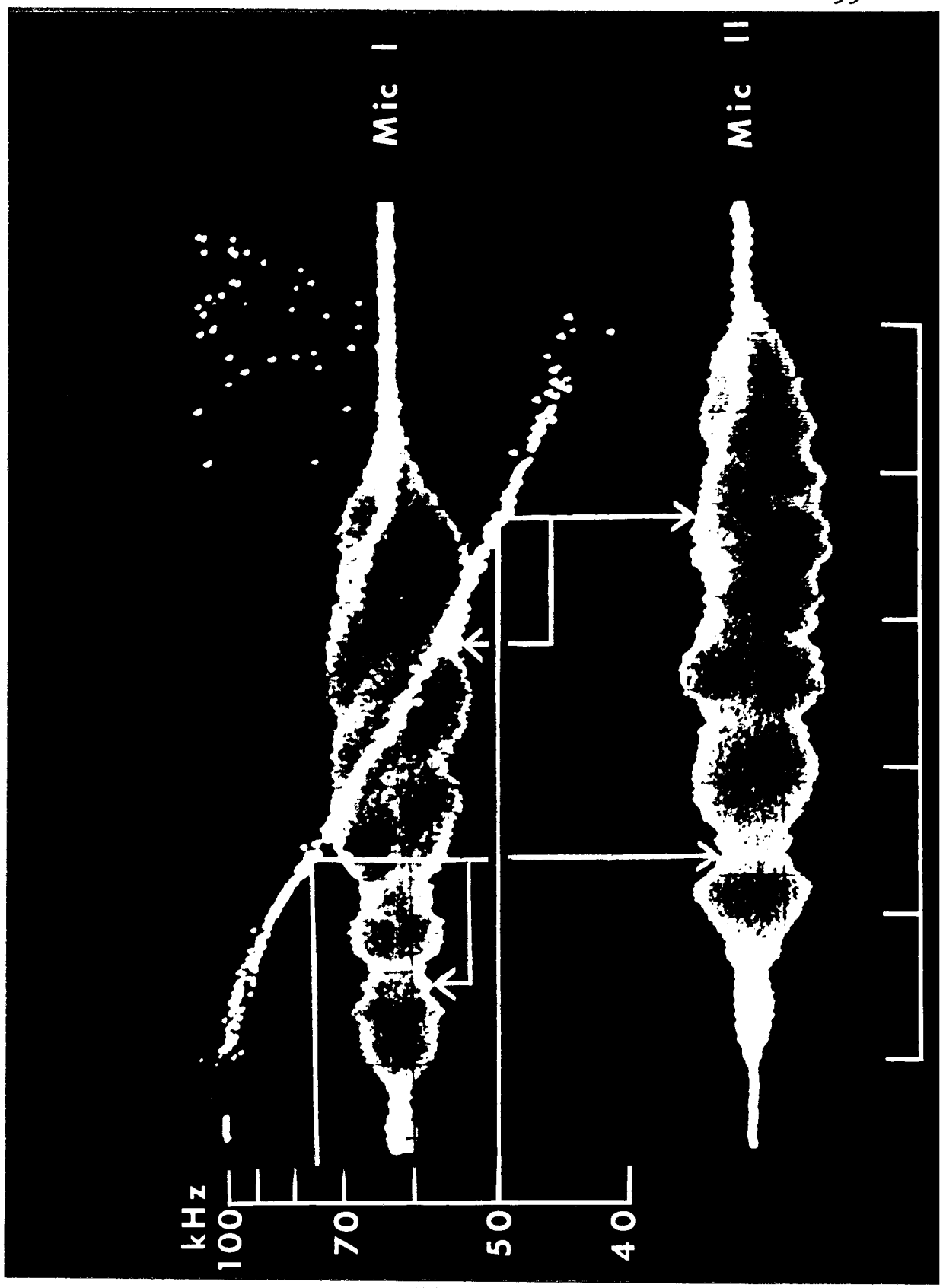


FIGURE 9.

Figure 10.

Relationship between the sound emission (upper trace) and mouth movement in M. grisescens (lower trace). The upward deflexion of the lower trace represents the mouth opening. Sounds are emitted around the maximum opening of the mouth. Dots on the left represent the electrical stimuli applied to the midbrain. All three pictures were obtained from one bat under the same stimulus conditions. The time scale is 20 msec.

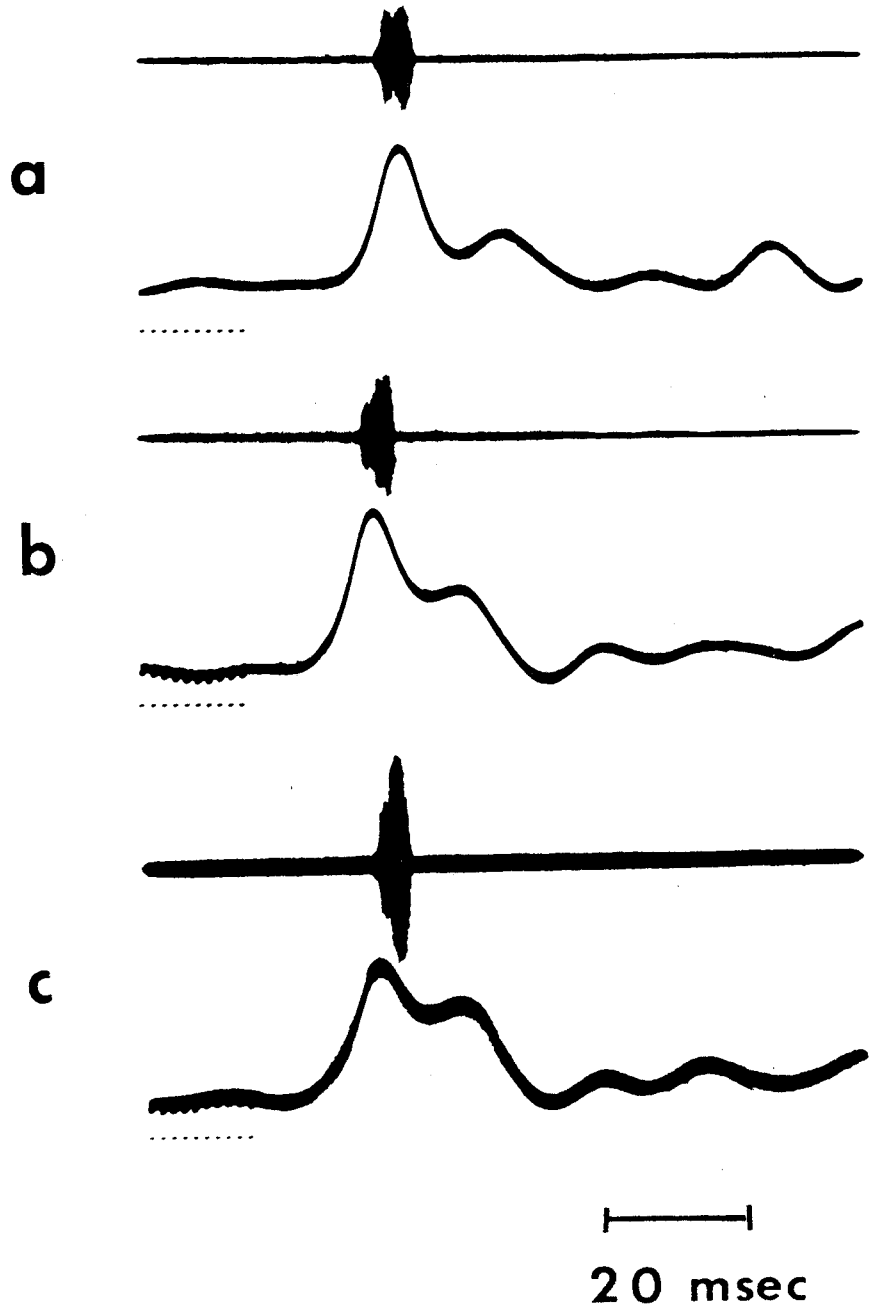


FIGURE 10.

Figure 11.

Radiation patterns of the 55, 75 and 95 kHz components in FM sound produced by M. grisescens. Each number near the ordinate crosspoints represents a sound pressure relative to that at the reference point (5° right and 5° down) in decibels. The contours were drawn by interpolation through these values. Zero degree is defined as the eye-nostril line. A, 55 kHz; B, 75 kHz; C, 95 kHz.

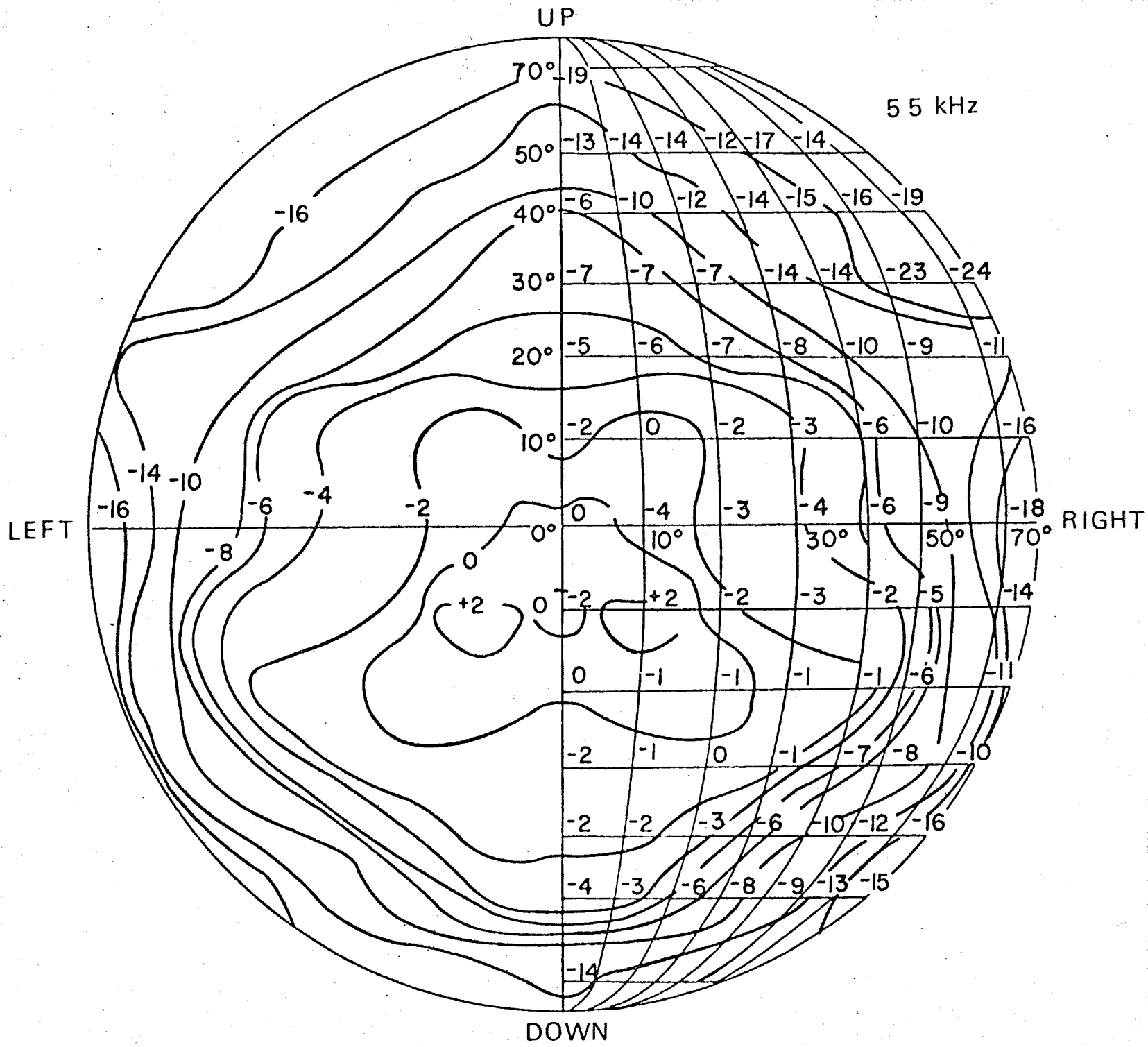


FIGURE 11, A.

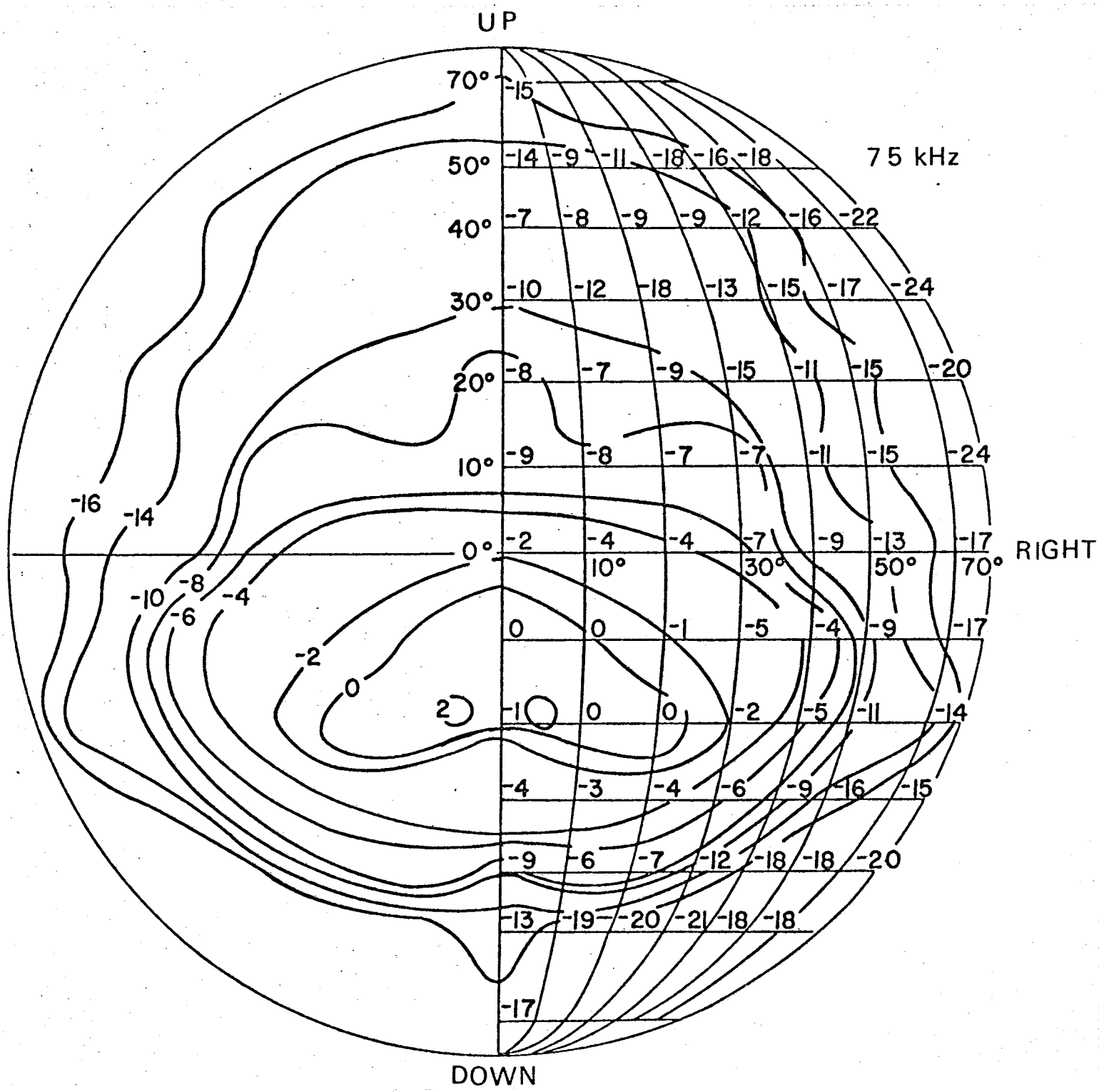


FIGURE 11.B.

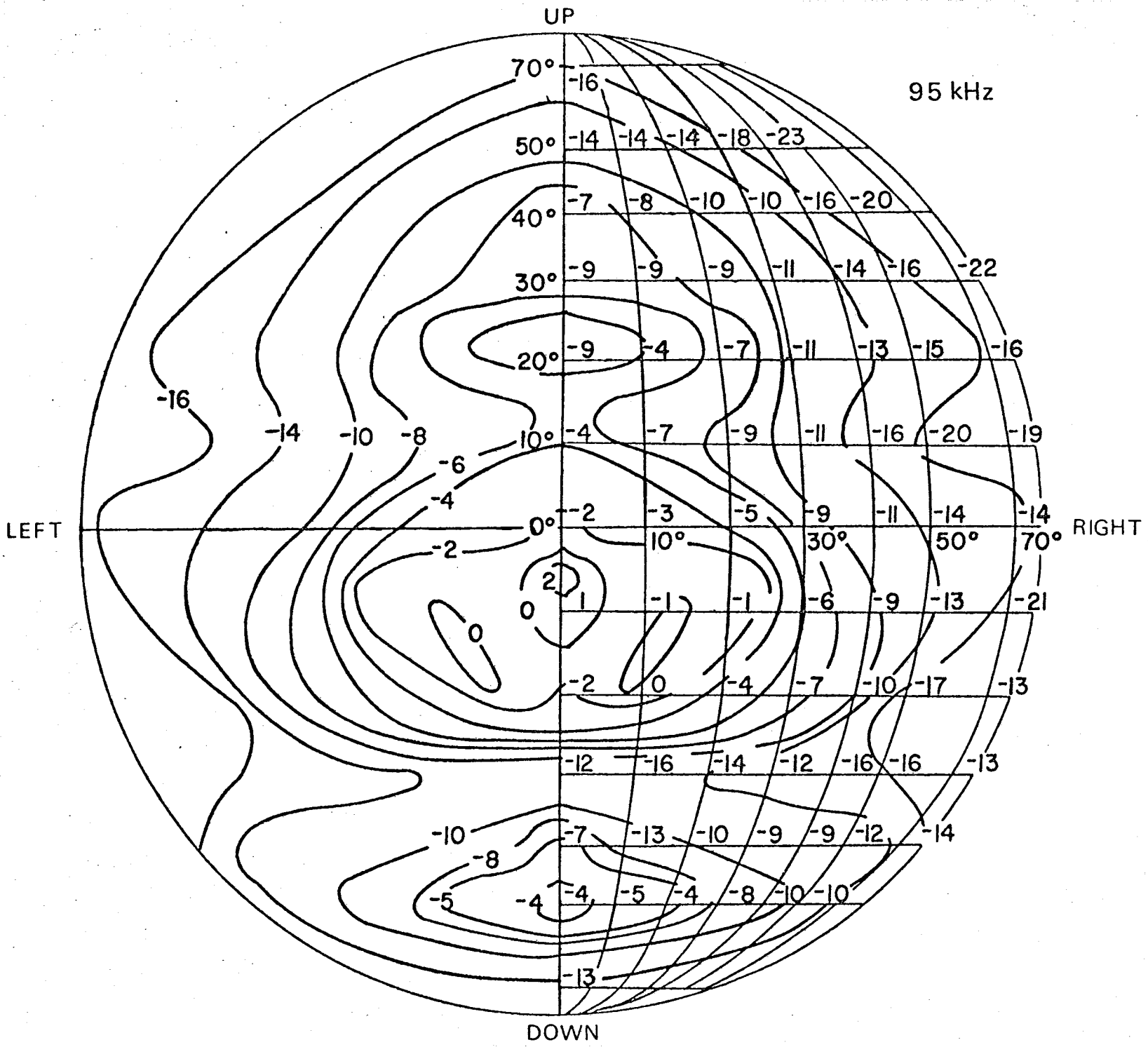

UP
95 kHz
LEFT
RIGHT
DOWN
70°
50°
40°
30°
20°
10°
0°
-16 -14 -14 -14 -18 -23
-7 -8 -10 -10 -16 -20
-9 -9 -9 -11 -14 -16 -22
-9 -4 -7 -11 -13 -15 -16
-16 -14 -10 -8 -6 -4 -4 -7 -9 -11 -16 -20 -19
-2 -3 -5 -9 -11 -14 -14
10° 30° 50° 70°
2 0 2 1 -1 -1 -6 -9 -13 -21
0
-2 0 -4 -7 -10 -17 -13
-12 -16 -14 -12 -16 -16 -13
-10 -8 -5 -4 -7 -13 -10 -9 -9 -12 -14
-4 -5 -4 -8 -10 -10
-13

FIGURE 11, C.

Figure 12.

Radiation pattern of the 55 kHz component of FM sounds emitted by M. grisescens in the horizontal (a) and vertical (b) planes in percentage of maximum (solid line and open circles) and in decibels (dashed line and filled circles).

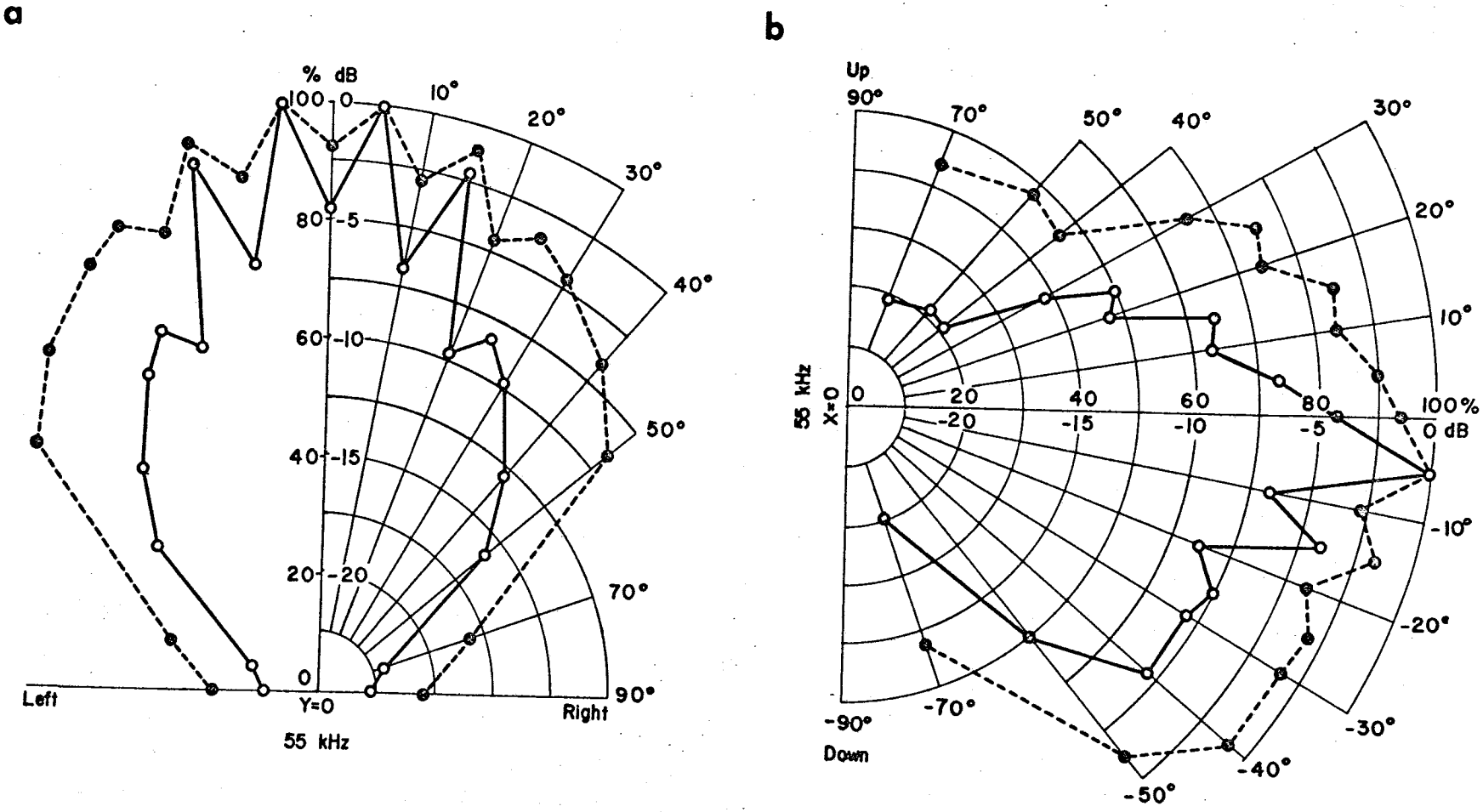

a
% dB
100
0
10°
20°
30°
80
-5
40°
60
-10
50°
40
-15
20
-20
70°
0
90°
Left
Y=0
Right
55 kHz
b
Up
90°
70°
50°
40°
30°
20°
10°
55 kHz
X=0
0
20
40
60
80
100%
-20
-15
-10
-5
0 dB
-10°
-20°
-90°
-70°
-30°
-40°
-50°
Down

FIGURE 12.

Figure 13.

Radiation pattern of the 75 kHz component of FM sounds emitted by the bat in the horizontal (a) and vertical (b) planes. All symbols are the same as those in Figure 12.

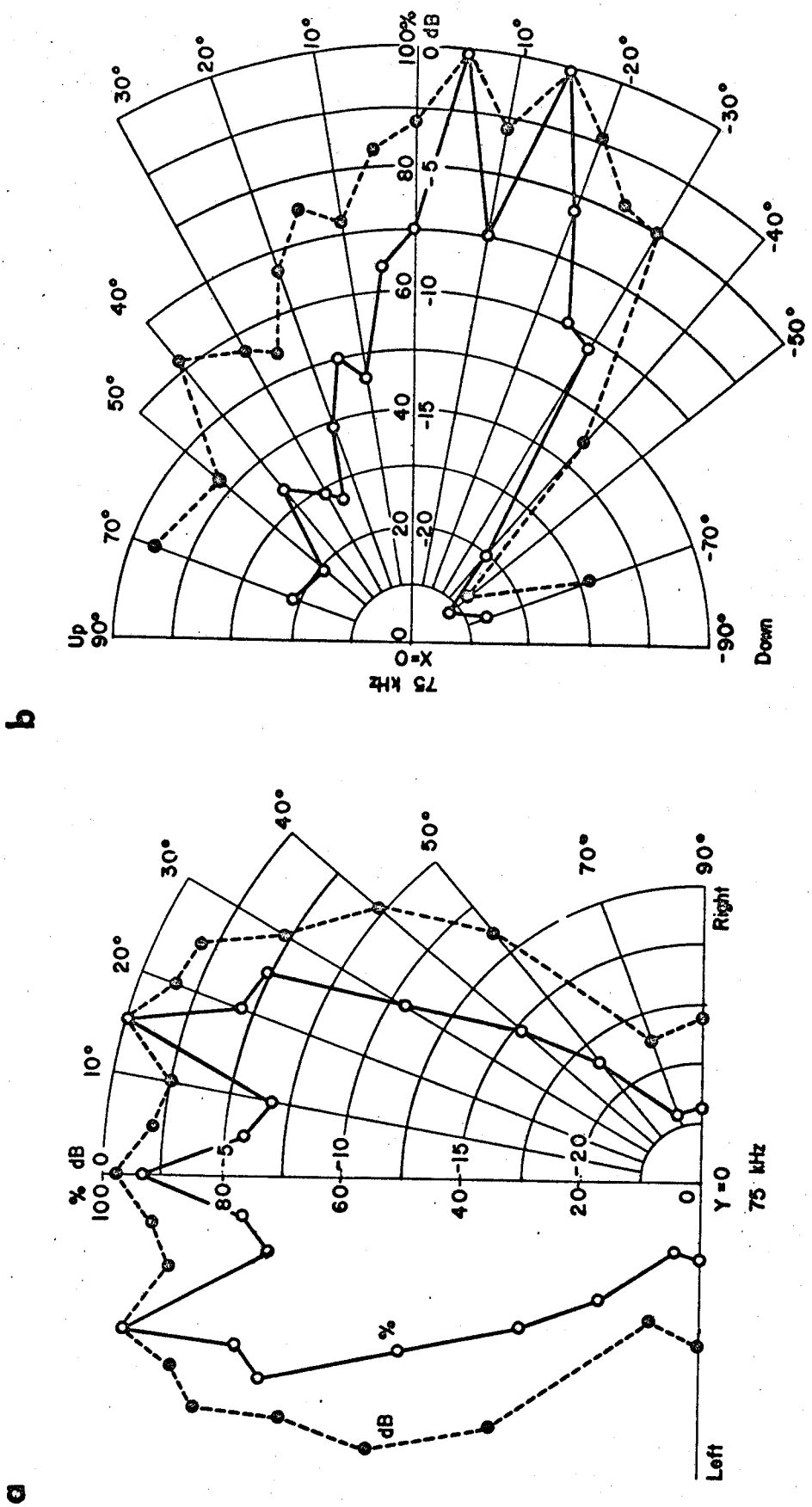

b
100% 0 dB
10° 20° 30° 40° 50° 70° 90°
-10° -20° -30° -40° -50° -70° -90°
80 5
60 -10
40 -15
20 -20
0
Up
Down
X=0
75 kHz
a
% dB
100 0
80 -5
60 -10
40 -15
20 -20
0
Y=0
75 kHz
Right
Left
%
dB

FIGURE 13.

Figure 14.

Radiation pattern of the 95 kHz component of FM sounds emitted by the bat in the horizontal (a) and vertical (b) planes. All symbols are the same as those in Figure 12.

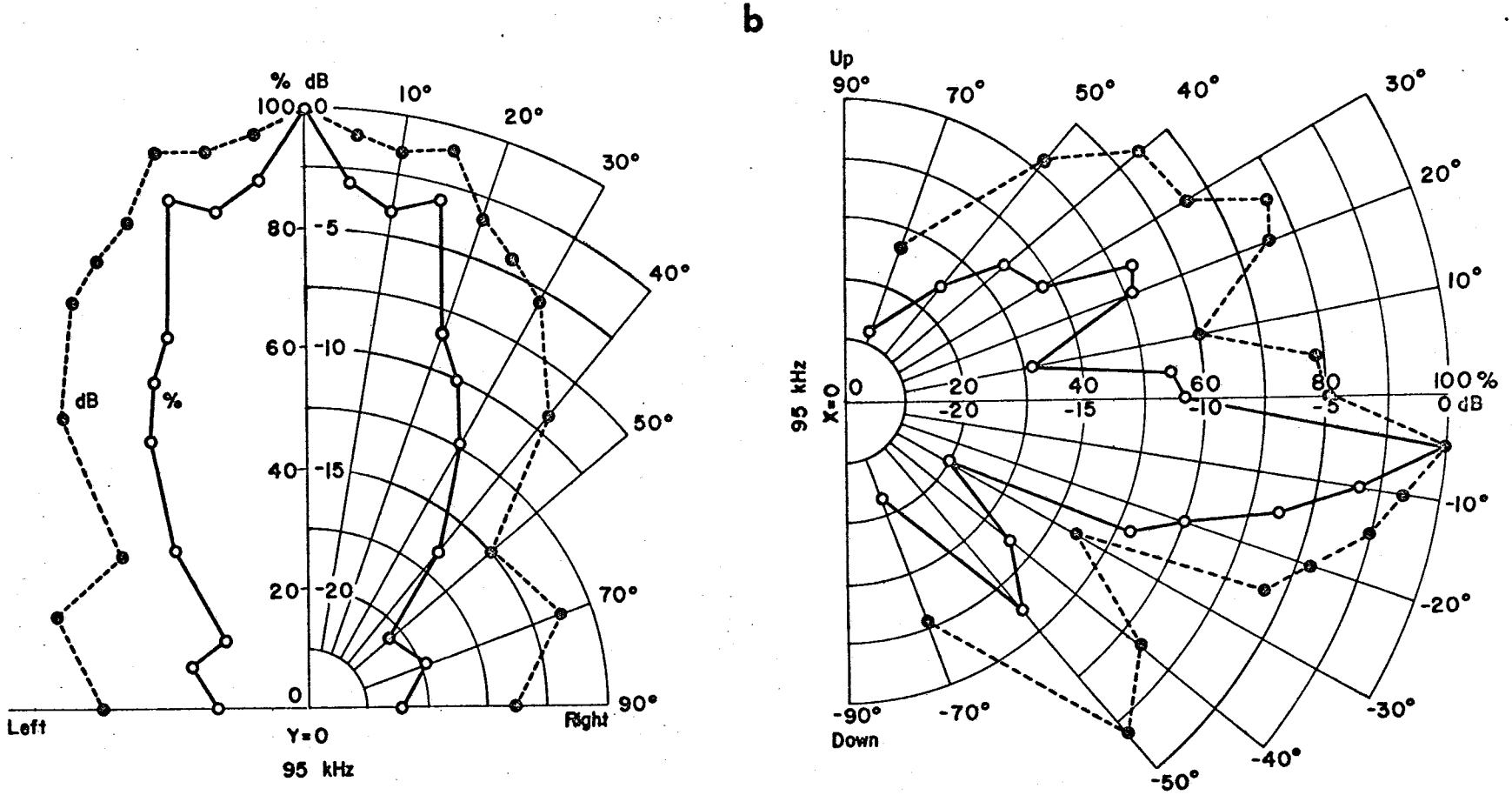
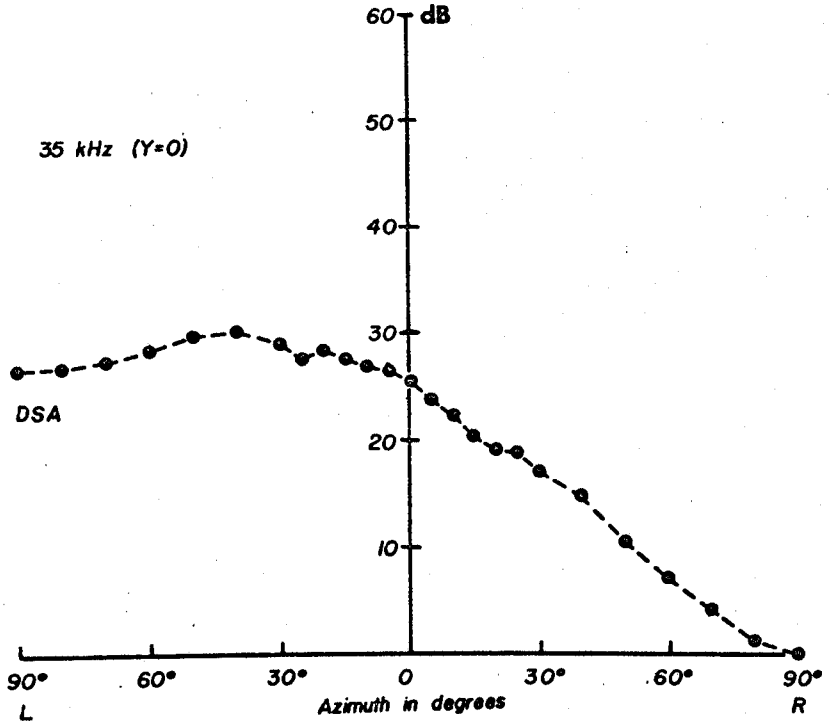

a
% dB
100
0
10°
20°
30°
80
-5
40°
60
-10
50°
40
-15
20
-20
70°
0
90°
dB
%
Left
Y=0
Right
95 kHz
b
Up
90°
70°
50°
40°
30°
20°
10°
95 kHz
X=0
0
20
40
60
80
100 %
0 dB
-20
-15
-10
-5
-10°
-20°
-30°
-90°
-70°
-40°
-50°
Down

FIGURE 14.

Figure 15.

The directional sensitivity of the auditory system (DSA) at 35 kHz. DSA, directionality of the orientation sound (DO) and directional sensitivity of the echolocation system (DSE) at 55 kHz. Each DSA curve is the average of measurements with 5 bats, while each DO curve is the average of data obtained from 2 bats. The ordinates and abscissae represent sensitivity in decibels and azimuth in degrees, respectively.


60
dB
50
35 kHz (Y=0)
40
30
DSA
20
10
90°
60°
30°
0
30°
60°
90°
L
Azimuth in degrees
R

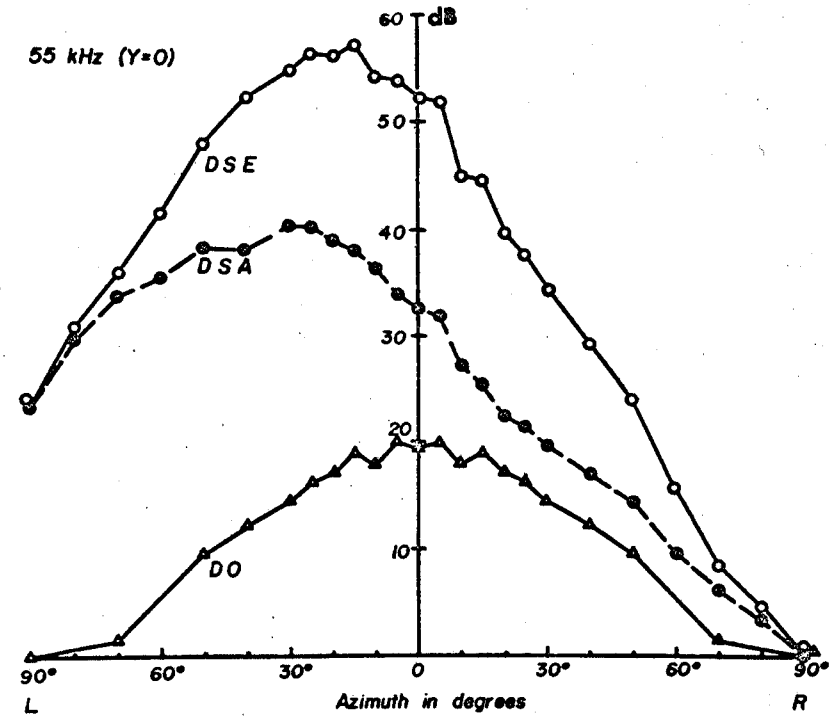

60
dB
55 kHz (Y=0)
50
DSE
DSA
40
30
20
DO
10
90°
60°
30°
0
30°
60°
90°
L
Azimuth in degrees
R

FIGURE 15.

Figure 16.

The DSA, DO and DSE at 75 and 95 kHz. Each DSA curve is the average of measurements with 5 bats, while each DO curve is the average of data obtained from 2 bats. All symbols are the same as those in Fig. 15.

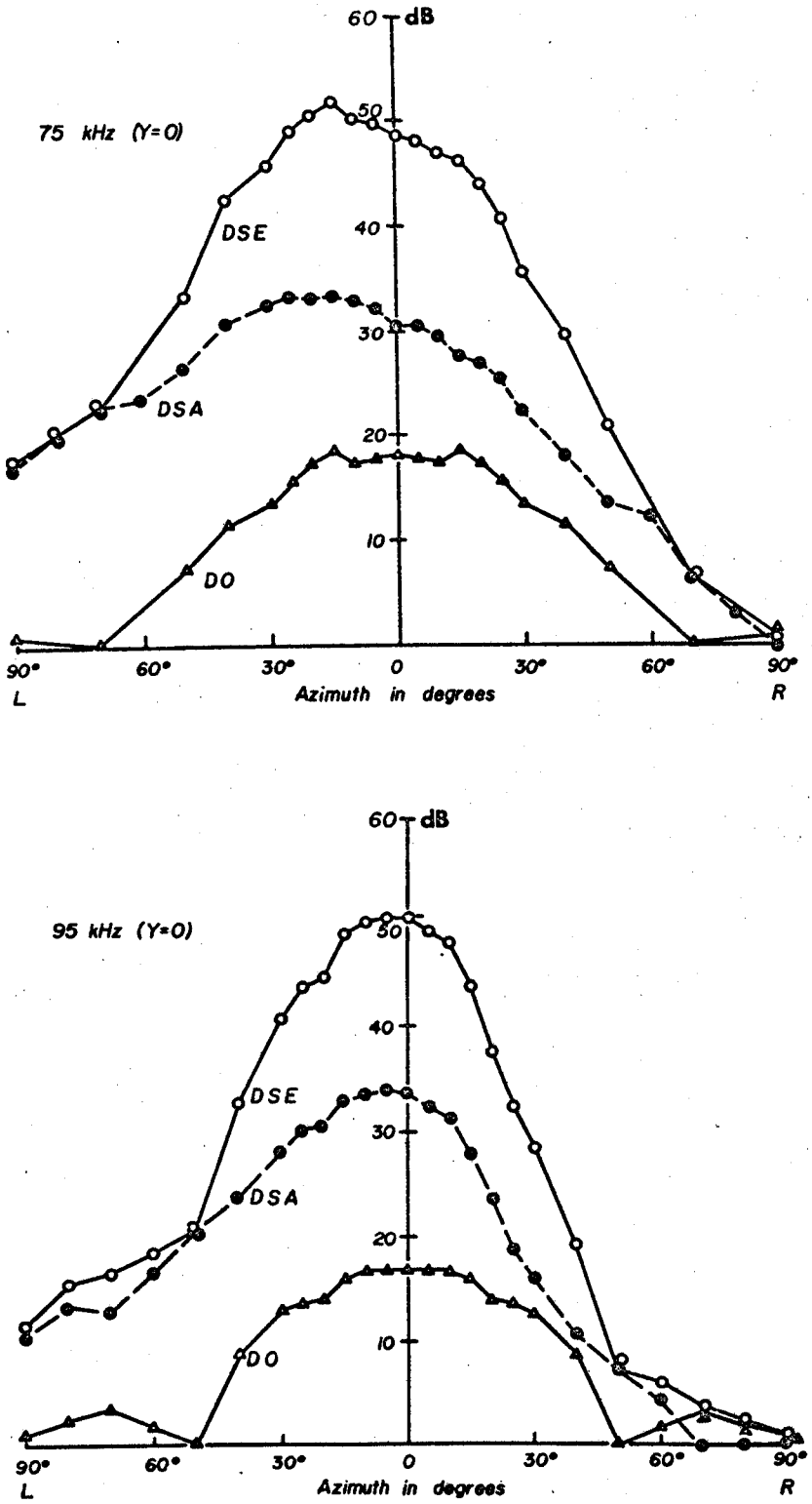

60 dB
75 kHz (Y=0)
50
40
30
20
10
DSE
DSA
DO
90°
60°
30°
0
30°
60°
90°
L
Azimuth in degrees
R
60 dB
95 kHz (Y=0)
50
40
30
20
10
DSE
DSA
DO
90°
60°
30°
0
30°
60°
90°
L
Azimuth in degrees
R

FIGURE 16.

Figure 17.

Interaural pressure differences (IPD) as a function of azimuth at either 35 and 55 kHz or 75 and 95 kHz. The ordinates and abscissae represent the IPD in decibels and azimuth in degrees, respectively. These curves were obtained from the data presented in Figures 15 and 16.

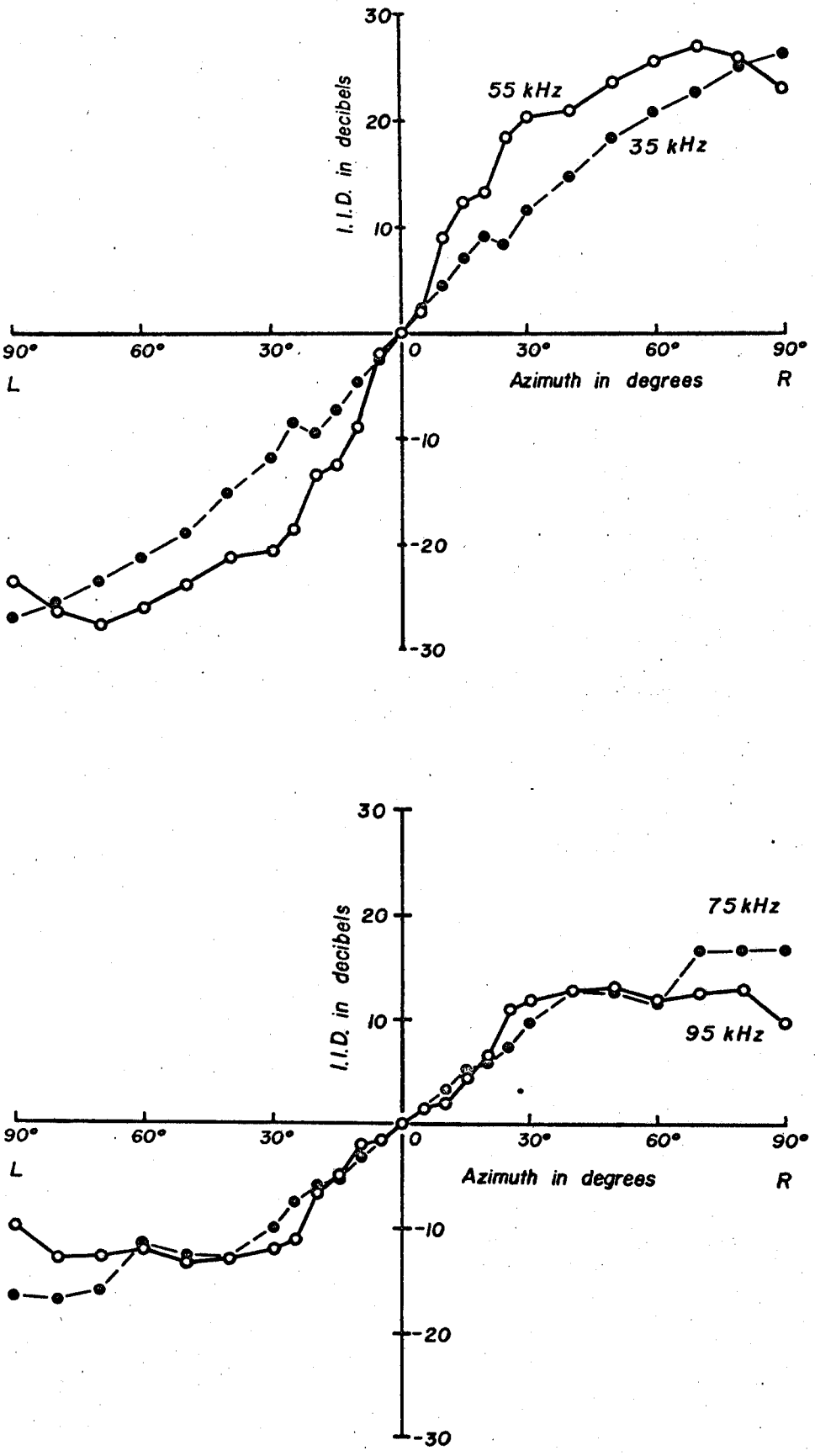

30
20
10
-10
-20
-30
I.I.D. in decibels
55 kHz
35 kHz
90°
60°
30°
0
30°
60°
90°
L
Azimuth in degrees
R
75 kHz
95 kHz

FIGURE 17.

Figure 18.

DSA, DO, DSE and IPD of Rhinolophus (R.f.) as a function of azimuth at 83 kHz. For comparison, the IPD curve of Myotis (M.g.) is also shown, which is the average of the curves for 75 and 95 kHz in Figure 17. All symbols are the same those in Figs. 15 and 17.

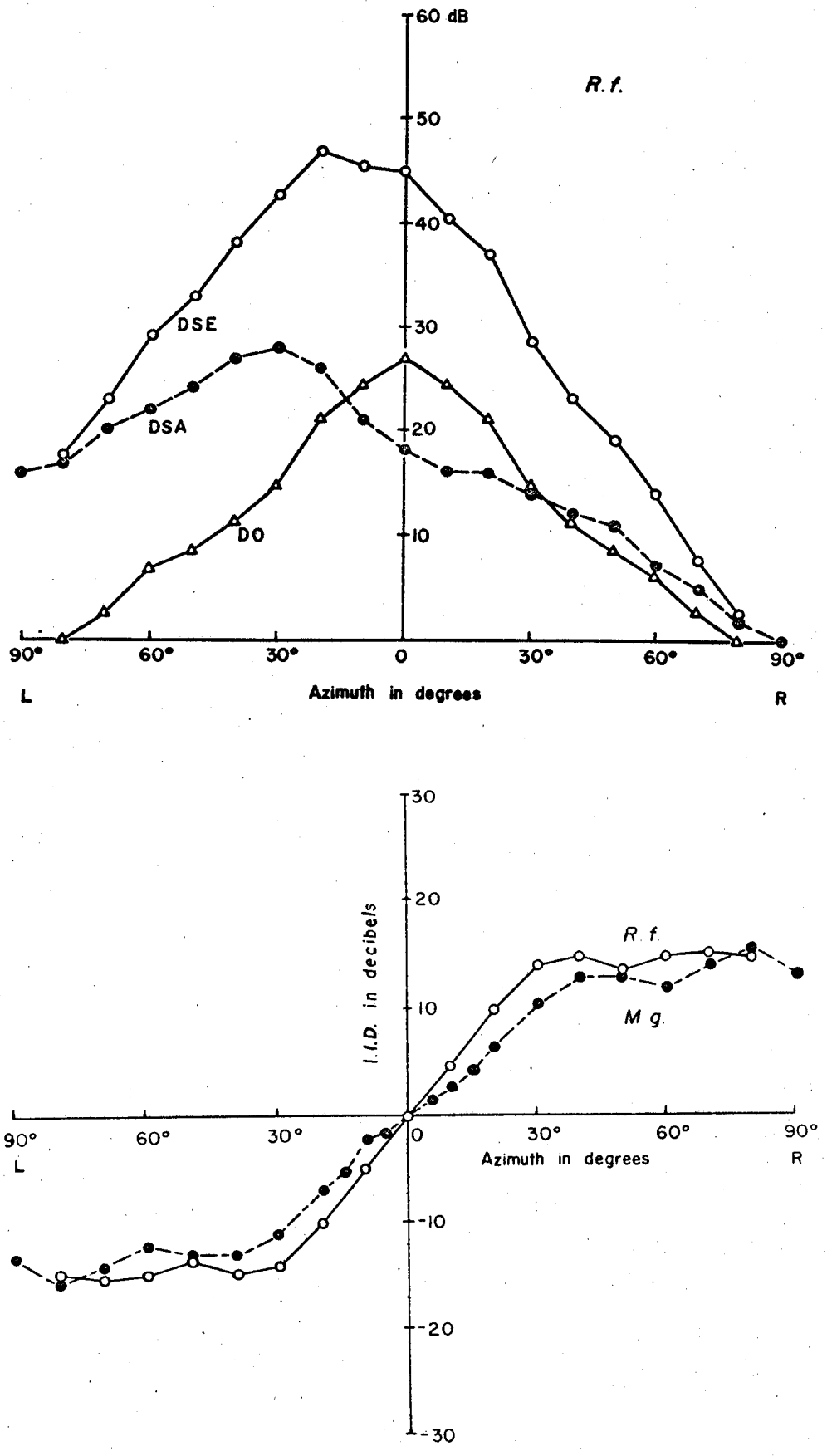

60 dB
R.f.
50
40
30
20
10
DSE
DSA
DO
90°
60°
30°
0
30°
60°
90°
L
Azimuth in degrees
R
30
20
10
-10
-20
-30
I.I.D. in decibels
R.f.
M.g.
90°
60°
30°
0
30°
60°
90°
L
Azimuth in degrees
R

FIGURE 18.

CHAPTER III

Attenuation Mechanism of Auditory Self-Stimulation by Neural Mean.

Sensory system usually receive stimulation produced by the animal's own activities. For instance, the retina is stimulated by movement of images introduced by eye and head movements, and the lateral-line system of fish is activated by water displacement caused by body movement. Such self-stimulation may not be absolutely necessary for monitoring the movements of the eye or body, and it may even disturb the perception of external sensory stimuli. Apparently, the visual and lateral-line systems have mechanisms for attenuating such self-stimulation. Visual perception in humans is suppressed before and during eye movements (Duffy and Lombroso, 1968). A comparable phenomenon has also been observed in arthropods (Palka, 1972). When fish and aquatic frogs move, the activity of sensory cells in the lateral-line organ is suppressed by efferent fibers before and during body movements (Russell, 1971).

The auditory system is stimulated by the animal's self-vocalized sound. This self-stimulation is important in controlling vocalization, as evidenced by the abnormal variability in intensity of speech sounds from deaf persons

(Davis and Silverman, 1970) and by the interference with normal development of songs in birds that are deafened (Konishi, 1963). Acoustic self-stimulation, however, would be unnecessarily intense for simple monitoring of vocalization, if the auditory system were not equipped with mechanisms for attenuating its sensitivity. Attenuation occurs both at the receptors and in the brain.

In the echolocating loud bats such as Myotis emitting a relatively strong (100-140 dB SPL) ultrasonic orientation sounds, echoes from short distances would not be detected, if such strong outgoing sound stimulate the ears, because the response is very poor to a sound that follows immediately after the strong one. In humans, cats,and bats, the muscles of the middle ear have been shown to contract synchronously with vocalization to attenuate self-stimulation (Carmel and Starr, 1963; Shearer and Simmons, 1965; Henson, 1965, and see Chapter IV). In addition to this muscular attenuation, neural attenuation by the olivo-cochlear bundle (OCB) is conceivable, but this has not yet been proved in bats. Data on the brain of gray bats, Myotis grisescens have been obtained which indicate the presence of a neural attenuating mechanism operating synchronously with vocalization (Suga and Schlegel, 1972). This neural attenuation in the brain was found by comparing the summated responses of primary auditory and lateral lemniscal neurons to self-vocalized frequency-modulated sounds (FM) with those evoked by the same sounds

played back through a tape recorder. The responses of lateral lemniscal neurons (LL) to the self-vocalized sounds were found to be much smaller than those evoked by the played-back sounds, even when the response of the primary auditory neurons (N_1) was nearly the same for both types of sounds. It is clear that the neural attenuation takes place between the auditory nerve and the inferior colliculus (IC), but its specific location has been unknown.

Between the primary auditory nerve and IC, there are the cochlear nucleus (CN), superior olivary complex (SOC), and nucleus of the lateral lemniscus (NLL). Each of these nuclei is further devided into subnuclei. Since the auditory pathway is complicated, the origin of the neural attenuation appeared difficult to identify. It is important to identify the site of the neural attenuation in order to understand the echolocation system of the bats. In this chapter the site of the neural attenuation (an interaction from the efferent system to the afferent one) is determined in the ascending auditory pathway.

Materials and Methods

The gray bats, Myotis grisescens was anesthetized with ether, and a smooth head of a nail of 1.8 cm in length was mounted on its skull with dental cement. A few hours after the surgery, the awake animal was placed on a plastic ball floating on water in a sound-proofed room, the inner wall of which was covered with fiber glass sheets in order to prevent the multiple echoes. The shank of the nail was locked into a metal rod with a set screw. Two small holes were then made in the skull without anesthesia. Tungsten-wire electrodes were inserted into the brain through these holes to record the summated activity of auditory neurons. The indifferent electrode was placed in the exposed temporal muscles which were removed from the skull bone. Seventeen bats were used. In the present experiment, mechanical stimulations were used to elicit vocalization instead of the electrical stimulation as employed in previous chapters. The bat was stimulated by touching its back or its tail or both with a brush or by moving the plastic ball on which the animal rested. FM orientation sounds or squeaks emitted by bat were monitored with a quarter-inch microphone (Brüel and Kjaer, 4135) placed about 10 cm in front of the bat's mouth and about 30° down from the eye-nostril axis, and were stored on a magnetic tape with a tape recorder (Ampex FR-100), which has a frequency response of 30 to 300,000 Hz at a tape speed of 60 inches per second. The microphone has a frequency response which is flat from

50 to 120,000 Hz within ± 1.0 dB. The responses, for example N_1, and N_3, evoked by thirty two emitted sounds were stored and averaged by a computer (Nicolet, 1070). The computer was triggered by electric pulses synchronized with the onset of the self-vocalized sound. The averaged response was plotted with an X-Y recorder. These are called "the self-evoked responses". Those electrical pulses were also stored in the magnetic tape and were used to trigger the computer for the responses to the played-back sound (see below). The tape-recorded sounds were played back at different amplitudes through a loudspeaker placed 68 cm in front of the bat's mouth between 4 and 60 minutes after the self-vocalized sounds were recorded. The output of the playback system, consisting of the recorder, amplifiers, and loudspeaker, had a flat frequency response curve from 20 to 100 kHz within ± 2 dB. The responses, say N_1 and N_3 evoked by these played-back sounds were averaged and plotted. These are called "the played-back responses". The self-evoked response for example N_3, was compared with the played-back response of N_3 in order to determine whether there was a difference in amplitude when N_1 was the same for both types of sounds.

Result

When recording and indifferent electrodes are placed on the dorsal surface of the IC and exposed temporal muscles, respectively, auditorily evoked potentials in response to a tonal stimulus can be easily recorded with five positive peaks. The five peaks of evoked potential are called N_1, N_2, N_3, LL (N_4), with the latencies of 0.8, 1.4, 1.8 and 2.8 msec, and IC responses to acoustic stimuli. With the recording electrode placed in the auditory nerve, the S-segment; the lateral superior olivary nucleus, or the nucleus of the lateral lemniscus, the N_1, N_3, or LL responses, respectively with the characteristic latencies, became diphasic or triphasic and became as large as 1 to 3 mV peak-to-peak, while others remained less than 0.2 mV. The N_1, N_3, and LL responses were apparently the summated action potentials mainly originating from the auditory nerve, the S-segment, and the nucleus of the lateral lemniscus. It was not easy to separately record a large N_2 response. The origin of N_2 was not clear, but it may have been the cochlear nucleus (see discussion). Since selective recording of an N_1, N_3, or LL response was possible, these evoked potentials were recorded in different combinations with two recording electrodes and examined the combinations in which the neural attenuation was found.

When the bat was stimulated mechanically, the animal emit FM orientation sounds or squeaks (probably a type of

communication sounds) if the mechanical stimulus was noxious. Thirty-two sounds were used to average the neural response by the computer. The sounds emitted by the bat ranged between 100 and 115 dB SPL.

When N_1 and contralateral N_3 were simultaneously recorded for self-vocalized and played-back sounds, the self-evoked N_3 was nearly the same as the played-back N_3 whenever the self-evoked and played-back N_1's were the same (Figure 19 A). In Figure 20 A, the relationship between the pressure level of the played-back sounds and the amplitudes of the evoked potentials is shown. The horizontal arrows and dashed lines represent the amplitudes of the self-evoked N_1 and N_3. These lines cross the curves for played-back N_1 and N_3 at about 85 dB SPL. Therefore, there is no neural attenuation between the cochlear nerve and the S-segment.

As another combination, the N_3 and contralateral LL were simultaneously recorded for self-vocalized and played-back sounds. As shown in Figure 19 B, the self-evoked and played-back N_3's are the same in amplitude, but the played-back LL is significantly much larger than the self-evoked LL (t-test for amplitude difference, $P < .01$). This indicates that the response of the nucleus of the lateral lemniscus is suppressed when the animal vocalizes. In Figure 20 B, the relationship between pressure level of the played-back sounds and the amplitudes of the evoked potentials is shown. The evoked N_3 crosses the curve for played-back N_3 at 70

and 74 dB SPL, while the dashed line indicating the amplitude of self-evoked LL crosses the curve for played-back LL at 60 dB SPL. Thus the amount of attenuation observed in Figure 20 B is equivalent to either 10 or 14 dB, with an average of 12 dB.

These experiments were repeated with 17 bats. In table 1, the amounts of the neural attenuation observed are shown for each type of vocalization with variety of combinations of the location of the recording electrodes. The neural attenuation observed for the emission of FM orientation sounds or squeaks (probably a kind of communication sound) was 0.0 $\pm$ 6.2 dB between N_1 and N_3 (16 animals) and 16 $\pm$ 12 dB between N_3 and LL (17 animals). In the previous experiment by Suga and Schlegel (1972), vocalizations were evoked by electrical stimuli applied to the midbrain, and the neural attenuation between N_1 and LL was measured as 25 dB in a mean value. In the experiments reported in this chapter, however, vocalizations were elicited without electrical stimulation. Consequently, the neural attenuation between N_1 and LL was remeasured; it was 15 $\pm$ 10 dB (11 animals).

Discussion

Nerve impulses are transmitted from the auditory nerve to the cochlear nucleus, then the superior olivary complex containing the S-segment, to the nucleus of the lateral lemniscus, and to the inferior colliculus. Present data indicate that the amount of neural attenuation found between the responses of the auditory nerve and the nucleus of the lateral lemniscus was not different from that between the responses of the S-segment and the nucleus of the lateral lemniscus (Table 1). The neural attenuation did not occur in either the cochlear nucleus or the S-segment, but it occurred in the nucleus of the lateral lemniscus. Further data supporting this conclusion remain to be obtained by recording single unit activity from the nucleus of the lateral lemniscus.

Since neurons in the cochlear nucleus (Suga, 1964, 1965), the S-segment (Jen, 1974), and the nucleus of the lateral lemniscus (Suga and Schlegel, 1973) of bats commonly show tonic on-responses to tonal stimuli, any evoked potential after N_1 could originate from one or more nuclei. Thus the origin of a summated evoked potential is very difficult to analyze. Nontheless, the statements on the origin of N_1, N_3, and LL are still valid, because only well-synchronized action potentials as an output of a nucleus can produce a large summated potential change recorded in the nucleus. For instance, the primary auditory neurons fire repetitively

during tonal stimuli, but these action potentials do not electrotonically contribute to the response which appears with a latency of 2.8 to 3.5 msec. Therefore, it is correct to describe the LL response as mainly originating from neurons in the nucleus of the lateral lemniscus. In the superior olivary complex of Myotis, the S-segment and the nucleus of the trapezoid body are large, while the accessary nucleus is absent (Harrison and Feldman, 1970).

From the measurements of the depth and orientation of the recording electrode, it is more likely that the N_3 response mainly originates from the S-segment. The nucleus of the trapezoid body and the preolivary nuclei, respectively, are too medial and ventral to the tip of the recording electrode to be considered as sources. The latencies of single-unit and mass responses of the S-segment of Myotis grisescens and M. lucifugus are 1.8 to 2.2 msec (Jen, 1974). It is believable that the N_3 response with a peak latency of 1.8 to 2.1 msec mainly originates from the S-segment. Thus, the N_1, N_3, and LL responses are apparently the summated action potentials mainly originating from the primary auditory nerve, the S-segment, and the nucleus of the lateral lemniscus.

Though, the evoked potentials are not simply proportional to the stimulus amplitude as shown in Figure 20, it reflects the population of the neurons which fire in a well synchronized volley of impulses. Thus it is useful as a measure of excitatory output of a nucleus.

Played-back sounds at the inner ear are similar, but not perfectly identical to the self-vocalized sounds, because that the output of the loudspeaker is not completely flat for all frequencies but uneven within ± 2 dB between 20 and 100 kHz, and that the signal-to-noise ratio of the played-back sounds was 30 to 40 dB, much smaller than that of the self-vocalized sounds for which it was more than 60 dB, and also because that self-stimulation always consisted of both bone and air conductions, but the played-back sounds consisted solely of air conduction. Therefore, there was slight difference between the responses to self-vocalized sounds and those to the played-back sounds. It, however, is reasonable to compare the responses to those two stimulus, because the two responses for example N_3 and LL are recorded simultaneously and compared in amplitude of the one when the other is the same in amplitude.

Since the computer was synchronized with the onset of either the self-vocalized or the played-back sounds, fluctuations in time were always present in averaging the responses, but the amount was the same for both the self-evoked and played-back responses. The standard deviation of the amplitudes of these evoked potentials was about ± 5 percent.

As shown in Table 1, the experiment showed that there is an attenuation of sensitivity to the acoustic signal synchronized with the vocalization, at the level of the nucleus of lateral lemniscus. It should be noted that there was differ-

ence in the amount of neural attenuation between N_1 and LL measured by Suga and Schlegel (1972) and that of Table 1, this difference suggests that there are some byproductive effects in the brain with the electrical stimulation (see also Chapter IV).

An alternative explanation of this neural attenuation could be a facilitation of responses to the played-back sounds following vocalization. This, however, appears unlikely because the time interval between vocalization and played-back sounds ranged between 4 and 60 minutes. If such a long-lasting facilitation occurs, responses to self-vocalized sounds would also be facilitated.

As already mentioned, the middle-ear muscles attenuate the self-stimulation by 20 to 25 dB (Henson, 1965, and see Chapter IV). Thus, the total attenuation by both the muscles and the neural events is 35 to 40 dB. This is a surprisingly large attenuation. Similar muscular and neural attenuation mechanisms would also exist in our communication system, because we never perceive our own speech sounds to be disturbingly loud, unless Eustachian tubes are abnormally patent.

Summary

1. Two tungsten-wire electrodes were inserted in a pair of auditory nuclei of the unanesthetized echolocating bats for recording a summated neural activity (evoked potential) to a self-vocalized orientation sound and also to a played-back sound.

2. The evoked potentials originating from cochlear nucleus (N_1) were compared with those originating from superior olivary complex; S-segment in echolocating bats (N_3). When N_1 showed the same amplitude to both the self-vocalized sounds and to the played-back sounds, N_3 also had the same amplitude to both those stimuli.

3. The evoked potentials originating from nucleus of the lateral lemniscus (LL) were always smaller in response amplitude to the self-vocalized sounds than to the played-back sounds, while the amplitude of played-back sound was adjusted to obtain the same amplitude of N_1 as in self-vocalized sound. In turn, the amplitude of played-back sound should be decreased by about 15 dB to obtain the same amplitude of N_3 as in self-vocalized sound.

4. Nucleus of the lateral lemniscus attenuates equivalently the ascending auditory activity synchronously with the vocalization both the orientation sounds and the squeaks (probable communication sounds).

5. The amount of attenuation observed was about 15 dB.

Thus the echolocating bats has a neural attenuation mechanism for the reduction of the self-stimulation in addition to the muscular mechanism in the middle ear which contracts and attenuates sound transmission by 20 dB. As a total amount of 35 dB at least is achieved in the echolocating bat for orientation sound emission.

6. There was an indication that the electrical stimulation would cause some byproductive effect in the brain, because there was slight difference in the amount of neural attenuation measured with the vocalizations elicited by the electrical and by mechanical stimulation of the animal.

Figure 19.

Summated responses of the auditory nerve (N_1), S-segment (N_3), and nucleus of the lateral lemniscus (LL) evoked by 32 self-vocalized sounds (Voc.) (a) and by these sounds played back (PB) with a tape recorder (b). Each response indicated by arrow is the average of 32 samples. The slow potential change following N_3 probably originated from the nucleus of the lateral lemniscus and the inferior colliculus. (A) N_1 on the left side and N_3 on the right side are simultaneously recorded. The self-evoked N_3 is very similar to the played-back N_3 whenever the self-evoked N_1 is nearly the same as the played-back N_1. The amplitudes of the played-back sounds are approximately 85 dB SPL. (B) LL on the left side and N_3 on the right side are simultaneously recorded. The self-evoked LL is significantly smaller than the played-back LL although the self-evoked N_3 is nearly the same as the played-back N_3. The amplitudes of the played-back sounds were approximately 75 dB SPL. The sounds produced by the bat were weaker than those in (A).

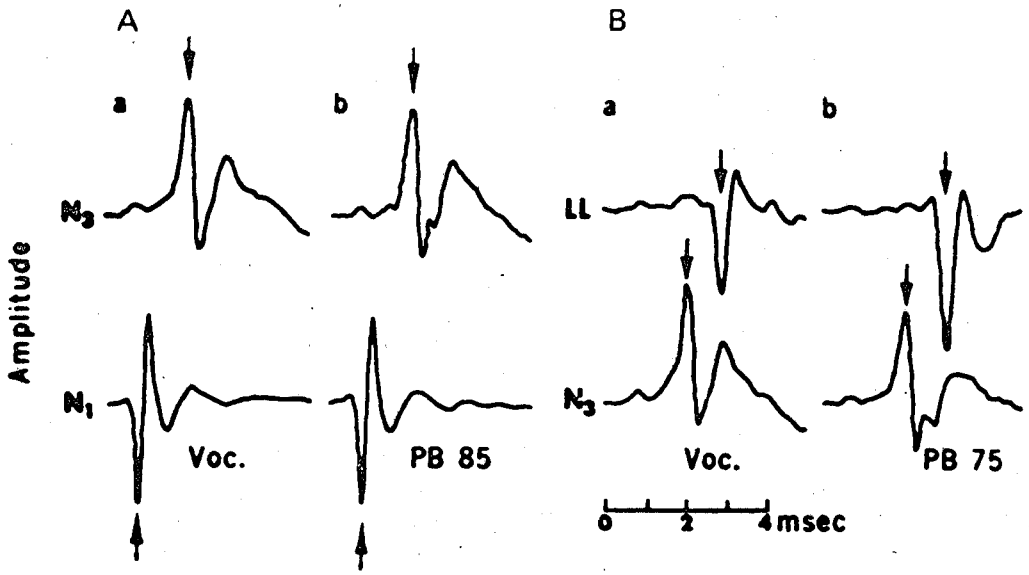


FIGURE 19.

Figure 20.

(A) and (B), relation between the amplitudes of the played-back N_1, N_3, LL (ordinates) and the approximate pressure level of the played-back sounds (abscissae). Each point represents the average of 32 responses. The ordinates represent the peak-to-peak amplitudes of the evoked responses plotted with an X-Y recorder. Ten units in the ordinates correspond to 0.2 to 0.3 mV. The amplitudes of the self-evoked N_1, N_3, and LL are indicated by the horizontal arrows and dashed lines. The graphs in (A) and (B) were obtained in the same recording conditions as those for the evoked potentials in Figure 19 (A) and (B), respectively. The amount of the neural attenuation (att.) is 10 to 14 dB, with an average of 12 dB in (B).

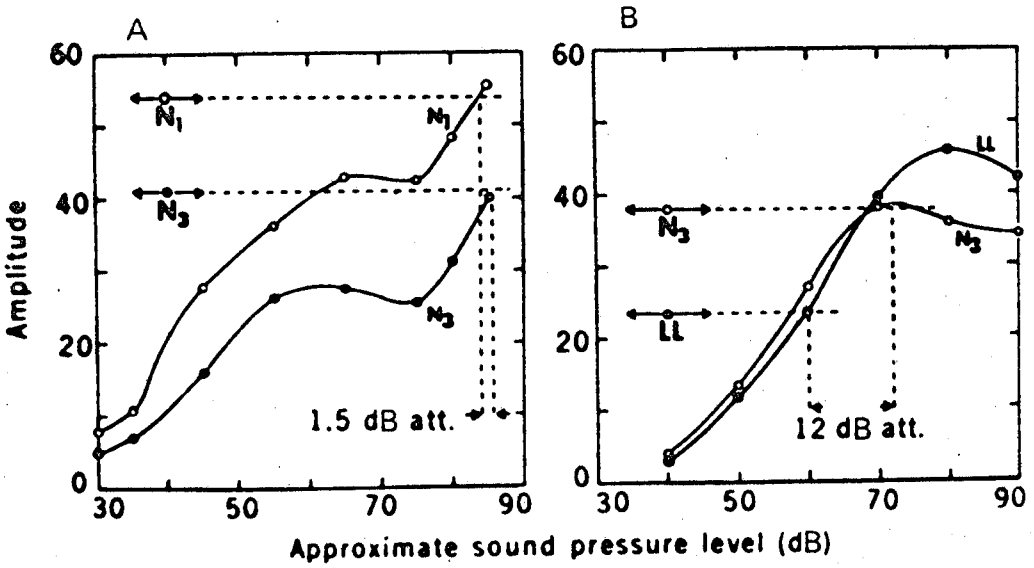

A
B
60
40
20
0
30
50
70
90
Amplitude
N1
N3
1.5 dB att.
LL
12 dB att.
Approximate sound pressure level (dB)

FIGURE 20.

Table 1.

Amounts of neural attenuation in dB (mean ± standard deviation) observed between three possible combinations (row) of three evoked potentials (N_1, N_3, LL). Amounts of attenuation in each combination of evoked potential were indicated for three types of vocalization (column). Each figure was calculated with a number (N) of the observed attenuations from different animal. The amount of attenuation was determined with the computer averaged potentials evoked by 32 vocalizations. The figures in the column of FM orientation signal was obtained from the 32 FM orientation sounds solely, the column squeak from the definitely human audible sounds, the right most column from the vocalizations consisting of both the FM orientation sounds and the audible squeaks. No sample was used twice for caliculation, but the same animal was used for the different measurements.

Neural attenuation in decibels

Types of sounds / Comb. of Evoked Pot.	FM orientation signal	Squeak	FM and/or squeak
$N_1 - N_3$	- 3.5 ± 3.8 N = 7	0.0 ± 4.7 N = 6	0.0 ± 6.2 N = 16
N_3 - LL	16 ± 12 N = 13	14 ± 11 N = 4	16 ± 12 N = 17
N_1 - LL	21 ± 7.6 N = 5	10 ± 9.0 N = 6	15 ± 10 N = 11

TABLE 1.

CHAPTER IV

Vocal Response to Acoustic Stimuli in Relation to Neurophysiology in the Auditory System

For echolocation, Microchiropteran bats emit species-specific orientation sounds. The bats which produce frequency-modulated (FM) signals are commonly called "FM bats", and the bats which use signals consisting of constant-frequncy (CF) and FM components are called "CF-FM bats". Among CF-FM bats, there are a few subtypes in terms of signal variations during the searching phase of insect hunting by sonar. For instance, Pteronotus parnellii (mustache bats) always emit a long CF sound ending with a short FM sound (Schnitzler, 1970). Pteronotus suapurensis (naked-backed bats) produce a sound consisting of very short CF component followed by a short FM component. Noctilio leporinus (fish-catching bats), on the other hand, independently change the durations of the CF and FM components within a range of 0-15 msec, so that some orientation sounds appear to consist of only CF or FM components (Suthers, 1965). Such differences in CF components among species may indicate that (1) the vocalization systems vary anatomically, and/or (2) the information obtained by the CF sound varies; that is, utility of the CF sound varies among species.

A CF sound is an ideal signal for Doppler measurement, e.g. the measurement of the relative velocity between a bat and a target, but it is poor for target ranging and for determining target characteristics because of its narrow spectrum band width. The CF sound is a good signal for echo-detection when the size of the target is similar to or larger than the wavelength of the signal and when the integration period for sensation is sufficiently long. The CF-FM bats presumably use the CF component for its inherent advantages. Rhinolophus ferrumequinum (Schnitzler, 1968; Schuller, 1974; Simmons, 1973) and P. parnellii (Schnitzler, 1970) adjust the frequency of the transmitted CF signal to receive a Doppler-shifted echo at a certain preferred frequency. In these animals the threshold curves of the auditory system show specialization for the reception of a sound at a certain frequency (Ajrapetianz and Vasilyev, 1971; Grinnell, 1970; Neuweiler, 1970; Pollak et al., 1972). The CF sound is undoubtedly not merely a byproduct of laryngeal activity. It is used for the measurement of target velocity, for target-detection, and perhaps for compensation of errors in echo-analysis with FM part caused by the Doppler effect. CF-FM bats which are known to alter vocalizations to receive Doppler-shifted echoes at a certain preferred frequency are well suited for behavioral and neurophysiological studies on the coupling between the auditory and vocalization system.

In Myotis, P. parnellii and P. suapurensis it has been

found that electrical stimulation of the dorsal part of the midbrain reticular formation elicits the emission of species-specific orientation sounds as already described in Chapter I. The neural circuit for the emission of orientation sound is apparently species-dependent. In this Chapter, it is described (1) how electrically elicited sounds for different species of CF-FM bats are modified by different types of acoustic stimuli and (2) what kind of specialization exists in the auditory system and its relation to vocal behavior.

Materials and Methods

Experimental subjects were ten Pteronotus parnellii and ten P. suapurensis from Panama and two Noctilio leporinus from Trinidad. The animals were anesthetized by ether only during surgery. On P. suapurensis, a nail 1.8 cm long was mounted on the exposed skull with dental cement and was fixed onto a metal rod with a set screw, to immobilize the head. P. parnellii and N. leporinus are larger animals, so that their heads were immobilized stereotactically with three blunt needles mounted on micromanipulators and pressed against their skulls.

In order to stimulate the brain with electric pulses, a small hole was made in the skull covering the inferior colliculus. A bipolar steel-wire electrode was inserted into the midbrain reticular formation through this hole. The tips of the electrodes were less than 50 μ in diameter and were 0.2-0.5 mm apart. The electrical stimuli used to evoke vocalization were short trains of monopolar electric pulses delivered at a rate of 0.5 per second. Each train consisted of 10 electric pulses which had 0.1 msec duration and 1 to 15 V amplitude. The estimated amount of current was 0.1-1.5 μ amps. The inter-pulse interval was 1.7 msec. Sounds emitted by the bat were picked up with a quarter-inch microphone (Brüel and Kjaer 4135) placed 8-10 cm anterior to the bat's mouth and were recorded with a tape recorder having a frequency response of 50-150,000 Hz at a tape speed

of 30 inches per second (Ampex FR-100). These sounds were roughly analyzed with a zero-crossing period meter for observation during the experiment. Later these were analyzed at 1/16 or 1/32 of the original tape speed with a Kay sonagraph.

The cochlear microphonic potentials (CM) and summated activity of the primary auditory neurons at the stimulus onset (N_1-on) and at the cessation (N_1-off) were recorded with a tungsten-wire electrode or a silver wire with insulation except the tip, placed and cemented at the rim of the round window through the dorso-posterior part of the auditory bulla. The summated activity of the lateral lemniscal neurons (LL) or the primary auditory neurons (N_1) at the stimulus onset or the cessation was recorded with tungsten-wire electrode which was placed in the nucleus of the lateral lemniscus or in the ventral side of the cochlear nucleus respectively through the inferior colliculus. Single unit activity of the primary auditory neurons was recorded with a glass micropipette electrode inserted into the auditory modiolus through the cochlear nucleus after aspirating the lateral part of the cerebellum (paraflocculus). The recording of all the above electrical activities was performed without anesthetic in a sound-proofed room, the inner wall of which was covered with fiber-glass to reduce echoes. Neural summated activity of the primary auditory nerve or the nucleus of the lateral lemniscus, and or single unit activities are stored and

averaged by a computer (Nicolet 1070) for 8 or 16, in some case for 32 acoustic stimuli. The electronic instruments used to generate acoustic stimuli were the same as those described by Suga (1968), (see Materials and Methods in General). For the measurement of threshold curves, pure tone pulses with a 0.5 msec rise-decay time and a 4 msec duration were repeatedly delivered at a rate of 1.5 per second, unless otherwise described. The amplitude of the tone pulse delivered from a condenser loudspeaker was measured with a quarter-inch microphone (Brüel and Kjaer, 4135) placed at the bat's ears, and it was expressed in dB SPL.

Results

Vocal responses to acoustic stimuli in the electrically activated bats.

When electrical stimuli were applied to the dorsal part of the midbrain reticular formation and/or the lateral part of the central gray matter, near the boundary between the superior and inferior colliculi, each bat emitted sounds very similar to its species-specific orientation sounds. The latency of this vocal response to electrical stimuli ranged between 25 and 60 msec. Each sound emitted by P. parnellii for each electrical stimulus consisted of a 10-30 msec CF component followed by a 2-3 msec FM component (Figures 21 A, and 7, 8 in Chapter I). The frequencies of the first, second and third harmonics in the CF component were about 31, 62, and 93 kHz, respectively. The second harmonic always predominated, while the first harmonic was sometimes faint. The third harmonic was always present. The second harmonic in the FM component swept downward from 62 to 50 kHz. Each sound emitted by P. suapurensis in response to electrical stimulation consisted of 1-3 msec CF and FM components (Figure 21 B). The duration of the CF component emitted by P. parnellii. The second, third and fourth harmonics were of approximately equal strength in the sounds of P. suapurensis. Noctilio leporinus emitted either an FM sound or a compound CF-FM sound for each electrical stimulus

(Figure 21, C and D). The frequency of the CF component was about 53 kHz. It was not clear whether there are separate areas in the brain for the emission of purely FM sounds and for the emission of CF or CF-FM sounds. During the searching phase of hunting, N. leporinus emits both CF-FM sounds and emits only FM sounds in the approaching and terminal phases of the echolocation of prey (Suthers, 1965). The electrical stimuli did not evoke purely CF sounds, although they often evoked a short CF sound followed by an FM sound, with an intervening silent period of about 4 msec (Figure 22 B).

When the intensity of the electrical stimuli delivered at a rate of 0.5/sec were decreased, the amplitudes of electrically evoked sounds became small, and the latencies of these vocal responses became long and showed large fluctuations. Under these conditions, 2 msec tone pulses at a repetition rate of 250 per second (tone burst), or continuous tones were delivered in addition to the electrical stimulation. Then, the electrically-evoked sounds became large in amplitude and more regular in occurrence. The number of individual sounds emitted for each electrical stimulus often increased. Hereafter, such a behavioral response is called "a vocal response to acoustic stimuli". The vocal response to acoustic stimuli varied with the parameters of the acoustic stimuli. For instance, an increase in amplitude of the tone burst caused an increase in amplitude of the emitted orientation sound of the vocal response of P. parnellii (Figure 22 A).

The frequency and amplitude of the pure tone burst (TB) were varied so that the tuning curve of the vocal response could be measured. The vocal response to acoustic stimuli (V.R.) was sharply tuned to 62 kHz (Figures 23 and 24). As the frequency of the stimulus increased toward the best frequency the threshold of the vocal response decreased at a rate of 1200 dB/octave. The threshold increased at a rate of 1300 dB/octave above the best frequency. The Q-10 dB value (the best frequency divided by the band-width of the tuning curve at 10 dB above the minimum threshold: a measure of sharpness of a tuning curve) was about 50. The minimum threshold was about 23 dB SPL. The threshold was higher than 65 dB SPL at frequencies below 50 kHz and above 75 kHz. When a continuous pure tone (CP) was delivered instead of tone burst, it was found that the threshold of the vocal response to a continuous pure tone was 10-25 dB higher than that for 2 msec tone burst, although the continuous pure tone had energy of 3 dB larger than the tone burst had (Figure 24).

A response representing the summated activity of the primary auditory neurons (N_1) appeared at both the onset and cessation of stimulus. N_1 at the stimulus onset is hereafter called "N_1-on" and N_1 at the cessation is "N_1-off". The threshold curve for N_1-on was quite different from that of N_1-off (Figure 23). N_1-on was tuned to about 30-45, 63-64 and 95-100 kHz, while N_1-off was sharply tuned at 24-27, 59-61 and 90-95 kHz. The tuning curve of the vocal response

to acoustic stimuli (V.R.) was similar neither to these neural tuning curves, but its single sharp notch did appear at 62 kHz, between the sharp tuning curve of N_1-on and N_1-off (Figure 23).

Instead of pure tone burst, both upward and downward sweeping FM tone burst were delivered as acoustic stimuli. The vocal response to acoustic stimuli in P. parnellii were significantly stronger to the FM sounds than to pure tone burst except for those around 62 kHz as shown by arrows in Figure 24. In particular, downward sweeping FM sounds were most effective in evoking vocal responses. The thresholds for downward sweeping FM were 10-20 dB lower than those for upward sweeping FM sounds at the same frequencies and about 30 dB lower than those for pure tone burst, except for frequencies immediately around 62 kHz. These data suggest that the vocalization system is coupled with the auditory system through particular types of auditory neurons specialized for CF or FM detection.

As already described, the N_1 threshold curve of P. suapurensis showed a broad notch tuned at about 50 kHz (Figure 25). There was no sharp peak as with P. parnellii. The Q-10 dB value was roughly 1.7. The threshold of the acoustic enhancement of the vocal response in this species of bat was high, being 70-80 dB SPL for upward sweeping FM sounds and 50-60 dB SPL for downward sweeping FM sounds (Figure 25). These data indicate that the vocalization system

of P. suapurensis is not strongly coupled through single neurons sensitive to a particular pure tone, but there is some coupling through neurons particularly sensitive to downward sweeping FM sounds.

N. leporinus often emits CF orientation sounds or sounds consisting of CF and FM components (Suthers, 1965). The threshold curves of N_1-on and of the vocal response were lower for an FM sound sweeping from 60 to 30 kHz than for pure tones or upward sweeping FM sounds over the same frequency band.

Vocalization and middle-ear muscle activity.

The middle-ear muscles (MEM) of mammals synchronously contract with the emission of sounds (Carmel and Starr, 1963; Henson, 1965; Salomon and Starr, 1963). In P. parnellii and P. suapurensis, cochlear microphonic potentials (CM) were recorded with an electrode implanted on the round window or in the internal auditory meatus. When a continuous pure tone, as a testing stimulus, was delivered from a loudspeaker while keeping the position of the bat's head and the loudspeaker constant, the cochlear microphonic was constant in amplitude, provided there were no gross body movements. However, the CM varied greatly when the animal moved. The middle-ear muscles thus apparently contracted together with gross body motion. When the bat vocalized, the CM evoked by an emitted orientation sound was consistently recorded in addition to

the CM evoked by the continuous pure tone. The CM evoked by the emitted sound could be eliminated with an electronic filter to a satisfactory extent. It was then found that the CM of the steady pure tone became smaller in synchrony with the vocalization, as first described by Henson (1965) in Tadarida.

In Figure 26 A, vocalization was initiated by electrical stimulation to the dorsal part of the reticular formation in midbrain of P. parnellii. When electrical stimuli failed to evoke vocalization, the middle-ear muscles did not contract (A1). When they evoked vocalization, however, the middle-ear muscles synchronously contracted with the vocalization (A2-A4). The muscles started to contract one millisecond earlier or nearly the same time as the onset of vocalization. When the vocalization was not strong, the muscle contraction reached a plateau after about 4 msec from its beginning (A2, A3). When the emitted sound was strong, however, the amount of contraction increased and reached a plateau only after 6-10 msec (A4). Since the CM evoked by the FM component of the emitted sound was not completely removed from the pure tone CM by the filter, it was not clear whether the muscle contraction further increased at the time of emission of the FM component. The middle-ear muscles more often appeared to begin to relax prior to the emission of the FM component, rather than to further contract. The middle-ear muscle contraction terminated within one millisecond after

the termination of the first vocalization. When the bat produced more than one sound for each electrical stimulus, the middle-ear muscle contraction lasted for more than 10 msec after the second and after the third sounds (A2-A4). The time-course of the contraction of the middle-ear muscles in P. parnellii was thus quite different from that observed in Tadarida brasiliensis, which emits FM signals for echo-location (Henson, 1965).

In P. suapurensis, the middle-ear muscles started to contract about 4 msec prior to vocalization and relaxed with 3 msec after it. The maximum contraction always occurred in the initial part of the vocalization and the relaxation started to occur during its later part (Figure 26 B). Thus, the time-course of the contraction of the middle-ear muscles of P. suapurensis was more similar to that of the FM bat, T. brasiliensis, than to its relative, P. parnellii. In the above experiments, the middle-ear muscles tonically contracted during the delivery of the continuous pure tone of test signal as an MEM reflex, and attenuate its transmission through the middle ear by 5-10 dB (see below). Consequently, the amount of attenuation of self-stimulation in the natural condition could not be properly studied. The above experiments, however, indicated that the amount of attenuation of vocalizations was more than 15 dB in P. suapurensis.

Since the loudest sounds which normally stimulate the ears are self-vocalized sounds, the animals have an efferent

nerve which reduces the amount of self-stimulation by contracting the middle-ear muscles. These muscles also contract as an acoustic reflex when intense sound is delivered. The shortest latency of this reflex in the bats was 6-7 msec, measured in terms of the attenuation of the cochlear microphonic evoked by a tone pulse (Figure 26 C). This reflex would play only a minor role in attenuating the amount of stimulation provided by orientation sounds emitted by other bats flying nearby and those self- emitted, because the duration of orientation sounds is short compared with the reflex time. In P. parnellii the threshold of the middle-ear muscle reflex was higher than 60 dB SPL, when measured 8 hours after cessation of ether administration (Figure 27). The reflex was not observed for sounds higher than 90 kHz, even when the amplitude was increased upto 100 dB SPL. As shown in Figure 27, the threshold of the reflex was high at 60 kHz but low near 56 and 62 kHz. The threshold curve for the middle-ear muscle reflex between 50 and 70 kHz was more like that of N_1-on than N_1-off (Figure 28).

In P. suapurensis the threshold curve of the middle-ear muscle reflex was broad with no abrupt peak and notch (Figure 29 A), different from P. parnellii. The threshold was about 63 dB SPL for sounds between 23 and 60 kHz. The reflex was observed for sounds upto 120 kHz. This high threshold of the reflex may partly be due to an after-effect of anesthetic and also to the ratio of the signal (CM) to noise. When the

amplitude of a 100 msec tone burst was kept at 90 dB SPL and the amount of the CM attenuation by the middle-ear muscles was measured at its maximum point, the curve in Figure 29 B was obtained. The amount of attenuation was large at 55-60 kHz, the frequency of the strongest harmonic of the CF component in the bat's sounds. Above these frequencies attenuation quickly diminished; the efficiency of the reflex declined. This is expected, because generally the lower the frequency of sound, the greater is the attenuation by the middle-ear muscles (Møller, 1965; Starr, 1969).

Changes in excitability of the auditory system accompanying vocalization.

Since echolocating bats emit sounds and then listen to immediately-occurring echoes, the responses of auditory neurons to sound following another have been studied in anesthetized non-vocalizing bats (Friend, Suga and Suthers, 1966; Grinnell, 1963; Grinnell and Hagiwara, 1972; Suga and Schlegel, 1973). In normal conditions, contraction of the middle-ear muscles (Henson, 1965) and simultaneous modification of the activity of neurons at a higher level in the auditory system (Suga and Schlegel, 1972; and see Chapter III), both occur synchronously with vocalization. The responses of auditory neurons to synthetic echoes following sound emission were studied in unanesthetized, vocalizing bats of the species, P. parnellii.

The frequency of the CF component in the second harmonic

of the orientation sound of P. parnellii is about 62 kHz, and the terminal FM component sweeps down from 62 to 45-50 kHz. When a pair of either pure tones at 62 kHz or FM sounds sweeping down from 63 to 45 kHz with a 0.5 msec duration were used for measurement, the recovery curve of the lateral lemniscal evoked potential (LL) was very fast (Figure 30 A). A 50 % recovery in response amplitude occurred at about 2 msec inter-stimulus delay, regardless of whether the stimulus was CF or FM sound. When a pure tone pulses was repetitively delivered as a test stimulus along with intermittent spontaneous vocalization by the bat, probably for echolocation, the recovery cycle in terms of the response amplitude to the test stimuli was much longer than the above (Figure 30 B). The bat's emitted sounds were obviously different from the artificial stimulus in spectrum and duration. A 50 % recovery of the LL response for a tone of 32 kHz occurred at about 25 msec delay from the beginning of vocalization, or about 9 msec delay from the termination of the vocalization. The N_1 response for a 62 kHz sound, however, was not reduced more than 76 % during vocalization. When the vocalization was evoked with electrical stimulation of the midbrain rather than spontaneously occurring, the recovery of the LL response was much slower than the recovery after the bat's autonomous vocalization (Figure 30 C). This might be due to the emission of a stronger sound and/or some effect of the electrical stimulus. As shown by the curves in Figures 30, B and C, the LL response

to a tone pulse delivered immediately before vocalization was smaller than the control value. Such a reduction of the LL response is very prominent before the vocalization initiated by electrical stimuli, indicating that the electrical stimulation could evoke an unnatural phenomenon in the auditory system.

Off-responses to pure tone pulse.

As previously described, the threshold curve of N_1-on in P. parnellii showed a sharp notch at 62-63 kHz, and the curve of N_1-off showed a sharp notch at 59-61 kHz, a frequency region where N_1-on had a relatively high threshold (Figure 28). Both the on and off responses were most prominent for a tone pulses with an abrupt (0.01 msec) rise-decay time. Apparently, the scatter of sound energy to adjacent frequencies at the sharp onset and cessation of the stimulus simultaneously excited many primary auditory neurons. The interval between N_1-on and N_1-off was identical to the duration of the stimulus, wtithin an error range of 0.1 msec (for example, see Figures, 33 A1 and B1). In lengthening the rise-decay times from 0.01 to 5 msec, the off-response usually disappeared completely or weakened, while the on-response still remained clearly. The off-response was more sensitive to a change in stimulus amplitude than was the on-response. The best frequency to evoke the off-response was 59-60 kHz regardless of the decay time.

Off-responses of LL to pure tone pulses were also found in P. suapurensis. As opposed to P. parnellii, the threshold curve of the off-response for P. suapurensis did not show any sharp peak or notch. Lengthening the decay time shifted the off-response curve toward lower frequencies (Figure 31). Off-responses in P. suapurensis appeared mainly to arise from physical properties of the acoustic stimulus (i.e., of acoustic transients and the ear). The threshold curve for on-response did not shift to lower frequencies with the lengthening of rise time from 0.2 to 1.0 msec (Figure 31).

The properties of the primary auditory neurons in P. parnellii should thus be studied in order to explore the neural basis for the sharp tuning curves of the N_1-on and N_1-off responses. Such a study was not performed here beause that this species was in short supply. For this reason only P. suapurensis was used in order to check whether the N_1-off response was due to the off-discharges of single neurons rebounding from neural inhibition. In the cochlear (auditory) nerve, the responses of 35 single neurons to 50 msec pure tone pulses with an 0.2 msec rise-decay time were studied. Neurons showed tonic on-responses to pure tone stimuli within their excitatory area and post-excitatory inhibition after the stimulus, but off-discharges were not evident. For pure tone stimuli at frequencies just outside the excitatory area of a given single neuron, about half of the neurons showed a clear off-response in addition to a phasic on-response.

Background discharges, if any, were not affected at times between the on- and off-discharges. As far as this observation is concerned in P. suapurensis, though the sample number is small (35), the off-response appears not to be a rebound from neural inhibition but rather the response to mechanical transients of the stimulus and/or of the ear.

When a 10 msec tone pulse with a 0.2 msec rise-decay time and with frequency sweeping 80 down to 40 kHz was delivered to P. parnellii, the on-response was prominent but the off-response was indistinct. The off-response to the 59-61 kHz component was not observed in addition to the N_1-on. In the natural orientation sound of P. parnellii, a CF component at about 62 kHz is always followed by an FM component sweeping from 62 down to 45-50 kHz. When the animal emitted such a sound or when a similar artificial sound was delivered from a loudspeaker, the N_1 and LL responses appeared at the onset and also at the termination of the stimulus. To what extent did the response to the terminal portion of the sound consist of the off-response to the CF component and to what extent was it the response to the FM component?

P. parnellii, in flight, adjusts the frequency of the emitted CF component in an orientation sound in order to receive Doppler-shifted echoes from moving target relative to the animal at a preferred frequency, about 62 kHz; the bat compensates for echo Doppler shifts by lowering its transmitted frequency. For example, the frequency of a CF component could

be 58 kHz in an emitted sound and 62 kHz in an echo (Schnitzler, 1970). Echoes almost always return soon enough to overlap the emitted sound, due to the length of the siganl (8-30 msec). Accordingly, when a 15 msec, 58 kHz tone pulse was delivered along with a 15 msec, 62 kHz tone pulse, timed so that the onset of the 62 kHz sound was delayed from the onset of the 58 kHz sound, the LL-off response to the 58 kHz sound was greatly attenuated (Figures 32, A1 and A2). An FM sound of 3 msec was attached to the end of the 58 kHz sound without an amplitude change occurring at the boundary. The response to this CF-FM tone pulse is shown in Figure 32 B1. The response to the terminal portion of the CF-FM sound was smaller than the off-response to the 58 kHz tone pulse. This response at the terminal portion was not appreciably reduced by simultaneous delivery of a 62 kHz sound (Figure 32 B2). These masking experiments indicate that the response to the terminal portion of the CF-FM sound primarily consisted of the response to the FM component and not the off-response to the CF sound. In Figure 32, one should also notice that the LL-off response to the 62 kHz sound was greatly reduced by 58 kHz sound and by the CF-FM sound.

The same masking experiments were repeated with recording of both N_1 and LL response in order to clarify whether the masking of off-responses by 62 kHz sound occurred at the periphery and/or in the midbrain; neural rebound. One recording electrode was placed near the auditory nerve in order to

record N_1, and the other was inserted into the nucleus of the lateral lemniscus in order to record the LL response. These two electrodes were connected to the differential inputs of a single amplifier. As shown in Figure 33 A, both the N_1-off and the LL-off responses to a 58 kHz tone pulse were greatly attenuated by the 62 kHz tone. Both N_1 and LL responses to the terminal portion of the CF-FM sound were not appreciably reduced by the 62 kHz tone (Figure 33 B). Thus, the observed masking phenomenon was due to peripheral events. The N_1 and LL responses to the terminal portion of the CF-FM signal were mainly the response to the FM component.

Discussion

Vocal responses to acoustic stimuli in electrically activated bats.

Vocalization occurred when the electrical stimuli were applied to the central gray matter and/or the midbrain reticular formation (Chapter I). Vocalization also occurs, however, in association with emotional distress such as resulted by mechanical stimulation (Chapter III). Consequently, there is a problem as to whether the electrical stimuli directly excite a part of the vocalization system or instead evoke an emotional change, which in turn initiates vocalization. In the experiments described here and in Chapter I, (1) the shortest latency of the vocal response was 25 msec, (2) the vocal response occurred synchronously with each electrical stimulus and immediately stopped when the stimulus stopped, (3) no gross body movements were associated with this vocalization when the electrodes were placed at low threshold area, and (4) the properties of electrically-evoked sounds were similar to those of the species-specific orientation sounds. Therefore it is reasonable to consider that the electrical stimulation directly excited a part of the vocalization system. The vocalization was appropriately different from species to species; the neural circuitry in the vocalization system in the midbrain or the larynx apparently differs systematically from species to species.

The change in the electrically-evoked vocalization in the presence of acoustic stimuli indicates that the bat may have attempted to echolocate with the electrically evoked sounds. When the acoustic stimuli were delivered, echoes from objects in front of the bat such as a loudspeaker and a microphone which should not be covered with cotton might be masked. The animal could increase the amplitude and perhaps the number of out-going sonar siganl in order to detect the masked echoes. In this situation, the vocal response to a masking signal should theoretically be related to the spectrum of the masking sound, to the information-bearing elements in the emitted CF-FM signals, and also to properties of the auditory neurons used for echo analysis.

The vocal response to acoustic stimuli that the electrically activated vocalization can still be controlled by an acoustic information ascending the afferent auditory system also suggests that the electrically evoked orientation sound is not a result of simple motor nerve stimulation but a result of the activation of the systematic neural circuits for the emission of orientation sound.

The observed thresholds for vocal responses to acoustic stimuli might be interpreted in the following way: in the CF component of the orientation sound of P. parnellii, the 62 kHz second harmonic is the principal information-bearing element, and the first and third harmonics in the CF component are not so important, because the threshold of the vocal

response was low at 62 kHz only. Theoretically it is conceivable that the threshold curve of the vocal response to acoustic stimuli has the same shape as that of the power spectrum of the orientation sound if the sonar of the bat is ideal. Thresholds for FM sounds sweeping down from high to low frequencies were lower than thresholds for upward sweeping FM sounds and for pure tones, except for tones at about 62 kHz. Thus, the FM components in the first, second and third harmonics of the orientation sound all appear to be significant to the bat.

The very sharp tuning curve for the vocal response at about 62 kHz and the low threshold for the downward sweeping FM sounds probably indicates that acoustic control of the vocal response was mediated by particular types of auditory neurons; one type of auditory neuron with a very narrow tuning curve at about 62 kHz and the other type being more sensitive to FM sounds than to pure tones (FM-specialized neurons). In bats of genus, Myotis, which emits only FM sounds for echolocation, the narrowest tuning curve obtained in the single neuron in the inferior colliculus showed a Q-10 dB value of 120, a low frequency slope of about 2000 dB/octave and a high frequency slope of about 1300 dB/octave (Suga, 1964). P. parnellii apparently can utilize the activity of such a neuron sharply tuned at a certain frequency and can ignore the activity of all other neurons tuned at other frequencies. In anesthetized bats of the genus Myotis, FM-specialized neurons

were found to be about 3 % of the total in the inferior colliculus and not more than 14 % of the total in the auditory cortex (Suga, 1965a, 1965b). In P. parnellii, the responses of single neurons to acoustic stimuli have not yet been studied, but there is little doubt that the bat has FM-specialized neurons, because the animal emits FM siganls for echolocation and because such neurons have been found not only in bats, but also in cats (Whitfield and Evans, 1965; Watanabe and Ohgushi, 1968). The low threshold vocal responses to FM sounds are probably mediated by FM-specialized neurons.

In P. suapurensis, the threshold of the vocal response was significantly higher for pure tone than for FM sounds, regardless of frequency. The threshold curve was not sharply tuned at a frequency. The frequency of the lowest threshold of the vocal response to acoustic stimuli was at about 60 kHz, while that of the N_1 was around 50 kHz. The relative significance or function to P. suapurensis of the CF component of the sounds for echolocation may be different than for P. parnellii. On the other hand, the FM components of the orientation sounds are probably equally essential, consisting the low thresholds for vocal response to FM signals in both species. N. leporinus often emits an orientation sound consisting of CF components. Its vocal responses were similar to those of P. suapurensis. Vocal responses to acoustic stimuli in these species of bats also indicate that vocalization system is coupled with the auditory system through particular types

of auditory neurons. The coupling may depend upon the information bearing elements in the signals with which the animals are most concerned at a given moment.

Vocalization and middle-ear muscle (MEM) activity.

As shown in Figure 26 C, middle-ear muscles (MEM) contract as a reflex to an intense sound, with a latency of 6-7 msec. This mechanical apparatus can provide about 20 dB attenuation for sound transmission to inner ear. The threshold of the MEM reflex is about 70 dB SPL in the range of frequencies of the orientation sound (Figures 27 and 29). When the bat emits the orientation sound, the MEMs contract synchronously with the vocalization. The contraction occurs a few milliseconds prior to sound emission. The self-vocalized sound stimulates the ear at about 90-100 dB SPL. Even after the attenuation by the synchronous contraction of the MEMs, thus the amount of the self-stimulation at the input of the inner ear is at least 70-80 dB SPL equivalently. Thus the amount of self-stimulation is large enough to cause the MEM reflex, but there is no reflex after the synchronous contraction of the MEM with the vocalization as illustrated in Figs. 26 B1 and B2. Therefore when the animal emits the orientation sound, the reflex arc is opened or the ascending auditory system was equipped with an attenuation mechanism to self-stimulation (neural attenuation) by the amount of more than 10 dB. The latter case is not the same mechanism of neural attenuation described

in Chapter III, because the acoustic reflex arc involved is ascending through the medial trapezoid body to the brain stem motor nuclei (Figure 2 in Introduction), so that the information through the nucleus of the lateral lemniscus may not be primary to the reflex.

Threshold curves on P. parnellii.

In some of the bats producing CF-FM signals for echo-location, it has been found that their auditory system is sharply tuned at a frequency of a predominant CF component in the signal (Ajraptianz and Vasilyev, 1971; Grinnell, 1970; Grinnell and Hagiwara, 1972; Neuweiler, 1970; Pollak, et al., 1972). Pollak et al. (1972) found that the CM threshold curve of unanesthetized P. parnellii was so sharply tuned at 62 kHz that a Q-10 dB value was 310. In the present data obtained from unanesthetized P. parnellii, 3-15 hours after stopping ether administration, the CM threshold curve was similar to theirs. The presence of a sharply tuned notch at 61 kHz was repeatedly confirmed. The Q-10 dB value was, however, about 50. For a 61 kHz, 4 msec tone pulse with a 0.2 msec rise-decay time, the envelope of the CM was quite different from the stimulus envelope; showing a slow increase in amplitude at the onset and a slow decrease after the cessation. Such a peculiar CM response was not observed for 50 and 70 kHz tone pulse. A similar CM response to the 61 kHz tone was also observed by Pollak and his co-workers (personal communi-

cation), this indicates that the ears of P. parnellii has some mechanical resonating element(s) sharply tuned at 61 kHz.

In P. parnellii, both the threshold curves of N_1-on and N_1-off were different from the CM threshold curve. N_1-on was sharply tuned at 63-64 kHz and broadly tuned at 30-40 kHz, but it was insensitive to 59-60 kHz sound. On the other hand, N_1-off was sharply tuned at 59-61 kHz. Some of the differences in the threshold curves may explained by the position of the recording electrode, but the differences between N_1-on and N_1-off threshold curves are not so explained, because they consistently existed regardless of the position of the recording electrode. In Rhinolophus, N_1-on is sharply tuned at 83.3 kHz, but N_1-off does so at 81.5 kHz, to which N_1-on is insensitive (Neuweiler, 1970; Neuweiler et al., 1971). Comparative studies on several species of neotropical bats indicated that on- and off-responses are tuned at the same frequency in Saccopteryx (Grinnell, 1970), but off-responses are tuned frequency higher than those for which on-responses are tuned in Hipposideros and Aselliscus (Grinnell and Hagiwara, 1972; Grinnell, 1973). The off-responses are not related to the insensitivity of on-responses in these bats.

Off-responses at the periphery.

Off-responses to tone pulse have been recorded from the peripheral auditory system of certain species of bats

(Grinnell, 1970; Grinnell and Hagiwara, 1972; Neuweiler, 1970). In order to discuss the functional role of the off-responses, studies on the properties of the primary auditory neurons were attempted, but preliminary data was only obtained because of the number of bats available. It may be nontheless important to discuss the implications of the data on single unit activity and summated evoked potentials and to speculate the origin of off-response.

Off-discharges as rebounds from inhibition caused by tonal stimuli have been found in the cochlear nucleus of Myotis lucifugus (Suga, 1964). Off-discharges have often been found at higher auditory nuclei in various types of animals (e.g. in bat P. parnellii, Grinnell, 1970; in monkeys, Katsuki et al., 1962; in cats, Whitfield and Evans, 1965). Off-discharges observed in the brain are excluded in the following discussion, because these are easily explained by a rebound from neural inhibition. In bats (Frishkopf, 1964), cats (Sachs and Kiang, 1968), and monkeys (Nomoto et al., 1964), the primary auditory neurons commonly show "two-tone suppression or inhibition" which is still observed after severing the efferent nerve fibers. Two-tone suppression appears to be due to non-linearity in the cochlea (Engebretzen and Eldredge, 1968; Pfeiffer, 1970). At the termination of two-tone suppression, primary auditory neurons show off-discharges as a rebound from the two-tone suppression (Sachs and Kiang, 1968; Arthur et al., 1971). The off-discharges observed in

the primary auditory neurons of P. suapurensis were not associated with such suppression. According to the present knowledge, it is unlikely that sensory hair cells located in a particular portion of the basilar membrane release inhibitory substances that cause hyperpolarization of afferent fibers during acoustic stimulus and then depolarization as a rebound from the hyperpolarization at the cessation of the stimullus. The off-responses observed in the present experiments are clearly not related to the activity of the olivo-cochlear bundle (OCB), because the latency of the N_1-off response was the same as the latency of the N_1-on response within 0.1 msec error. Furthermore, the shape of the threshold curves on N_1-on and N_1-off can not be explained by this. The second possibility is that the off-response is the response to an acoustic transient at the cessation of the stimulus. The number of the primary auditory neurons studies here was small, but these data suggest that the N_1-off response was neither a rebound from neural inhibition nor from two-tone inhibition. In P. suapurensis, N_1-off responses appeared to be due to a mechanical transient in a receptor site and/or a stimulus.

Summary

1. The mustache bat (Pteronotus parnellii), the naked-backed bat (P. suapurensis) and the fish-catching bat (Noctilio leporinus) all from Central America, produce orientation sound containing a constant-frequency (CF) component followed by a frequency-modulated (FM) component. The functions of the CF component may differ among these species. Several different aspects of echolocation system in these species of bats were neurophysiologically studied.

2. Electrical stimulation of the midbrain reticular formation and/or the central gray matter elicited vocalizations which were indiscriminable from the orientation sounds used for echolocation in each species. When the tips of the stimulating electrodes in these areas were close to the boundary between the superior and inferior colliculi, only minimal body movements were associated with the vocalization.

3. The electrically elicited vocalization was enhanced by the delivery of acoustic stimuli. This behavioral vocal response to acoustic stimuli was related to particular features of orientation sound of each species. In P. parnellii, the vocal response to acoustic stimuli was sharply tuned at the frequency of the CF component in the second harmonic of the orientation sound and also to downward sweeping FM sounds. In P. suapurensis and N. leporinus, the vocal responses were very prominent only to downward sweeping FM sounds, but

those were very poor to pure tone pulses and were not sharply tuned. The differences in the vocal responses indicate that the CF component is more essential to echolocation in P. parnellii than to that in P. suapurensis and N. leporinus.

4. In P. parnellii, N_1-on (auditory nerve response at the onset of the stimulus) was very sharply tuned at about 63 kHz and broadly tuned at 30-64 kHz, while N_1-off (auditory nerve response at the cessation of the stimulus) was sharply tuned at about 60 kHz, a point at which N_1-on was insensitive. The threshold curve of P. suapurensis was quite different from that of P. parnellii. N_1-on was broadly tuned at a 40-60 kHz. N_1-off greatly varied with a decay time of the stimulus. In N. leporinus, N_1-on also showed broad tuning. The auditory system of P. parnellii, a bat which produces a long CF component, showed specialization for the reception of certain CF sounds.

5. Preliminary studies on primary auditory neurons performed with P. suapurensis indicate that N_1-off is not due to a rebound from neural inhibition but to some mechanical transient.

6. Masking experiments with P. parnellii indicate that N_1-off and LL-off (lateral lemniscal response at the cessation of a tone pulse) for a CF-FM sound similar to its orientation sound mainly consisted of the responses to the FM component and not the off-response to the CF component.

7. The latency of the middle-ear muscle (MEM) reflex

in terms of the attenuation of the cochlear microphonic potential (CM) was 6 msec in the shortest case in P. parnellii and P. suapurensis. The middle-ear muscles always started to contract a few milliseconds prior to vocalization. The contraction lasted during vocalization and attenuated the amount of self-stimulation by more than 10 dB. The MEM reflex arc is opened when the animal emits the orientation sounds. The primary function of the middle-ear muscles in these bats is, probably to attenuate the amount of self-stimulation, because acoustic signals used by the bats for echolocation are usually short.

Figure 21.

Sonagrams of electrically elicited sounds from P. parnellii (A), P. suapurensis (B) and N. leporinus (C) and (D). The ordinate and abscissa represent frequency in kilohertz and time in 10 msec/division. The envelopes of the sounds are shown below the sonagrams.

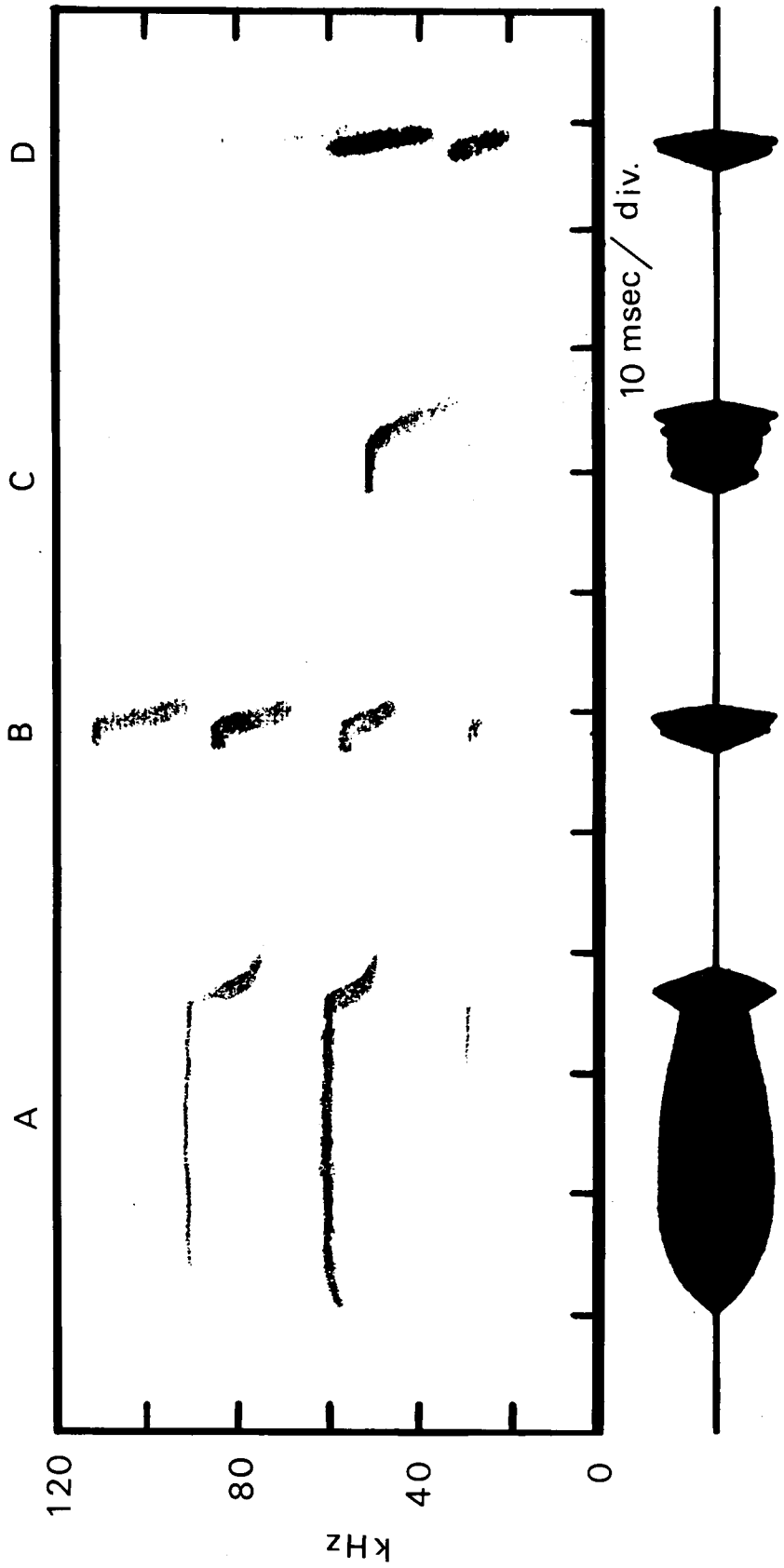


FIGURE 21.

Figure 22.

Change in electrically elicited sounds produced by acoustic stimuli delivered at a rate of 250 per second. A: Electrically elicited sounds from P. parnellii increased in amplitude (ordinate) when the amplitude of 61 kHz tone burst (abscissa) was raised. The duration of the tone pulse was 2.0 msec. The arrow indicates the amplitude of the electrically elicited sounds without the acoustic stimuli. B: Electrically elicited sounds from N. leporinus (a) increased in both amplitude and duration when 40 kHz tone burst was delivered at 84 dB SPL (b). The output of the zero-crossing period meter is shown above each sound with a calibration on the left for the rough estimation of the sounds CF or FM.

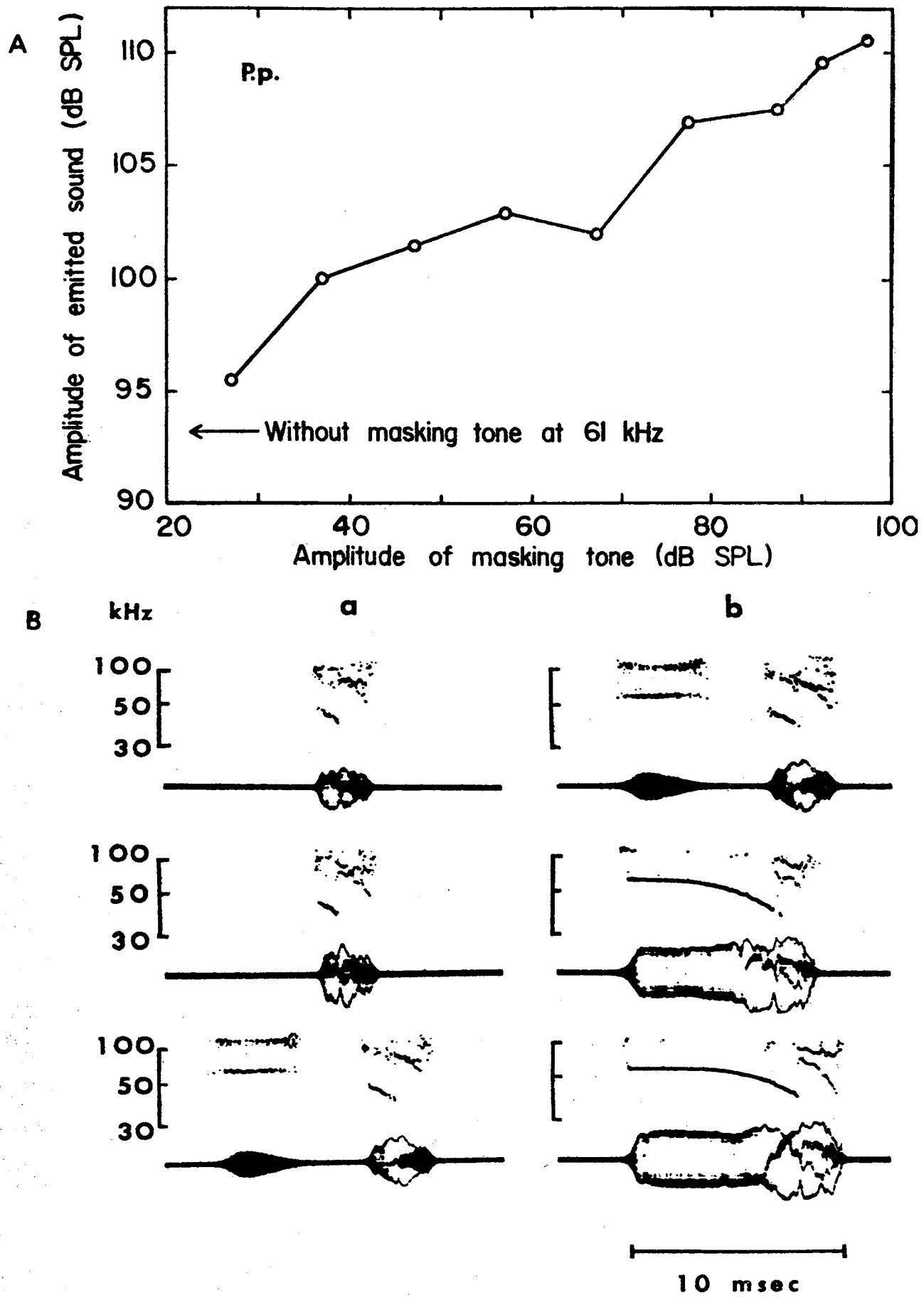

A
P.p.
Amplitude of emitted sound (dB SPL)
110
105
100
95
90
20
40
60
80
100
Amplitude of masking tone (dB SPL)
← Without masking tone at 61 kHz
B
kHz
a
b
100
50
30
100
50
30
100
50
30
10 msec

FIGURE 22.

Figure 23.

Threshold curves of vocal response of P. parnellii to acoustic stimuli. V.R.: a threshold curve of the vocal response measured with 2 msec pure tone burst delivered at a rate of 250 pulses per second. Threshold curves for N_1-on and N_1-off responses are also presented for a comparison. The uppermost dotted line in each graph hereafter indicates the frequency-response curve of the loudspeaker. The ordinates represent stimulus amplitudes at threshold in dB SPL. The abscissae represent the frequencies of the acoustic stimuli in kilohertz with a logarithmic scale. At the right a part of the threshold curves is shown on linear expanded frequency axis.

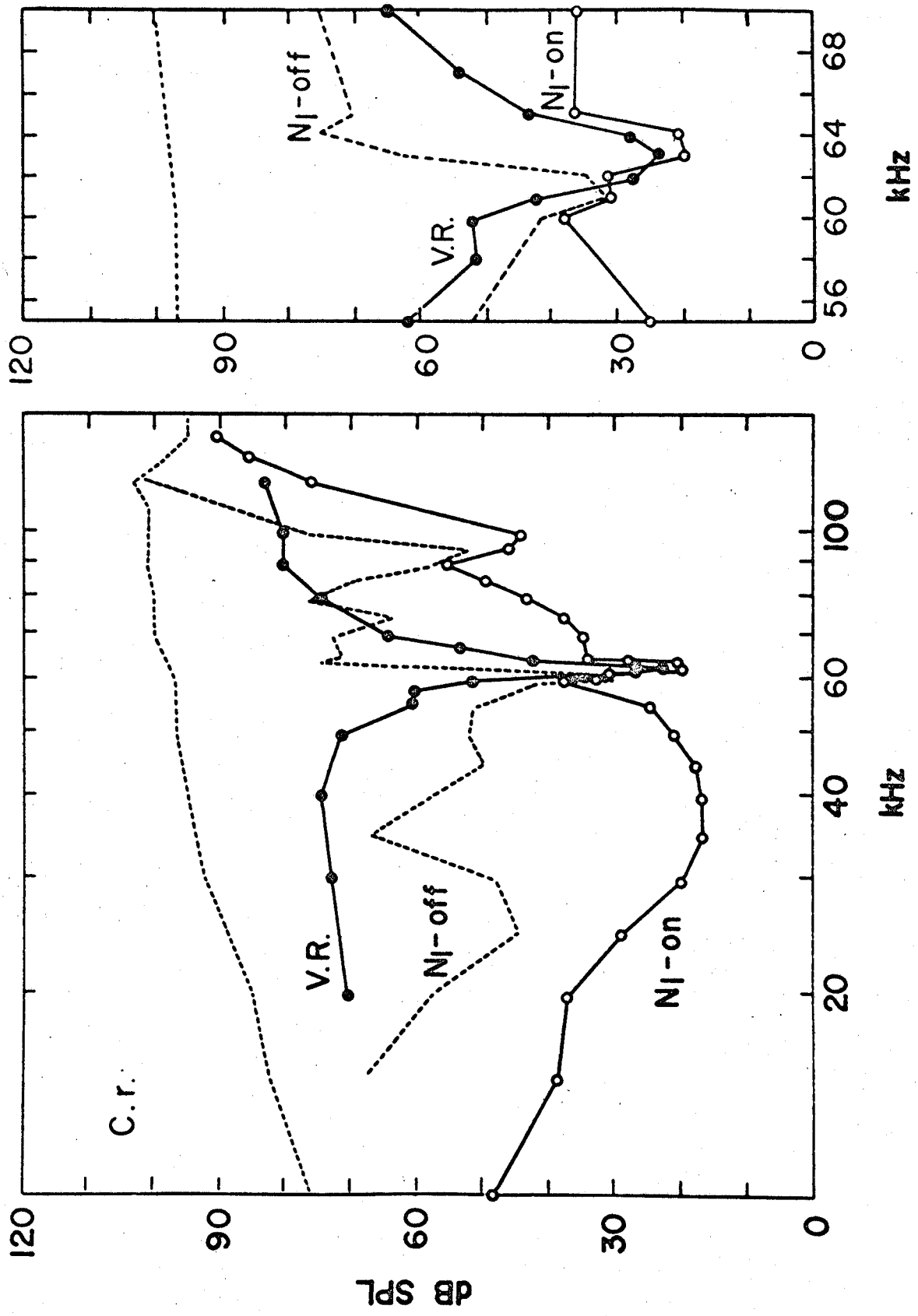

dB SPL
120
90
60
30
0
C.r.
V.R.
NI-off
NI-on
20
40
60
100
kHz
56
60
64
68
kHz

FIGURE 23.

Figure 24.

Threshold of vocal responses of P. parnellii to acoustic stimuli. TB: a threshold curve measured with 2 msec pure tone burst delivered at a rate of 250 pulses per second. CP: a threshold curve measured with a continuous pure tone. Each arrow indicates the direction of frequency sweep in FM tone pulses by its head, the range of frequency sweep by its length and the threshold of the vocal response to the FM tone burst by its vertical position. Lower dashed-line curve represents N_1-on threshold. Symbols are the same as in Figure 23, see also the explanations for Figure 23.

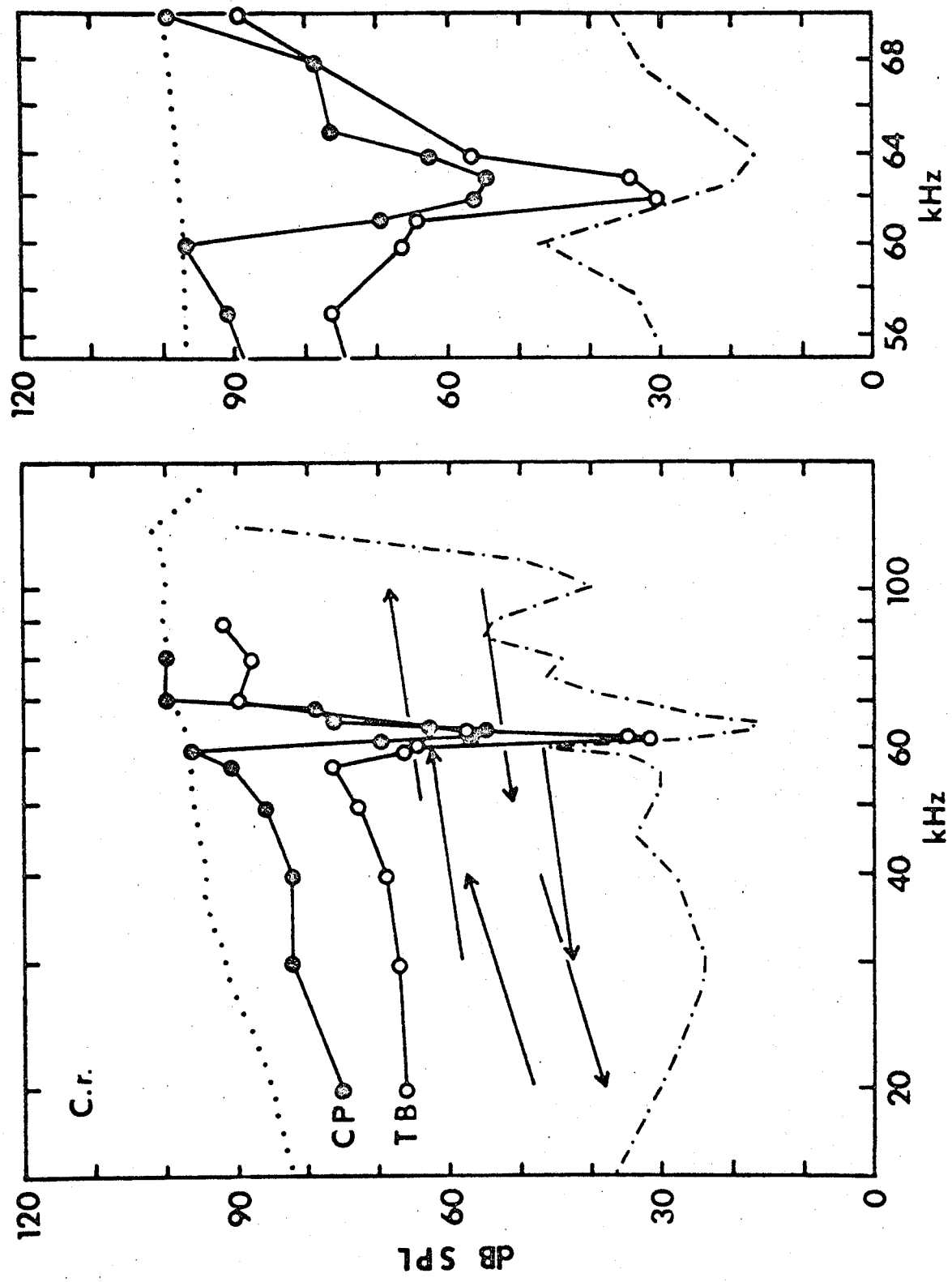

120
90
60
30
0
56
60
64
68
kHz
120
90
60
30
0
20
40
60
100
kHz
dB SPL
C.r.
CP
TB

FIGURE 24.

Figure 25.

Threshold curves for N_1-on (filled circles) and the vocal response to pure tone burst (open circles) in the electrically activated P. suapurensis. The arrows represent thresholds of the vocal responses to FM tone burst. see also Figure 23 legends.

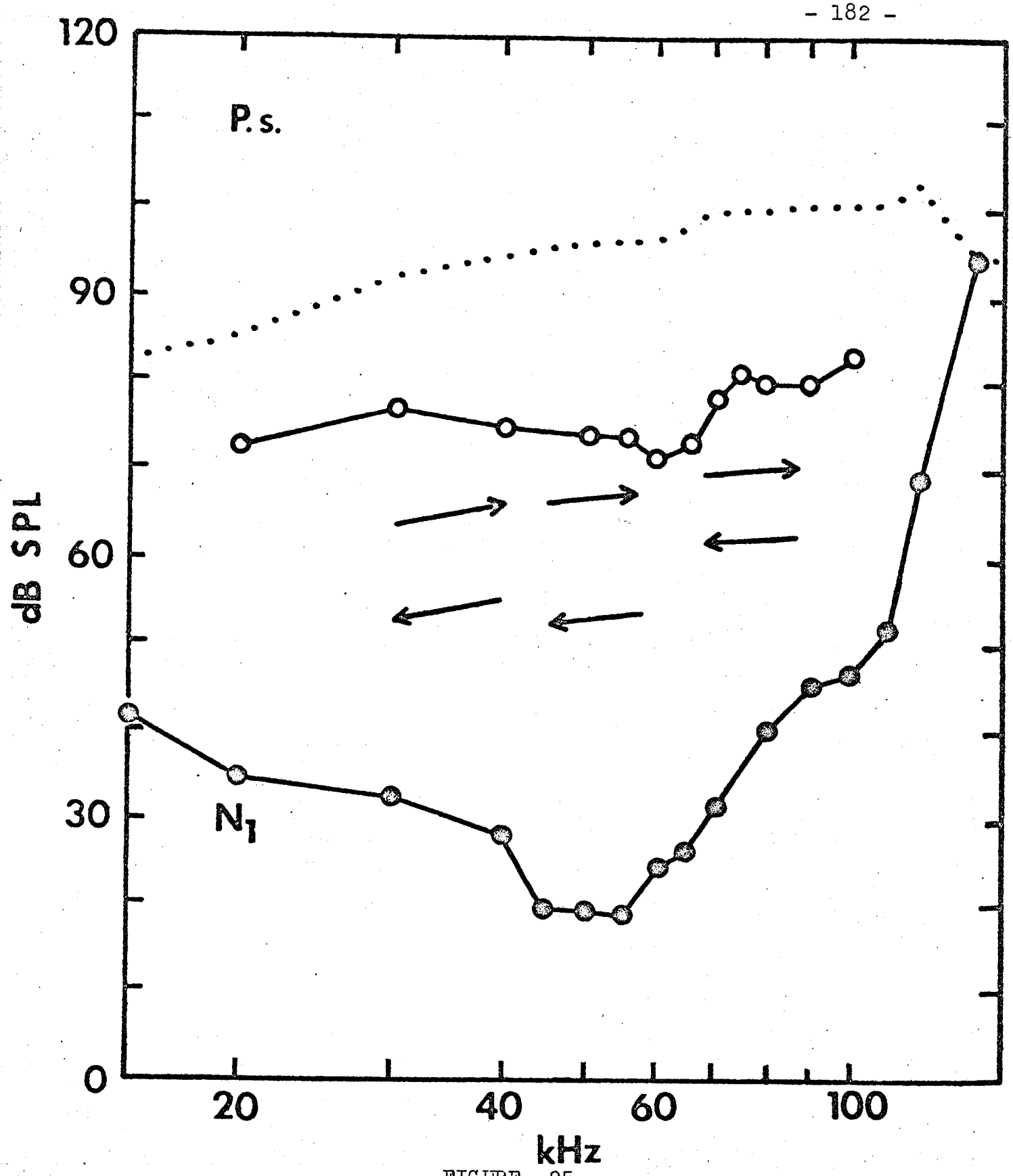


FIGURE 25.

Figure 26.

Attenuation of cochlear microphonic potentials (CM) by the middle-ear muscle contraction. A: Vocalization (lower trace) of P. parnellii elicited by electrical stimuli. The CM for a continuous tone (upper trace) is attenuated by the muscles whenever the bat vocalizes (2-4). The continuous tone is 20 kHz and 80 dB SPL. The time scale is 20 msec and the vertical white lines to the left within each CM are stimulus artifacts. B: The CM of P. suapurensis also is attenuated by the muscles whenever the animal vocalizes. The continuous tone is 26 kHz and 89 dB SPL. The time scale is 10 msec. C: the CM of P. parnellii evoked by a tone pulse is attenuated by the middle-ear muscle reflex. The tone pulse is 35 kHz and 93 dB SPL in 1, and 63 kHz and 96 dB SPL in 2. The time scale is 10 msec.

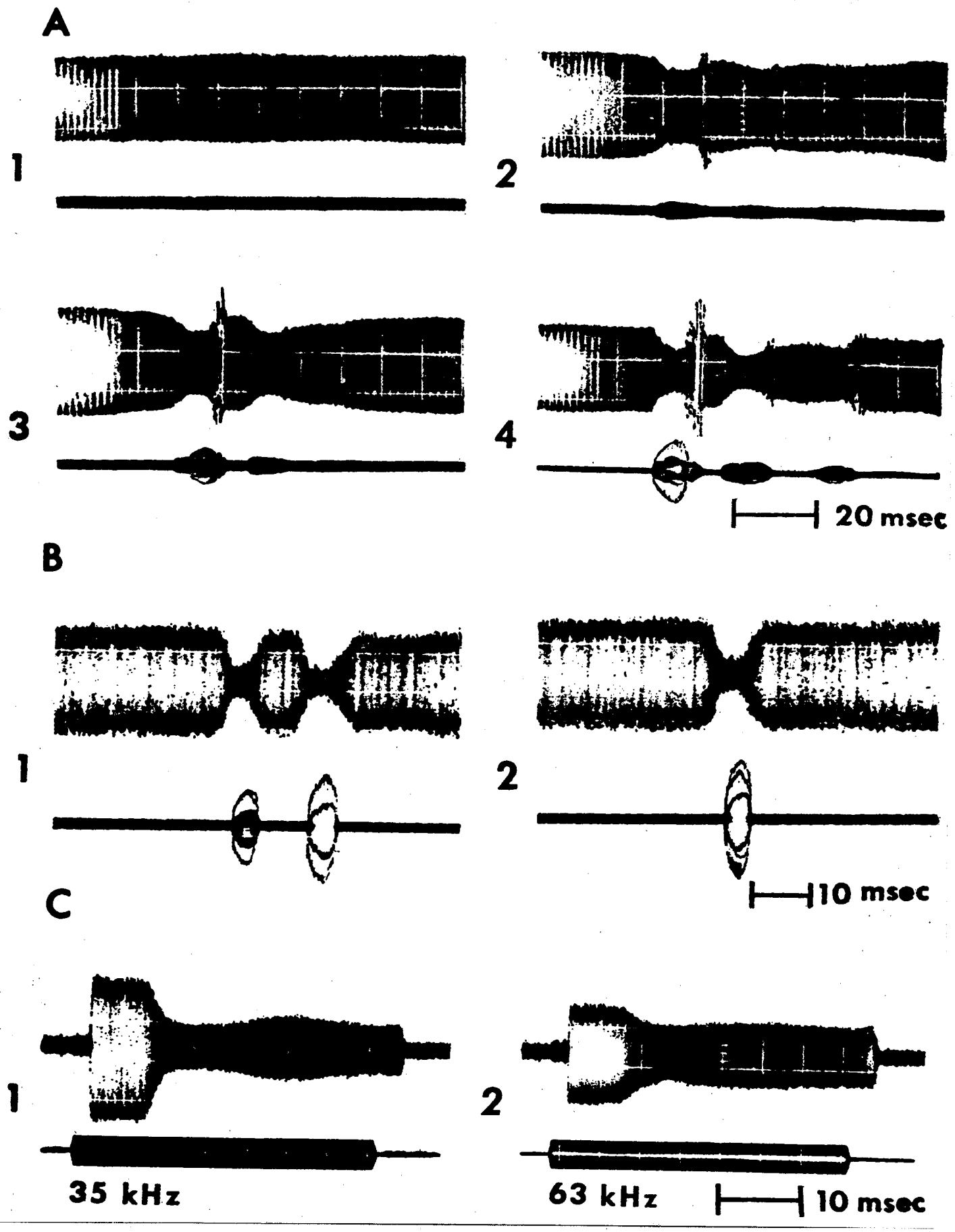


FIGURE 26.

Figure 27.

Threshold curves of the cochlear microphonic (CM), the middle-ear muscle reflex (MEM) in terms of attenuation of the CM, N_1-on and N_1-off obtained from P. parnellii. The sound used had a duration of 100 msec and a rise-decay time of 0.5 msec. See also Figure 23 explanations.

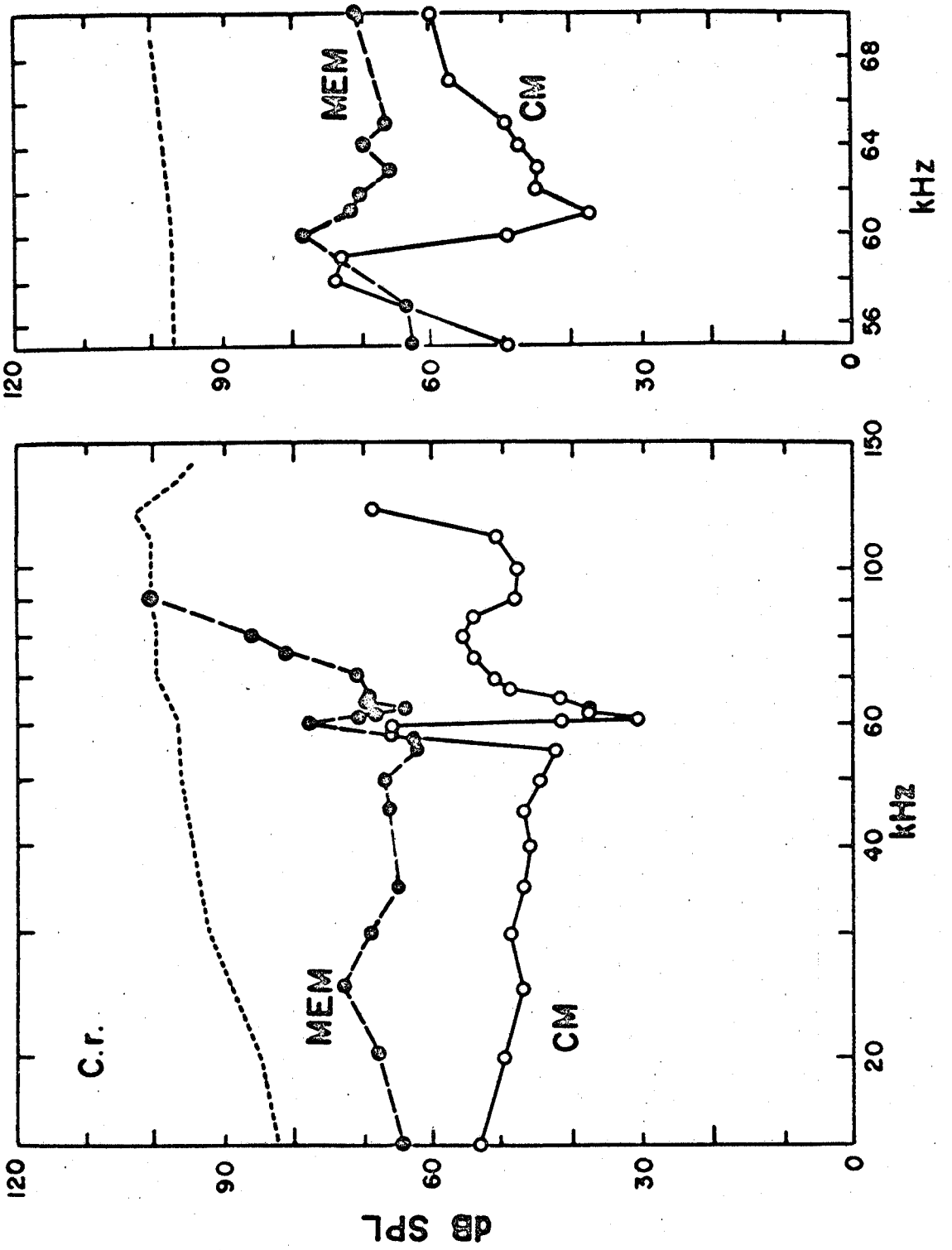


FIGURE 27.

Figure 28.

Threshold curves of N_1-on and N_1-off obtained from P. parnellii. The sound used had a duration of 100 msec with a 0.5 msec rise-decay time. Also see Figure 23 legends.

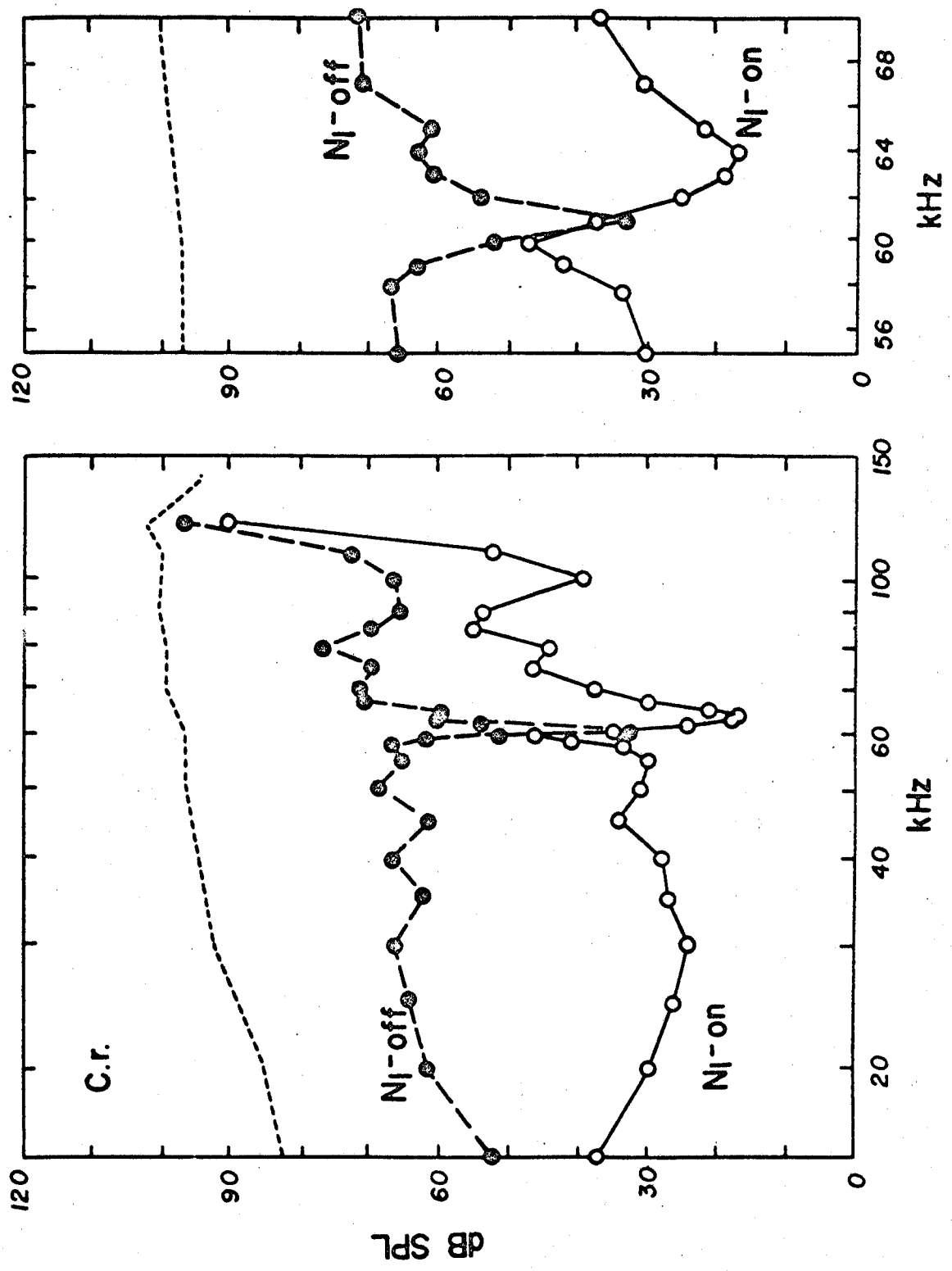

dB SPL
120
90
60
30
0
C.r.
Ni-off
Ni-on
20
40
60
100
150
kHz
Ni-off
Ni-on
56
60
64
68
kHz

FIGURE 28.

Figure 29.

Threshold curve of middle-ear muscle reflex in terms of attenuation of CM (A) and the amount of attenuation of CM by the middle-ear muscles (B), obtained from P. suapurensis. The sound used had a duration of 100 msec and 0.5 msec rise-decay time. The ordinate indicates the threshold in dB SPL in (A) and the amount of attenuation in dB in (B). The abscissae show frequency in kilohertz. In B, the stimulus amplitude was kept at 90 dB SPL.

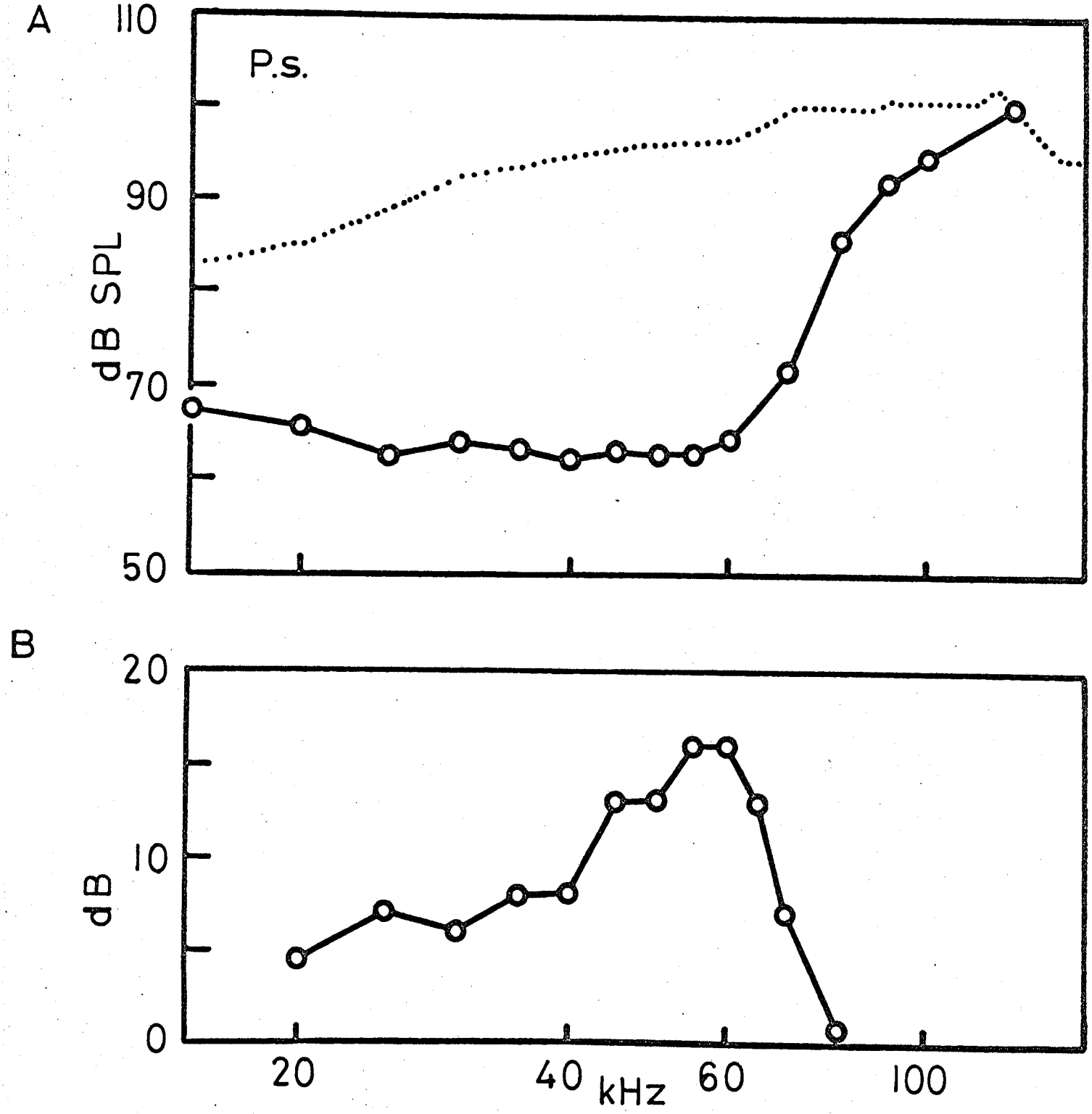


FIGURE 29.

Figure 30.

Recovery curves of N_1 (auditory nerve response) and LL (lateral lemniscal response) of P. parnellii. A: Recovery curves measured with a pair of identical 80 dB SPL pulses. The first sound serves as a conditioning signal. The sound was either a 62 kHz pure tone pulse or an FM tone pulse sweeping from 63 to 45 kHz. B: Recovery curves of N_1 and LL during and after voluntary vocalization. N_1 was evoked by a 62 kHz, 97 dB SPL tone burst, and LL was evoked by a 32 kHz, 93 dB SPL tone burst. C: Recovery curves of LL during and after vocalization elicited by electrical stimulation. LL was evoked by either a 40 kHz, 87 dB SPL or 62 kHz, 87 dB SPL tone burst. The duration and rise-decay time of the tone pulse were 0.5 and 0.2 msec, respectively. The ordinates represent the percent amplitudes of N_1 and LL. The 100 % point was the amplitude of N_1 or LL which was evoked by the test tone alone without the conditioning tone or vocalization. The abscissae represent the time in milliseconds after or before the onset of the conditioning tone or the vocalization.

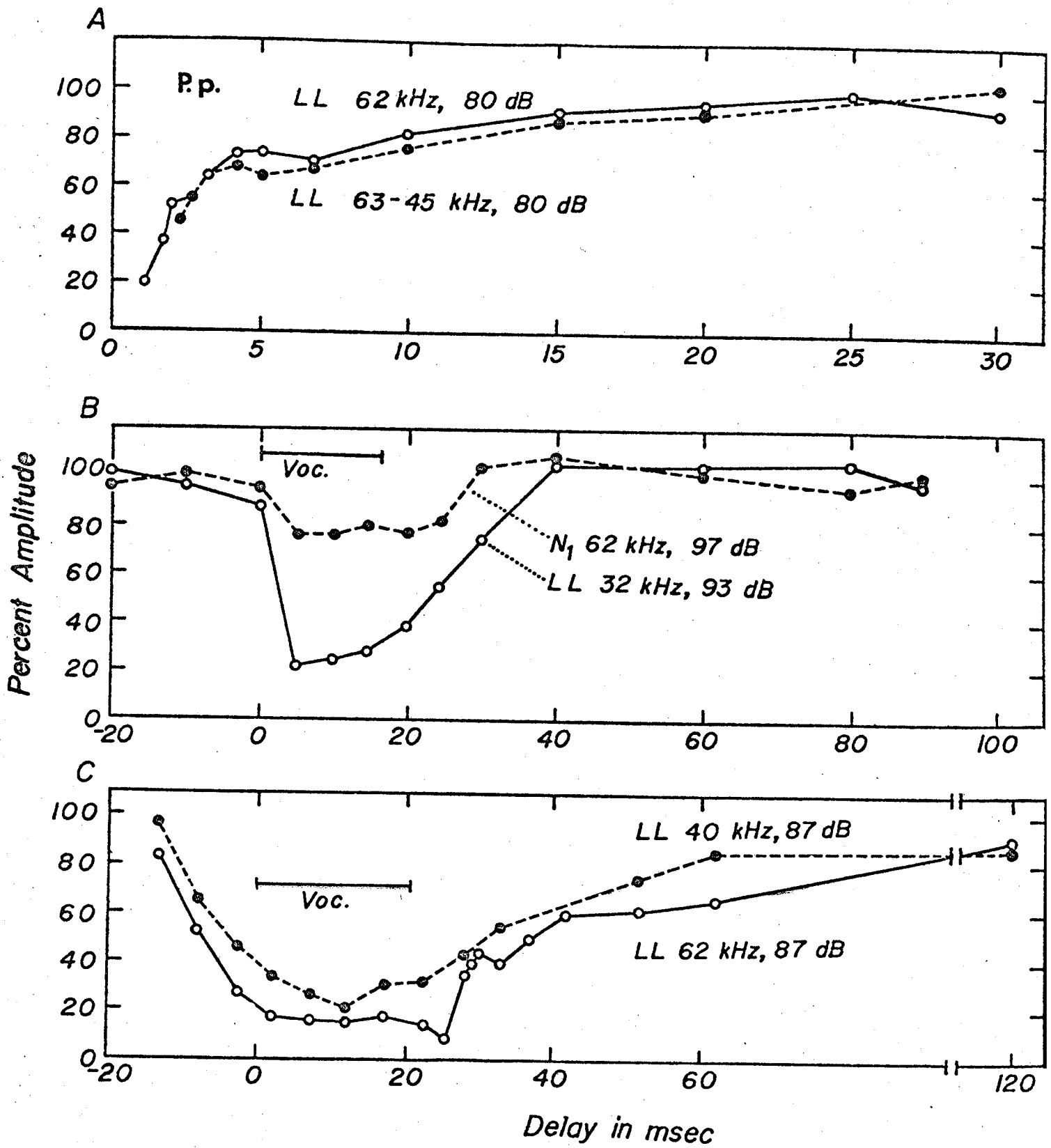

A
P.p.
LL 62 kHz, 80 dB
LL 63-45 kHz, 80 dB
B
Voc.
N1 62 kHz, 97 dB
LL 32 kHz, 93 dB
C
LL 40 kHz, 87 dB
Voc.
LL 62 kHz, 87 dB
Percent Amplitude
Delay in msec

FIGURE 30.

Figure 31.

Threshold curves of LL-on and LL-off in P. suapurensis measured with tone pulses with either a 0.2, 0.5 or 1.0 msec rise-decay time. The ordinate and abscissa represent the amplitude of the tone pulse at a threshold and the frequency of the tone respectively.

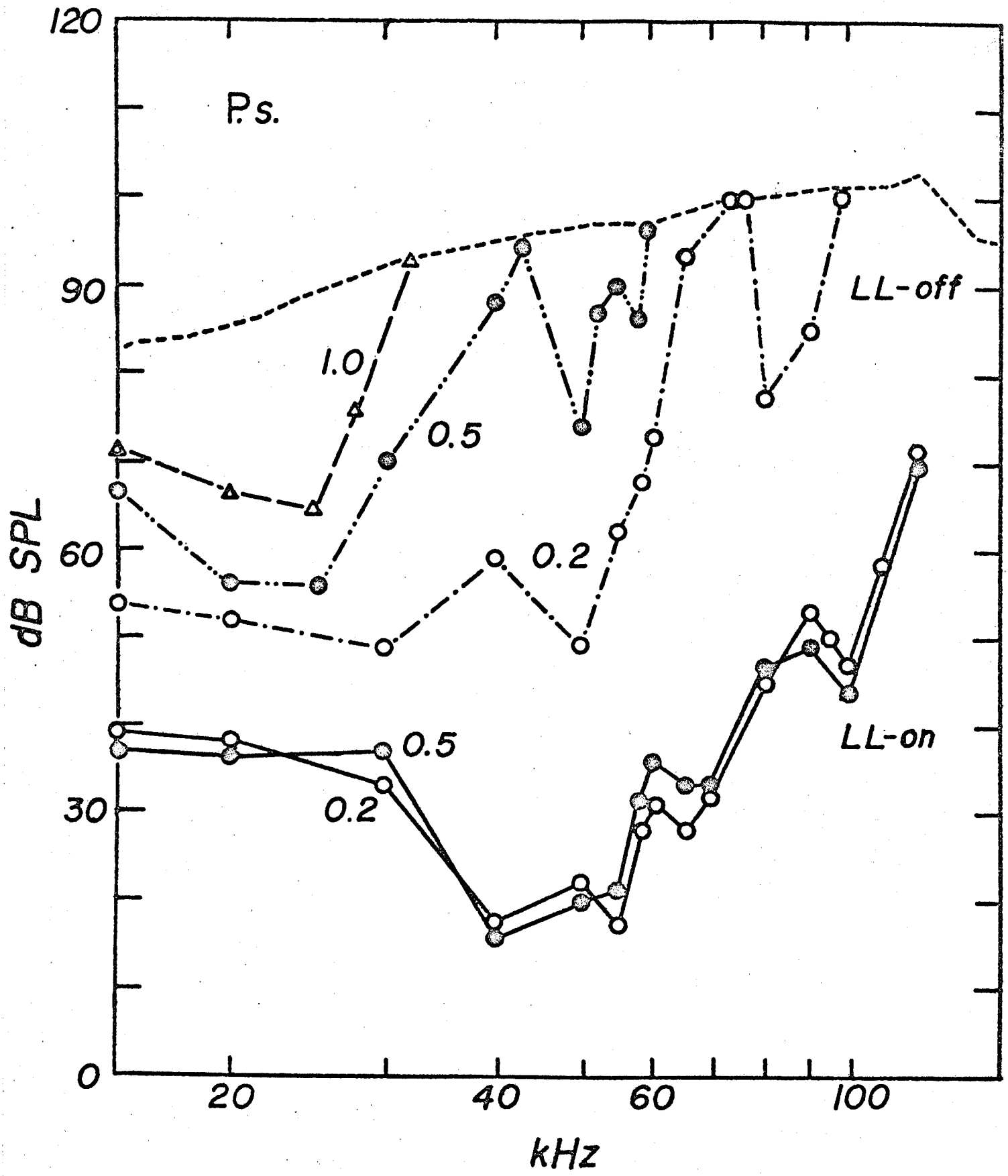


FIGURE 31.

Figure 32.

The off-response of lateral lemniscal evoked potential to a 58 kHz tone pulse is suppressed by a 62 kHz tone pulse (A), while the response to the terminal FM part of a 58 kHz CF with a 58-45 kHz FM sound is not (B). Each evoked potential is the average of 32 responses to the same stimulus delivered 32 times. The horizontal bars represent tone pulses, the frequencies of which are indicated by the figures below the bars. The short horizontal bar is the time scale of 5 msec.

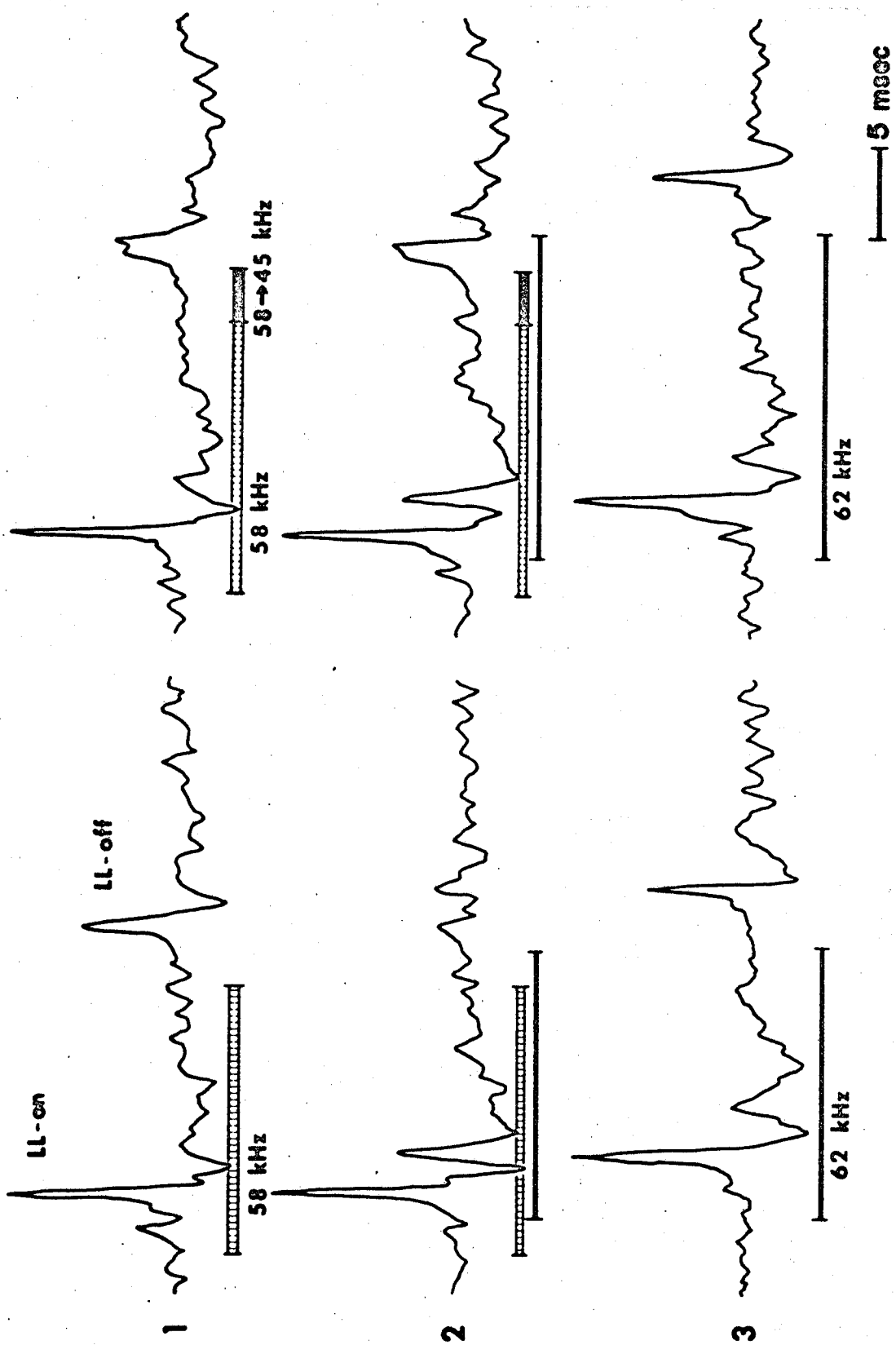


FIGURE 32.

Figure 33.

The off-responses of LL and N_1 to a 58 kHz tone pulse are both suppressed by a 62 kHz pure tone (A), while the response to the FM sound (58-45 kHz) attached to 58 kHz CF tone pulse is not masked by a 62 kHz tone (B). See also Figure 32 for explanations.

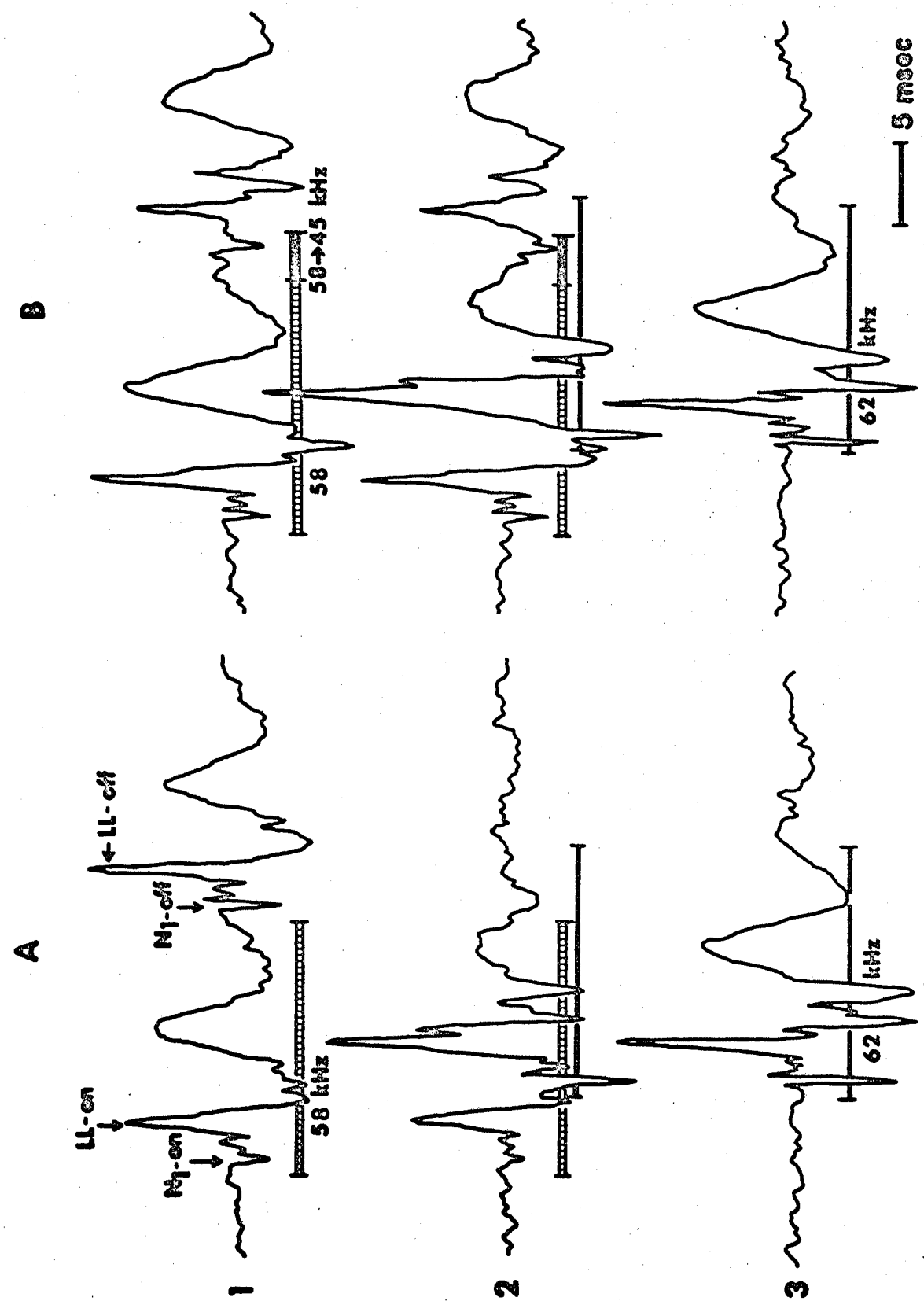

A
B
LL-on
N1-on
LL-off
N1-off
58 kHz
58→45 kHz
58
62
62
kHz
kHz
5 msec
1
2
3

FIGURE 33.

DISCUSSION

The echolocating bat emits rather intense orientation sounds. The ears of the animal receive inevitably a fraction of the strong out-going sonar signal just before faint echoes from surrounding objects come back. Therefore, in order to keep the auditory sensitivity to echoes normal there must be an interaction from vocalization system to auditory one in synchrony with the sound emission for the protection from the neural over-drive. On the other hand, it is evident from observation of the animal behavior that an appropriate program of the sound emission must be activated in the circuitry of the brain concerned with the emission of orientation sound in order to achieve an optimum performance under the situation of echolocation. The selection of the emission pattern of sonar sound must depend on the informations extracted in afferent auditory system. The auditory and vocalization system are thus strongly coupled each other in echolocating bat. Each interaction has been separately described in a variety of animals, e.g., vocal to auditory in cat (Carmel and Starr, 1963) and in bat (Suga and Schlegel, 1972), auditory to vocal in bird (Konishi, 1963) and in man (Davis and Silverman, 1970). Echolocating bat has to positively utilize those coupling pathways in order to perform the optimum operation of the echolocation system as a whole for

the efficient insect hunting. Present studies revealed some of the basic properties of the coupling in both directions between auditory and vocalization system in several species of the echolocating bats including both FM and CF-FM bats.

In order to investigate the coupling of the auditory and vocalization system, the animal must emit orientation sound and listen to echoes, and furthermore it is preferred that the animal may attempt to echolocate. Since neurophysiological studies on echolocation system of the bat have been performed with anesthetized bat, there was little knowledge about the coupling in those two systems even though undoubtedly plays an essential role as a system for echolocation (e.g. Grinnell, 1970; Neuweiler, 1970; Suga, 1968). Therefore in the present studies all subjects used were awake animals which had been surgically operated more than two hours before experiment. Ether anesthetic was applied only during surgery. The experimental animal was held by a supporting apparatus without disturbance to vocal musculatures and innervations or to hearing one's so that the animal was held only at its skull. The movement of the animal were absorbed by a plastic ball floating on water because the skull could not be strong enough against the impact forces such as kicking or jumping of the animal. Because of the structure of the auditory system it is easier to approach to the auditory system from the ventral side than from the dorsal side, however, the ventral side approach would give serious damages

on the vocal musculatures and innervations. The holding method employed in the present experiments was thus designed to allow animal to be unrestrained but its head at a certain direction. However, under such an experimental condition in which all of the animal's voluntary endeavors become futile and reactionless, the animal was depressed in voluntary echolocation. In the squirrel monkey (Jürgens and Ploog, 1970) and in quail (Potash, 1970), vocal responses can be elicited by the electrical stimulation of the brain. In both cases the position of the effective stimulation for vocal response showed wide distribution in the midbrain.

When electrical stimulation was given at the dorso-lateral part of central gray matter or at the lateral part of the reticular formation in the midbrain at the level of the boundary between inferior and superior colliculi of the echolocating bat, the animal emits sounds very similar to the orientation signal used in echolocation. The latency of this vocal response was in the range of 25-60 msec, this value was, however, very short compared with those observed in monkeys and the birds, several hundreds milliseconds in both cases. In the cases of the monkey and the bird, some of the repertoire of the animal's vocalizations were elicited by the electrical stimulation with a different electrode location or with a different parameter of the stimulus. On the other hand, the present experiment about electrical stimulation of the bat's brain, revealed that certain parts of the midbrain

near the inferior colliculus were only concerned with the emission of species-specific orientation sounds, though probable communication sounds were vocalized in response to the electrical stimulation of the thalamic area. All other area seemed to be unrelated with vocalization of the orientation sound because of large twitchings and abnormal frequency sweeps. Each bat using different orientation sound for echolocation emits the sound specifically similar to each species sonar signal in response to the stimuli regardless the type of the sonar signal; FM or CF-FM. Thus the electrical stimulation applied to the parts of the central gray matter and/or of the reticular formation appeared to be activating directly the systematic vocalization circuitry. The difference of the latency and specificity of the evoked sound between the bat and the other animals may be attributed to the difference in type of the sound utilized by the animals; one is orientation sound of which emission is highly autonomously controlled, and the others are communication sounds which must be controlled emotionally or by cerebral activity in large amount.

The phenomenon that the vocalization elicited by the electrical activation of the certain parts of the midbrain could be enhanced in intesity and stabilized in occurrance by the presence of acoustic stimuli suggests that the circuitry activated electrically was not a simple motor system but organized neuron pool for the sound emission. Furthermore,

the activated neurons have the connections with the auditory neurons, because the vocalization could not be elicited by any acousitc stimuli alone but could be enhanced when electrical activation in the parts of the midbrain was accompanied. Furthermore again, because the threshold curve to the enhancement in the electrically activated vocalization by the acoustic stimuli has a sharp peak at the frequency of the orientation sound in a case of CF-FM bat (P. parnellii), it is undoubtedly evident that the sounds emitted in response to the electrical stimuli to the parts of the midbrain of the echolocating bat was orientation sound for echolocation and the animal would supposedly attempt to echolocate with the sound elicited by electrical stimulation. Then the vocal response to acoustic stimuli can be understood as equivalent as the behavioral response observed in the jamming experiments performed by Griffin (1963). According to Griffin's observation, Myotis can pass through the wire of 0.18 mm in diameter without any problem under quiet condition. When the jamming noise was presented to interfere the flying animal, the animal increased the intensity of orientation signal emitting in order to obtain the echoes in increased amplitude. The enhancement of the electrically elicited sound emission thus can be quite compatible with the above cited behavioral response. This vocal response to acoustic stimuli was the one of the emphasized subjects of the present studies. An interaction from ascending auditory system to vocalization efferent was

thus uncovered with several biologically and behaviorally positive significances. It became at first possible to clarify the coupling by using acoustic stimuli associated with the electrical stimulation to the brain of the behaviorally vivid animal, echolocating bat.

This type of investigation should be performed in other animals and sensory systems whether an activated neural response by a stimulus (in this case electrical stimuli) can be modified by the other one in order to shed a light for the integration mechanism of the information in biological system.

Electrical stimulation to unanesthetized animal is thus a powerful experimental technique, however, the electrical stimulation might bring with unnatural effects. One of the indication of the unnatural effects was observed in the difference of the sensitivity change curve accompanied with vocalization (Chapter IV, Figure 30 B and C). And the other indication is difference in the amount of neural attenuation to the self-emitted sounds elicited electrically and voluntarily (Chapter III). In spite of those unnatural effects elicited in the brain, paradoxically, the electrical stimulation of the brain was useful in the investigation of coupling between auditory and vocalization system because the difference in phenomena accompanied with the voluntary vocalization and the electrically evoked one can be compared and examined with suggestions on the neural circuitry. Furthermore the electrical stimulation technique made it possible to measure

the sound field generated by the bat for echolocation. Sound fields have been measured with the orientation sound emitted voluntarily by flying or roosting animals (Simmons, 1969; Schnitzler, 1968). Those measurements were performed without fixing the head of the animal because the tethered animal would not attempt to echolocate or to emit orientation sound. By the electrical stimulation technique employed in the present work, the orientation sound field was measured for the sufficient number of samples of the sound. The directional sensitivities of the echolocating system could be obtained thus for the different frequency components of the FM orientation sound of Myotis. The measurements revealed that Myotis emits the sound beam slightly downward from the eye-nostril line. The high frequency components were not necessarily superior to the low frequency components in the FM orientation sound in terms of interaural pressure difference (IPD) for echo-localization. Myotis would be able to discriminate about one degree azimuth difference of echo source by IPD. At the terminal phase of the insect pursuit, the dynamic range of the echolocation system in horizontal plane would increase compared with that of the searching phase, since Myotis shifts downward its frequency sweep of the orientation sound in terminal phase, while Rhinolophus seems to be scanning the surroundings by moving its pinnae synchronously with the sound emission (Schnitzler, 1968) instead of widening the range by the frequency change as in Myotis.

The other pathway of the coupling is the interaction from efferent system to afferent one. This coupling can be considered as the protective mechanism to the intense self-vocalized sound, but on the other point of view, this may be necessary to monitor the out-going sonar signal as the "re-afferent copy" at the appropriate level of neural activity. One way of the attenuation of incoming acoustic signal is achieved in echolocating bat by bending the pinnae down and covering the external auditory meatus. This reaction has been observed in bat exposed to an extremely intense sound field (Wever and Vernon, 1961), under such condition the animal squat down refusing to fly. However this pinnae reaction is not observed in synchrony with the orientation sound emission. The next attenuation apparatus is equipped in middle-ear. The middle-ear muscles of tensor tympani and stapedius have been reported to contract synchronously with vocalization (Carmel and Starr, 1963; Salomon and Starr, 1963; Henson, 1965). In the present work described in Chapter IV, the followings are confirmedly observed and measured. The MEM starts to contract a few milliseconds prior to the emission of the orientation sound. The amount of attenuation by MEM contraction would be at least 20 dB. The MEM reflex to intense sound requires about 6 msec latency so that the reflex can not act as a protective mechanism to self-vocalized sound. According to the present data, the MEM reflex arc is opened when the animal vocalizes. The reflex arc consisted of the pathways from the

medial trapezoid body to the brain stem motor nuclei thus receives an efferent copy like activity in synchrony with sound emission.

Even after the attenuation by MEMs, the self-emitted orientation sound stimulates the auditory neurons too intensely as a simple monitor of sonar signal. Though there is olivo-cochlear bundle of Rasmussen whose inhibitory action on the sensory hair cells was confirmed in cat (Fex, 1962), it may not play a role for this attenuation mechanism, since it takes time larger than 50 msec at least. In addition to this reason, the olivo-cochlear bundle was not the subject of experiment in the present studies because of little knowledge about the anatomy in bat and difficulty of surgical operation on the middle-ear muscles. This is the one of the points remained to be investigated in future for the entire description of the coupling.

Grinnell (1967) have reported that intense acoustic signal to anesthetized Myotis often elicits smaller LL potentials than do much fainter signals. Grinnell suggested that this evoked potential could be explained by the "closed" single unit with upper and lower thresholds, and that the "over-driving" above the upper threshold may protect the excessive input to higher order nucleus. However, the closed neuron is completely suppressed by the intense sound given before faint one without excitation (Suga, 1968), so that it can not be the origin of the smaller LL potential to the louder

sound followed by the faint one. This phenomenon of LL evoked by the successive two sound pulses is probably attributed to the middle-ear muscle activity. In order to compare simply the sensitivity of the auditory system during sound emission and that of resting state, the magnetic tape recorder was utilized. The neural responses to the self-emitted stimulus were recorded from the active animal, and later the same stimulus was given to the same but resting animal. Therefore the difference between those two records could be attributed to the efferent control to the afferent system accompanied with the efferent activity. Self-evoked responses to the self-stimulation were obtained only once, and by changing the intensity of the played-back stimulus which is essentially the same as self-emitted sound, it is possible to find out the intensities at which one of the two responses becomes equal to the self-evoked one. The recording and playback system of the orientation sound used had an uniform characteristics sufficient enough to the purpose of the experiment.

Suga and Schlegel (1972) showed that there is a neural attenuation mechanism to the sound elicited by the electrical stimulation of the brain. The attenuation was observed between the primary auditory nerve and the inferior colliculus. In the present work the site of the neural attenuation was determined by the same logic, as described in Chapter IV. The nucleus of the lateral lemniscus was the site of the attenuation of auditory signal accompanied with the vocaliza-

tion of both the orientation sound and communication sound. Some differences were seen in the amount of attenuation between those measured by Suga and Schlegel (1972) and those described here. This may be due to the electrical stimulation applied to the midbrain as described elsewhere in the early part of this discussion. The electrical stimulation was not employed in the present work for the neural attenuation for the sake of examination of the byproductive effects of the stimulation.

The experiment to determine the time course of the neural attenuation was attempted in order to examine whether the neural attenuation is simply attenuation to the self-stimulation or accompanied with the "neural facilitation" for the improvement of the echo-detection. But the experiments was not completed because the number of orientation sound becomes enormously large to plot even one curve so that the bat can not bear such a heavy work even by the electrical stimulation, and more essentially because proper delaying device of the acoustic signal was not available which covers 0-10 msec at least in the frequency range of 20-100 kHz.

In the auditory system of the CF-FM bat, the presence of the off-response to the pure tone or FM tone stimulus has been reported (Grinnell and Hagiwara, 1972; Grinnell, 1973). Off-response of N_1 and LL were examined in Chapter IV by the masking technique, even though this topic is purely the physiological studies of the ascending auditory pathway

instead of coupling with efferent system. It was confirmed in Pteronotus that N_1-off response was evoked by the mechanical transient phenomenon occurring most probably in cochlea, but not neural rebound as in the higher auditory nuclei, though single unit recording was only preliminary.

The main coupling between auditory and vocalization system thus occurs in the level of the midbrain of the echolocating bat in both direction.

CONCLUSIONS

Microchiropteran bat emits the ultrasonic orientation sound ranging 100-20 kHz in frequency and 140-70 dB SPL in intensity. The animal can locate the surrounding objects by hearing echoes and analyzing them. Man-made radar and sonar systems are equipped by the "transmit-receive circuit" which protects the delicate input of the receiver in synchrony with the powerful emission of out-going pulse, and which also disconnects the transmitter device after the emission of the pulse to increase the sensitivity of the receiver to faint echoes.

In the echolocating bat the middle-ear muscles contract and attenuate the sound wave by 20 dB in synchrony with the emission of orientation sound (Chapter IV). This muscular mechanism can be contrasted to the transmit-receive circuit in the above cited man-made radar. In addition to the muscular attenuation, the neural activity caused by the self-stimulation with the self-vocalized sound was suppressed at the nucleus of the lateral lemniscus (Chapter III). This suppression is equivalent to about 15 dB attenuation of sound intensity. Thus the inferior colliculus as the analyzing center for echolocation is protected from the over-drive by the self-emitted sonar signal by total amount of 35 dB attenuation, and is able to maintain the reactivity to

the faint echoes in normal level.

Another aspect of the transmit-receive circuit is, however, still remained unkown that whether the auditory system has a super-normal sensitivity to the echoes after the suppression to the self-emitted sound or not.

Echolocating bat, in contrast to man-made radar, can change the properties of the orientation signal in relation to the situation. Thus the bat controls the vocalization system to emit the optimum orientation sound in order to perform the biological task at a given moment of echolocation, such as the approaching phase of insect hunting. This adaptive control must be provided by the coupling pathways from the auditory system where the information born by echoes were analyzed, to the vocalization system.

For the investigation of the coupling or interaction from auditory to vocalization system, the animal must essentially be able to emit the orientation sound. In order to obtain the orientation sounds from the tethered experimental animal and also in order to investigate the neural activation necessary for sound emission, the electrical stimulation was performed in the brain of the echolocating bat. When the lateral part of the central gray matter and/or the dorsal part of the reticular formation to which the inferior colliculus is juxtaposed, are stimulated with the short train of electric pulses, bats regardless the type of the orientation sound whether FM or CF-FM, emit the sounds very similar to species-

specific orientation signal. This is confirmed with four FM bats Myoits austroriparius, M. grisescens, M. lucifugus, Eptesicus fuscus, and with three CF-FM bats Pteronotus parnellii, P. suapurensis, Noctilio leporinus (Chapter I).

This electrical stimulation technique provided the possibility to measure the sound field of the orientation sound. In this technique the animal is fixed in place but is able to emit orientation sound. The sonar signal of Myotis is emitted in the beam with 20-30 degrees in half amplitude width, and slightly downward from the eye-nostril line. Irradiation angle is wider at lower frequencies in FM sound. The directionality of the echolocating system is not necessarily superior at higher frequency. The lower frequency sweep of the orientation sound of Myotis at the terminal phase of echolocation is not necessarily disadvantageous but advantageous for widening the dynamic range of echo-localization (Chapter II).

When acoustic stimuli are delivered in addition to the electrical stimulation of the brain, bat increased the intensity of the electrically activated sound. This behavioral response to the acoustic jamming stimuli offers the evidence of the coupling from the auditory system to vocalization one. In the case of P. parnellii the coupling seems to be mainly provided by the pure-tone-specialized neurons tuned at a certain frequency. The frequency corresponds to the CF component of the orientation sound used in the species

(Chapter IV). FM specialized neuron as seen in inferior colliculus seems to play a significant role for the coupling from auditory to vocalization in all species examined. This is the first behavioral confirmation that the neurons observed in inferior colliculus are utilized by the animal for echolocation.

GENERAL SUMMARY

1. The brains of several representative species of echolocating bats were found to contain regions concerned with the emission of species-specific orientation sounds. When the dorsal part of the reticular formation in the midbrain or the lateral part of the central gray matter juxtaposed to the inferior colliculus which is the essential center for echolocation in bat is stimulated with a short train of electric pulses, the bat emits sounds identical to orientation sounds with a latency of 25 to 60 msec.

2. Radiation patterns of the different frequency components in frequency-modulated (FM) orientation sound emitted by the gray bat, Myotis grisescens in response to the electrical stimulus to the midbrain were measured. The directional sensitivity of the echolocation system was calculated using both the directional sensitivity of auditory system in terms of amplitude of the lateral lemniscal evoked potential (LL) and the radiation pattern of the orientation sound measured by using the electrical stimuli.

3. The directional sensitivity of the echolocation system is sharper at the higher frequencies of FM orientation sound, while the higher frequency components in FM orientation sound were not superior to the lower frequency ones in terms of the interaural pressure difference which is the essential

cue for echo-localization.

4. The site of the neural attenuation to the self-stimulation by the out-going sonar signal was determined as the nucleus of the lateral lemniscus, since the evoked potentials originating from the nucleus of the lateral lemniscus (LL) were always smaller in response amplitude to the self-vocalized sounds than to the played-back sounds when the amplitude of played-back sound was adjusted to obtain the same amplitude of N_1 as in the self-vocalized sound. The amount of neural attenuation observed was about 15 dB for both the orientation sound and squeaks.

5. The middle-ear muscles contract prior to or during vocalization to attenuate the self-stimulation by 20 dB. As a total amount of attenuation, 35 dB at least is thus achieved for the protection of the inferior colliculus as an analyzing center for echolocation from the excessive input generated by the animal itself.

6. The middle-ear muscle reflex requires about 6 msec latent period so that the reflex can not act as a protective mechanism from the strong orientation sound emitted by the bat flying nearby because the duration of the orientation sound is usually short. The reflex arc is opened when the animal emits the orientation sound to avoid the attenuation of the echoes coming back several milliseconds after the emission.

7. The electrically elicited vocalization was enhanced

by the delivery of acoustic stimuli. This behavioral response to acoustic stimuli accompanied with the electrical stimulus can be considered as equivalent response as that observed in jamming experiment.

8. The response to acoustic stimuli by the electrically activated Pteronotus parnellii was sharply tuned at the frequency of the CF component in the second harmonic of the orientation sound and also to downward sweeping FM sounds. In P. suapurensis and Noctilio leporinus, the vocal responses were very prominent only to downward sweeping FM sounds, but those were very poor to pure tone pulses and were not sharply tuned as in P. parnellii. The difference in the vocal responses indicates that the CF component is more essential to echolocation in P. parnellii than to that in the other two species.

9. N_1-off response observed in P. suapurensis is not due to a rebound discharge from a neural inhibition but to some mechanical transient. Masking experiments with P. parnellii revealed that N_1-off and LL-off for a CF-FM sound mainly consisted of the responses to the FM component and not the off-response to the CF component. Thus the FM component of the orientation sound is an important information bearing element in the mustache bat. The auditory system of P. parnellii showed a specialization for the reception of certain CF sound at the frequency slightly higher than that of orientation sound in terms of N_1-on sensitivity.

LIST OF ABBREVIATIONS

CF	:	constant-frequency (sound)
CF-FM	:	constant-frequency followed by frequency-modulated component
CM	:	cochlear microphonic potential(s)
cm	:	centimeter(s) as a length scale
CN	:	cochlear nucleus
CP	:	continuous pure tone
C.r.	:	Chilonycteris rubiginosa (mustache bat)
CRT	:	cathode ray tube (oscilloscope)
dB	:	decibel(s)
dB SPL	:	sound pressure level in decibels
DCN	:	dorsal cochlear nucleus
DO	:	directionality of orientation sound
DSA	:	directional sensitivity of auditory system
DSE	:	directional sensitivity of echolocation system
FM	:	frequency-modulated (sound)
HC	:	hair cell
HON	:	higher order nervous system
Hz	:	hertz as a frequency measure
IC	:	inferior colliculus
IPD	:	interaural pressure difference
ITD	:	interaural time difference
kHz	:	kilohertz = 10^3 Hz

LL	:	evoked potential originating from the nucleus of the lateral lemniscus
LSO	:	lateral superior olive (S-segment)
m	:	meter as unit length
MEM	:	middle-ear muscle(s)
M.g.	:	Myotis grisescens (gray bat)
MGB	:	medial geniculate body
M.l.	:	Myotis lucifugus (little brown bat)
mm	:	millimeter(s) = 10^{-3} m
msec	:	millisecond(s) = 10^{-3} sec
MTB	:	medial trapezoid body
MTR	:	magnetic tape recorder
mV	:	millivolt(s) = 10^{-3} V
μ	:	micron(s) = micro-meter = 10^{-6} m
μ amp	:	micro ampere = 10^{-6} Amp
μbar	:	micro bar(s) = 10^{-6} bar
μsec	:	microsecond(s) = 10^{-6} sec
N	:	Newton(s) as a force unit, nucleus
N.l.	:	Noctilio leporinus (fish-catching bat)
NLL	:	nucleus of lateral lemniscus
nm	:	nano-meter(s) = 10^{-9} m = milli-micron
N_1	:	evoked potential from the primary auditory nerve
N_3	:	evoked potential from the SOC
N_4	:	lateral lemniscal evoked potential (equivalent to LL)
OCB	:	olivo-cochlear bundle of Rasmussen
PB	:	played-back (sound)

P.p.	:	Pteronotus parnellii (the same as C.r.)
P.s.	:	Pteronotus suapurensis (naked-backed bat)
PST	:	post-stimulus-time (histogram)
Q-10 dB	:	quotient of frequencies at the minimum threshold and the width of 10 dB above it
RF	:	reticular formation
R.f.	:	Rhinolophus ferrumequinum (horse-shoe bat)
S.D.	:	standard deviation
sec	:	second(s) in time scale
SOC	:	superior olivary complex
TB	:	tone burst
T.P.G.	:	tone pulse generator
V	:	volt as a unit of electro-motive force
VCN	:	ventral cochlear nucleus
Voc.	:	vocalization

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