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THE POST-METAMORPHIC DEVELOPMENT OF THE PERIPHERAL AUDITORY SYSTEM PF THE BULLFROG, Rana catesbeiana: AN ANATOMICAL AND PHYSIOLOGICAL STUDY

BY

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THESIS

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Narrow Band Noise

Mass $\propto B$

Inertial $\propto \rho$

$c = \sqrt{B/\rho}$

$Z = \rho c = \rho\sqrt{B/\rho}$

$\rho\sqrt{\frac{B}{\rho}} = \sqrt{\rho B} = Z$

$c = \sqrt{B/\rho}$

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SECTION I. INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Hearing plays an important role in many behaviors of animals. In some animals, the auditory system may function as an alarm system for the detection of predators, while in others audition may serve in the detection of prey. Acoustic communication among individuals can function in social interactions such as mating behavior, parental recognition, territorial defense and the formation of a social hierarchy.

Acoustic communication involves both an emitter and a receiver. The emitter must possess a mechanism for generating a mechanical disturbance in the surrounding medium which can be propagated as an acoustic pressure wave through the environment. The receiver must possess a mechanism for the detection and recognition of this emitted signal. Furthermore, the spectral parameters of the acoustic signal must fall within the capacity of the receiving system to detect and analyse these cues before acoustic communication can take place.

The physical properties of the receiving system determine the spectral energies which will be detected and analysed. The auditory periphery is a mechanical system which transduces acoustic energy into the electrical events of the nervous system. This mechanical system is comprised of structures possessing mass, stiffness and resistance, and these physical parameters will affect the frequency response of the system. For example, an increase in mass will tend to attenuate high frequencies, while an increase in stiffness will attenuate low frequencies. An increase in resistance will attenuate the energy transmitted and decrease the sharpness of resonance. The physical properties of the structures of the ear will determine the frequency selectivity and sensitivity of the auditory periphery, and thus will dictate which spectral energies of the calls will be encoded and analysed by the central auditory system.

There has evolved two basic strategies which underlie the recognition of species-specific vocalizations. Birds and mammals have evolved a generalized ear that is not only capable of detecting frequencies which fall into the range of spectral energies present in their calls, but it is also sensitive to other frequencies as well. As a result, much of the neural processing of the species-specific call is carried out by the central nervous system (CNS). The CNS must separate the neural information encoded for the biologically signifigant features of the call from neural information representing other extraneous acoustic signals.

On the other hand, the spectral energies of anuran vocalizations not only fall within the frequency range of their auditory system, but the dominant spectral energies in the calls are so well matched to the frequency selectivity of the auditory system, that the ear appears to act as a "sensory filter" to their calls (Frishkopf etal., 1968). Thus, unlike the generalized ear of birds and mammals, the anuran peripheral auditory system is specialized for the selective detection of acoustic signals that have the same spectral features as those found in their calls. The CNS is primarily integrating neural information encoded for the acoustic parameters present in anuran vocalizations. Since the central auditory pathways of anurans follow the basic design found in higher vertebrates, the anuran auditory system is a useful model for investigating the neural basis of acoustic communication and complex feature detection.

This thesis is an investigation into the development of the anuran "sensory filter". Developmental changes in the morphology of the peripheral auditory system may alter the mechanical properties of its structures resulting in changes in frequency selectivity and sensitivity. Changes in the filter characteristics of the auditory periphery with age may have an affect on the detection and recognition of species-specific calls, and thus, may be behaviorally signifigant.

THE ANURAN AUDITORY PERIPHERY

General Structure

The peripheral auditory system of anurans is divided into an external ear, middle ear and inner ear. The external ear consists of the tympanic membrane and the surrounding cartilaginous ring (tympanic annulus). The middle ear is comprised of an air filled cavity containing three fused middle ear bones: plectrum, columella and operculum (Figure 1). The distal end of the plectrum attaches to the tympanic membrane, and the proximal end of the plectrum, which is called the extra-columella, is fused to the distal end of the columella. The proximal end of the columella is fused via the columellar footplate to the operculum, which is connected to the oval window of the inner ear. The opercularis muscle is present in anurans (Lombard and Straughan, 1974; Wever, 1979; Baker, 1969) and connects the operculum to the suprascapula. The function of the opercularis is equivocal, but has been suggested to function in the maintenance of body posture (Baker, 1969) and to serve in the transmission of acoustic energy to the inner ear (Lombard and Straughan, 1974; Wever, 1979).

The fluid filled inner ear is suspended within the bony otic capsule by loose connective tissure (Figure 1). The sensory epithelia of the receptor organs are bathed in endolymphatic fluid, which is separated from the perilymphatic fluid by the membrane of the perilymphatic sac. Vibrations set up in the perilymph by the movement of the oval window are transmitted to the endolymph through the contact membranes. Unlike higher vertebrates, anurans possess two distinct auditory organs, namely the amphibian and basilar papillae. The amphibian papilla is a unique structure found only in amphibians,

whereas the basilar papilla is the presumptive homolog of the mammalian cochlea (van Bergeijk and Witsch, 1957). More recently, however, it has been suggested that the basilar papilla is also a unique amphibian structure and is not homologous to any other vertebrate inner ear organ (Lombard, 1980). The three semicircular canals, utricle, lagena and saccule are vestibular organs. The saccule has also been shown to resond to high intensity acoustic stimulation (Moffat and Capranica, 1976).

The sensory organs of the inner ear are innervated by the bipolar fibers of the VIIIth cranial nerve, which has its ganglion located just within the wall of the otic capsule (Figure 2). The VIIIth nerve is segregated into anterior and posterior rami such that no interweaving of the anterior and posterior fibers occurs (Boord etal., 1971). Fibers innervating the two auditory papillae are found exclusively in the posterior ramus (Boord etal., 1971) and project to the dorsal medullary nucleus (Gregory, 1972; Matez, 1979; Lewis etal., 1980; Fuzessery and Feng, 1981). Efferent fibers from the central nervous system are found to terminate on the amphibian papilla (Flock and Flock, 1966), but not on the basilar papilla (Frishkopf and Flock, 1974).

The Middle Ear

The middle ear of terrestrial vertebrates functions as an impedance transformer between air and the fluid filled inner ear. Since the characteristic impedance of the inner ear fluid is much greater than that of air, most of the acoustic energy that falls incident on the air-fluid interface will be reflected rather than transmitted. The transformer action of the middle ear increases the sensitivity of the ear by 40 dB as demonstrated experimentally by observing the sensitivity of the microphonic potential of the inner ear before and after sectioning the middle ear (Strother, 1959).

The impedance transformer action of the columellar middle ear of anurans appears to be accomplished by three mechanisms (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). The primary mechanism is that the area of the tympanic membrane is greater than the area of the oval window resulting in a hydraulic lever. In addition, a columellar lever appears to exist having its fulcrum at the hinged extracolumella-columella junction. This columellar lever acts to amplify the force transmitted to the oval window through the mechanical advantage of the system. A third more subtle lever due to the curvature of the tympanic membrane also appears to exist in the anuran middle ear. These three lever systems are the same basic lever mechanisms that operate in the ossicular middle ear of mammals (Møller, 1974). Indeed for the frequency range that the anuran auditory periphery responds to, the sensitivity of the columellar middle ear is equal to that found in mammals (Saunders and Johnstone, 1972; Moffat and Capranica, 1978).

Mechanical measurements of the vibrations of the anuran tympanic membrane indicate that the middle ear acts as a low pass filter, and the upper cut off frequency of this low pass filter can be directly related to the mass of the middle ear structures (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). Larger species of anurans having more massive middle ear structures have a lower upper cut off frequency than do smaller species. One study indicates that the middle ear acts as a damped resonator in which the volume of the mouth cavity, Eustachian tube and middle ear cavity determines the resonant frequency of the middle ear response (Chung etal., 1978; 1981). However, the mode of stimulation employed by Chung etal differs from that of the previous studies (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). Furthermore, the displacement amplitude of the middle ear response observed by Chung etal (1978; 1981) is well beyond the calibration of their system, and thus their results are highly questionable.

Structure of the Anuran Auditory Papillae

Basilar Papilla

The basilar papilla is a tubular evagination of the ventrocaudal wall of the saccule (van Bergeijk and Witschi, 1957; Geisler etal., 1964; Wever, 1973). The organ lacks a basilar membrane, and the sensory epithelium is a semicircular crest of stationary hair cells and supporting cells anchored in the medial wall of the surrounding cartilaginous ring. The stereocilia of the sensory hair cells project into a gelatinous tectorial membrane which stretches across the sensory epithelium. Using mechanical scale models of the basilar papilla to qualitatively study the vibrations of the tectorial membrane, van Bergeijk (1957) concluded that traveling waves occur in the tectorial membrane resulting in a place mechanism of frequency analysis in this organ. However, recent electrophysiological evidence suggests that the basilar papilla acts as a simple tuned resonator (Capranica and Moffat, 1977) and lacks a tonotopic organization (Lewis etal., 1982a).

Based on the morphology of the stereocilary bindles, three types of hair cells are typically distinguished in the basilar papilla (Lewis and Li, 1975). Type A hair cells have short, graded bundles of stereocilia of small diameters and a long unbulbed kinocilium. This type of hair cell is found on the lateral edges of the sensory epithelium and appears to have a morphogenetic relationship to other hair cell types (Lewis and Li, 1973; Li and Lewis, 1974). Type D hair cells are found in the medial regions of the sensory epithelium and are characterized by short, graded bundles of stereocilia with a short bulbed kinocilium. Type F hair cells have long, graded stereocilia with a long unbulbed kinocilium. Evidence indicates that only the hair cells possessing bulbed kinocilia are in contact with the tectorial membrane, while hair cells having unbulbed kinocilia are free standing (Lewis, 1977a).

Comparative studies of the surface morphology of the basilar papilla have shown that the total number of hair cells appears to be species related in that the number of hair cells is directly proportional to the size of the anuran species (Alfs and Schneider, 1973; Lewis, 1978). Moreover, the orientation of the kinocilia of the hair cells also differs among the various anuran species (Lewis, 1977a; 1978).

Amphibian Papilla

The amphibian papilla is a more complex auditory organ than the basilar papilla and is located on the medial wall of the saccule just ventral to the utricle (Geisler etal., 1964; Wever, 1973). Like the anuran basilar papilla, the amphibian papilla lacks a basilar mambrane. The hair cells of the sensory epithelium are anchored to the roof of the amphibian papilla with the stereocilia projecting ventrally into a gelatinous tectorial membrane. The size and shape of the epithelium differs among anuran species ranging from a simple patch of epithelium found in the primitive tailed frog, Ascaphus truei (Lewis, 1981a) to the S-shaped epithelium consisting of a rostral triangular patch and an extended caudal S-segment of epithelium observed in more derived anurans (Lewis, 1977b; 1978).

The sensory epithelium contains three types of hair cells based on stereocilia morphology (Lewis and Li, 1975). As in the basilar papilla, Type A and type D hair cells are found along the lateral edge and medial regions of the epithelium, respectively. Type E hair cells are found in the central regions of the amphibian papilla and are characterized by long, graded bundles of stereocilia with a long bulbed kinocilium. The bulbed kinicilia of type E and type D hair cells appear to be firmly attached to the tectorial membrane (Lewis, 1976).

The tectorial membrane of the amphibian papilla is a delicate gelatinous structure which shrinks during histological processing. Lewis (1981b) has examined the tectorial membrane as a whole mount in the wet state under phase contrast microscopy. The tectorial membrane completely fills the amphibian papilla chamber and is most massive in the rostral region of the organ, while being less massive in the caudal region. Lewis (1981b) suggests that the difference in the size of the tectorial membrane between the rostral and caudal ends of the papilla may be in part responsible for the tonotopic organization along the epithelium of the organ (Lewis etal., 1982a; 1982b). Lewis and Leverenz (in press) suggest that the tonotopy of the amphibian papilla may be due to a local resonant network. The local resonant frequency is due to the local mass of the tectorial membrane and the local stiffness which results from the number of stereociliary bundles associated with the tectorial membrane at a given locus. Interestingly, the tectorial membrane and its associated structures in the mammalian cochlea also appears to act as a distributed resonance system (Zwislocki, 1980).

Electrophysiological Studies of the Anuran Auditory Periphery

The first attempt to record the electrical activity of the anuran auditory system was made by Adrian etal (1938). They observed the compound action potential from the VIIIth nerve of decapitated frogs in response to mechanical vibrations and speech sounds, and they concluded that frogs are sensitive to intense sounds only. Strother (1959) was the first investigator to demonstrate that the anuran auditory periphery is sensitive to sound by observing the microphonic potential from the inner ear of intact bullfrogs in response to pure tones.

Much of what is presently understood about the physiology of the peripheral auditory system of anurans is based on the results of single unit studies of the VIIIth nerve from a variety of anuran species: Rana catesbeiana (Frishkopf and Goldstein, 1963; Frishkopf and Geisler, 1966; Liff and Goldstein, 1970; Feng etal., 1975); Rana pipiens (Liff, 1969; Liff and Goldstein, 1970; Mudry etal., 1977; Feng, 1980; 1982; Feng and Shofner, 1981; Megela and Capranica, 1981); Rana clamitans (Sachs, 1964); Scaphiopus couchi (Capranica and Moffat, 1975); Eleutherodactylus coqui (Narins and Capranica, 1976; 1980); Bufo americanus (Capranica and Moffat, 1980); Hyla cinerea (Ehret and Capranica, 1980; Megela and Capranica, 1981). From these studies, several common response properties of anuran auditory fibers can be observed, and these responses are summarized below to give a general survey of frequency analysis in the anuran auditory periphery.

Anuran auditory fibers exhibit frequency selectivity in response to single pure tones at various frequencies of constant intensity, and this frequency selectivity is reflected in the V-shaped excitatory tuning curves possessed by all VIIIth nerve fibers. A tuning curve is characterized by its best excitatory frequency (BEF) which is the frequency that the fiber has its lowest threshold of excitation. The BEFs of anuran auditory fibers typically fall into three distinct populations. The low frequency population of auditory fibers is generally in the range of 100-500 Hz in all species, and these fibers exhibit nonlinear responses to combination tones. The mid and high frequency fibers do not show nonlinearities in their neural responses. It has been demonstrated in the bullfrog that the high frequency fibers are derived from the basilar papilla, while the low and mid frequency populations originate from the amphibian papilla (Frishkopf and Geisler, 1966; Feng etal., 1975; Lewis etal., 1982a; 1982b).

The distribution of BEFs for the mid frequency and high frequency selective auditory fibers are species-specific and typically correspond to the dominant spectral energies present in anuran calls. In addition, there exists a relation between the body size of the anuran and the frequency selectivity of these two populations. For example, in the bullfrog, the mid and high frequency populations range from 500-900 Hz and 1000-1700 Hz, respectively (Feng etal., 1975). In the green tree frog, which is considerably smaller than the bullfrog, the distribution of the mid and high frequency auditory fibers are 500-1200 Hz and 3100-3800 Hz, respectively (Capranica, 1976). Furthermore, in the Puerto Rican tree frog there exists a sexual dimorphism in body size, and signifigant differences between the mean BEFs of the mid and high frequency populations are found in males and females. The mid and high frequency populations of auditory fibers have means of 890 Hz and 2290 Hz, respectively in females, while in the smaller males these populations have means of 1060 Hz and 2990 Hz, respectively (Narins and Capranica, 1976; 1980). Thus, in larger anurans the mid and high frequency populations of auditory fibers are distributed over a lower frequency range than in smaller anurans. These differences in the frequency selectivities of these two populations among the various sized anurans may reflect differences in the physical properties of the middle ears and/or auditory organs.

The degree of frequency selectivity exhibited by the tuning curves can be measured quantitatively by the $Q_{10\ dB}$ value (Kiang etal., 1965). The $Q_{10\ dB}$ is defined as the ratio of the BEF to the frequency bandwidth at 10 dB above threshold. Sharper tuning curves, which show a greater degree of frequency selectivity, have higher values of $Q_{10\ dB}$ than do broad tuning curves. Typical values of $Q_{10\ dB}$ for anuran auditory fibers range from 1-4 (Capranica, 1976) and are similar to values obtained for avian (Sachs etal., 1974) and mammalian (Kiang etal., 1965; Evans, 1972) auditory fibers for the same range of BEFs.

The thresholds of excitation at BEF for anuran auditory fibers range widely from 10 dB SPL to 100 dB SPL (Capranica, 1976; Narins and Capranica, 1976). The sensitivity of the high frequency population of auditory fibers appears to be related to body size in that smaller species tend to have higher thresholds of excitation than do larger species (Loftus-Hills and Johnstone, 1970; Capranica etal., 1973; Loftus-Hills, 1973). As the intensity is increased above threshold at BEF, the firing rate of the fiber increases monotonically over a dynamic range of 20-40 dB (Liff and Goldstein, 1970; Capranica and Moffat, 1975; Capranica, 1976; Feng, 1982). In addition to the increase in firing rate, the latency of the neural response decreases as intensity is increased over a 30-40 dB range (Feng, 1982). Furthermore, high frequency and low frequency selective auditory fibers generally show little adaptation during a maintained tone burst at intensities above threshold, while mid frequency fibers exhibit rapid adaptation (Megela and Capranica, 1981). However, if the tone is presented under conditions where the background noise is of sufficient energy, the excitatory neural response will be to the noise rather than to the tone (Ehret and Capranica, 1980). Thus, the excitatory response to the tone is suppressed or masked.

When a low frequency auditory fiber is stimulated by an excitatory tone, the addition of a second tone of sufficient energy and frequency will also suppress the excitatory neural response. This is called two-tone inhibition (two-tone suppression) and is one type of nonlinear response to combination tones exhibited by the anuran auditory periphery. If the excitatory tone is held at a constant intensity, an inhibitory tuning curve can be generated by varying the frequency and intensity of the second tone. The inhibitory tuning curve is characterized by its best inhibitory frequency (BIF) which is the frequency that the fiber has its lowest threshold of inhibition. The

BIF and inhibitory tuning curve always lie outside of the excitatory tuning curve and above the BEF (Frishkopf and Goldstein, 1963; Liff and Goldstein, 1970; Capranica and Moffat, 1980). No inhibition is observed below the BEF of the fiber.

Recently, Capranica and Moffat (1980) have shown that low frequency selective auditory fibers also respond to the intermodulation distortion product, f_2-f_1 (where f_2 is greater than f_1 and both f_2 and f_1 lie outside of the excitatory tuning curve). The response to distortion products is excitation, but this excitation can be inhibited by the addition of a third tone i.e. shows two-tone inhibition. Thus, the distortion product acts as a single excitatory tone. The excitatory response to the distortion product is the second type of nonlinear response that low frequency fibers exhibit to combination tones. Two-tone inhibition and intermodulation distortion are not under efferent neural control (Frishkopf and Goldstein, 1963; Liff and Goldstein, 1970; Capranica and Moffat, 1980) nor do these responses appear to be due to nonlinearities in the middle ear (Capranica and Moffat, 1980). These nonlinear responses appear to be due to the mechanical nonlinearities within the inner ear. Since the anuran auditory organs lack a basilar membrane, which is thought to give rise to nonlinearities in the mammalian cochlea (Rhode and Robles, 1974; Rhode, 1977), Capranica and Moffat (1980) have suggested that the mechanism underlying these nonlinear responses in the anuran ear may involve the tectorial membrane.

DEVELOPMENT OF THE ANURAN AUDITORY PERIPHERY

This section describes the development of the peripheral auditory system in anurans. Since different investigators use different systems for the identification of the embryonic and metamorphic stages of development, stage numbers presented in this section follow those of Witschi (1956) and are shown in

Figure 3 in order to present a coherent summary of the development of the anuran auditory periphery.

The anuran auditory periphery begins to differentiate in the late neurula (stage 16) and early tailbud stages (stage 17) when the ectodermal otic placode invaginates to form the otic vesicle (Witschi, 1949). The medial wall of the otic vesicle gives rise to neuroblasts which will aggregate and differentiate into the VIIIth nerve ganglion (Witschi, 1949). From stage 20 when the anuran larva hatches to stage 23 when the operculum fold is apparent, fibers from the VIIIth nerve ganglion project into the medulla forming the ventral (vestibular) root of the VIIIth nerve (Larsell, 1934). At this time (stage 23), the amphibian papilla begins to differentiate off the medial wall of the saccule, but no dorsal (acoustic) root fibers of the VIIIth nerve have formed (Larsell, 1934).

When the hindlimb buds begin to form (stage 25), the dorsal root of the VIIIth nerve begins to develop as a few fibers connect the medulla with the amphibian papilla (Larsell, 1934). As the hindlimb buds grow, the amphibian papilla epithelium begins to evaginate and the tectorial membrane begins to appear (Larsell, 1934). When the length of the hindlimb bud is equal to its width, the tectorial membrane of the amphibian papilla thickens and the basilar papilla begins to differentiate as a short tube on the ventrocaudal wall of the saccule (Larsell, 1934). A tectorial membrane in the basilar papilla also begins to develop around this time.

Interestingly, the sensory epithelium of the amphibian papilla develops from two separate patches of epithelium (Li and Lewis, 1974). These two patches correspond to the rostral triangular patch of epithelium and the caudal S-segment. The patches are separate in tadpoles where the hindfoot paddle is formed (stage 27) and begin to fuse when the hindfoot is fully developed (stage 29). In contrast, the sensory epithelium of the basilar papilla develops from a single epithelial patch (Li and Lewis, 1974).

The anuran ear undergoes an extensive transformation during metamorphosis. The tympanic membrane and middle ear have not yet differentiated in tadpoles, but in some larval anurans a bronchial columella is found. The bronchial columella is a solid rod made up of mesodermal fibers and connects the bronchial membrane of the bronchus to the round window of the inner ear (Witschi, 1949; 1955). The function of the bronchial columella has not been demonstrated, but it is thought that the lungs serve as pressure detectors and that the bronchial columellae transmit vibrations from the lungs to the inner ear in an analogous manner as the swim bladder and Weberian ossicles in the ostariophysian fish (Witschi, 1949). The presence of the bronchial columella in larval anurans appears to be limited to Ranid anurans (Witschi, 1955).

The bronchial columella begins to differentiate when the hindlimb buds of the tadpole first form(stage 25). The columella is fully developed between the time the hindlimbs differentiate (stage 26) until the time the forelimbs are fully developed (stage 31). During the climactic period of metamorphosis (stages 31-32), the bronchial columella degenerates and the tympanic columella develops connecting the tympanic membrane to the oval window.

The post-metamorphic development of the peripheral auditory system has been investigated in only one study. Sedra and Michael (1959) have examined the post-metamorphic development of the middle ear in the Egyptian toad. In the newly metamorphosed toad, the columella is differentiated and its distal end is a mesenchymal mass which will differentiate into the plectrum. The operculari muscle has not yet attached to the operculum, and the tympanic membrane begins to differentiate. A few weeks following metamorphosis, the plectrum begins to become cartilaginous. While the tympanic membrane is further differentiated, the tympanic annulus is not fully developed being only sickle-shaped. The columella has increased in size and is well chondrified. One year after metamorphosis when the toad is half grown, the middle ear is similar to that of adult toads. The tympanic annulus is a full cartilaginous

ring, the tympanic membrane is fully differentiated, the plectrum is completely chondrified and is attached to the tympanic membrane. The middle ear cavity and Eustachian tube are also fully developed at this time. These observations suggest that there may be a period of time during post-metamorphic growth when the middle ear of the toad may be inefficient in transmitting sounds to the inner ear resulting in a relative insensitivity to airborne sounds (Capranica, 1976).

One electrophysiological study has attempted to investigate the development of auditory sensitivity in anurans. Weiss etal (1973) recorded the microphonic potentials from the inner ear of bullfrog tadpoles in response to pure tones of varying frequency and intensity. Comparison of their results with those of adult bullfrogs (Strother, 1959) shows that the tadpoles are typically 20-40 dB less sensitive than adults. Unfortunately, the tadpoles in the study were presented with airborne acoustic stimulation rather than with waterborne sounds. Thus, it is difficult to interpret the results of Weiss etal, since the auditory periphery of the bullfrog tadpole possesses a bronchial columella and would thus be specialized for the reception of waterborne sounds. Since the tadpole does not possess an impedance transformer for airborne sounds, one might expect tadpoles to be less sensitive than adults to airborne sounds.

STATEMENT OF THE PROBLEM

During the post-metamorphic development of anurans there is typically an increase in the body size of the animal. Given the results of comparative studies relating frequency selectivity and sensitivity of the auditory periphery to body size as well as the findings of Sedra and Michael (1959), it suggests that changes in the frequency selectivity and sensitivity may also occur in the peripheral auditory system during post-metamorphic development. This thesis is an investigation of the physiological and anatomical development of the anuran auditory periphery following metamorphosis. The North American bullfrog, Rana catesbeiana, is used in this study because (1) there is a large increase in body size during post-metamorphic development as shown in Figure 4; (2) there exists an abundance of useful background literature on the anatomy and physiology of the auditory periphery in adult bullfrogs; (3) there is also background literature available on the structure of adult bullfrog vocalizations and the behavioral response to the mating call.

Figure 1. Schematic diagram illustrating a caudal view of the peripheral auditory system of anurans.

AMPHIB. PAPIL. Amphibian papilla

BAS. PAPIL. Basilar papilla

COLUM. Columella

ENDOLYM. Endolymph

ENDOLYM. SAC Endolymphatic sac

ENDOLYM. DUCT Endolymphatic duct

LAG. Lagena

MED. OBL. Medulla oblongata

OPERC. Operculum

OTIC CAP. Otic cpasule

O.W. Oval window

PERILYM. CIST. Perillymphatic cistern

PERILYM. DUCT Perilymphatic duct

PERILYM. SAC Perilymphatic sac

PLECT. Plectrum

POST. VIIIth GANG. Posterior VIIIth nerve ganglion

POST. VIIIth N. Posterior VIIIth nerve

POST. VERT. CAN. Posterior vertical canal

R.W. Round window

SACC. Saccule

TYMP. Tympanic membrane

UTR. Utricle

UTR. SAC. FOR. Utricular-saccular foramen

(From Frishkopf and Golstein, 1963)

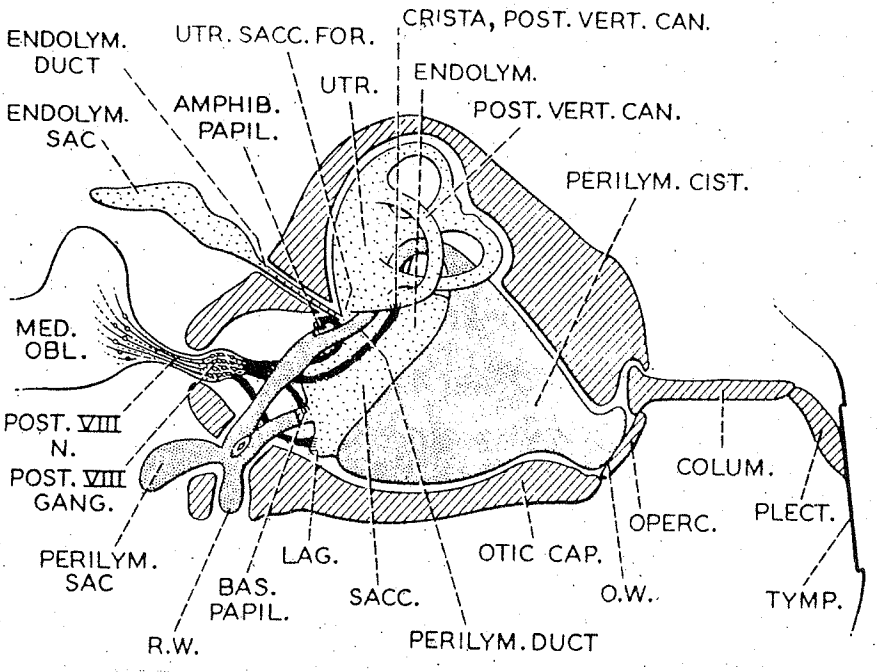

ENDOLYM. DUCT
UTR. SACC. FOR.
CRISTA, POST. VERT. CAN.
ENDOLYM.
UTR.
ENDOLYM. SAC
AMPHIB. PAPIL.
POST. VERT. CAN.
PERILYM. CIST.
MED. OBL.
POST. VIII N.
POST. VIII GANG.
COLUM.
OPERC.
PLECT.
PERILYM. SAC
LAG.
OTIC CAP.
BAS. PAPIL.
SACC.
O.W.
TYMP.
R.W.
PERILYM. DUCT

Figure 2. Schematic diagram illustrating the innervation of the inner ear organs in anurans. The anterior ramus of the VIIIth nerve (VIIIn.) innervates the anterior vertical canal (AVC), horizontal canal (HC), utricle (U) and saccule (S). The postior ramus of the VIIIth nerve (VIIIn.) innervates the lagena (L), amphibian papilla (AP), basilar papilla (BP) and posterior vertical canal (PVC). The ganglion of the VIIIth nerve (VIII n. gang.) lies just within the wall of the otic capsule (OC).

(From Feng etal., 1975)

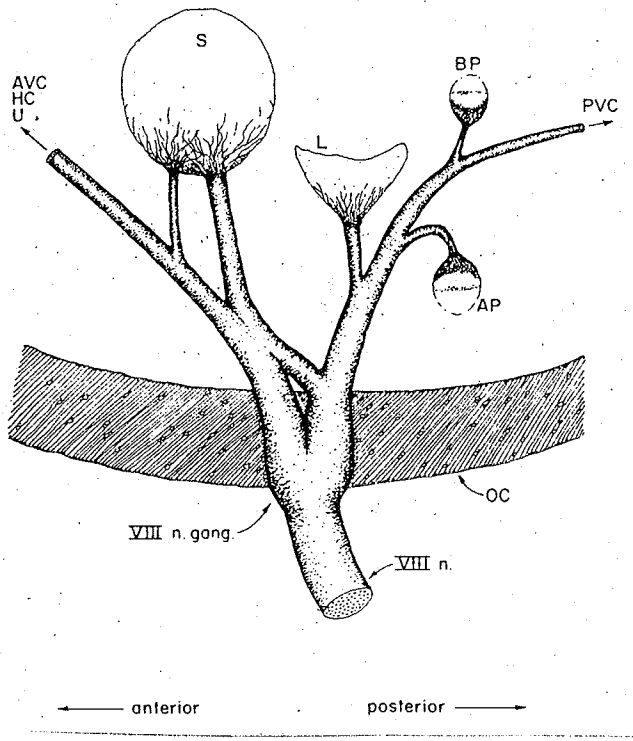

S
AVC
HC
U
BP
PVC
L
AP
OC
VIII n. gang.
VIII n.
anterior
posterior

Figure 3A. Schematic diagrams showing the embryonic stages (1-21) of anuran development.

a: frontal view

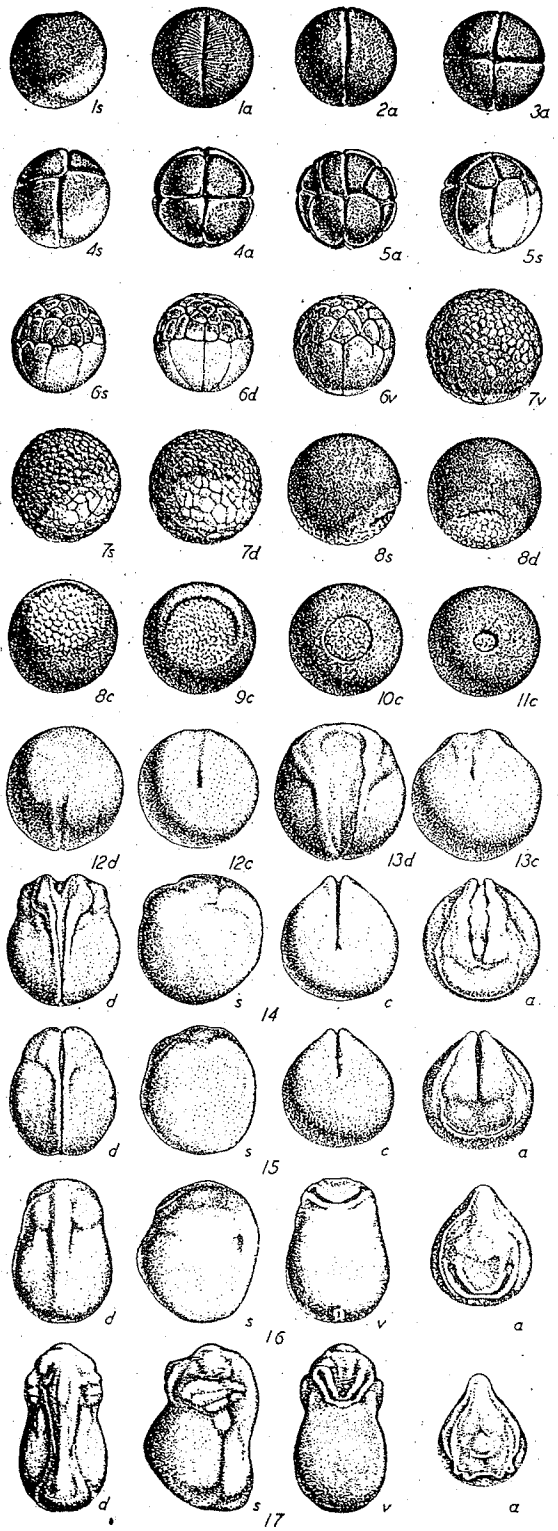
c: caudal view

d: dorsal view

s: lateral view

v: ventral view

(From Witschi, 1956)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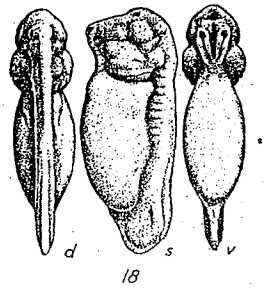
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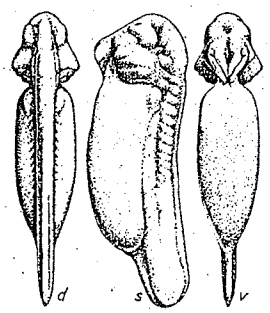
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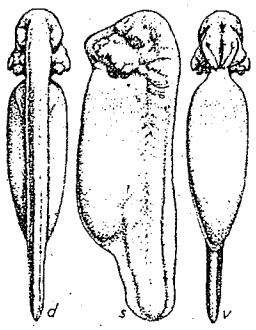
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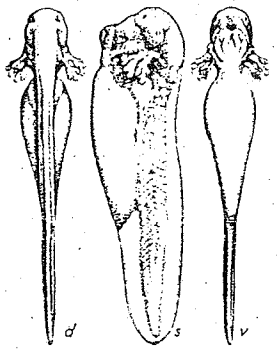
d
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v

21

Figure 3B. Schematic diagrams showing the late embryonic (22-25) and early metamorphic (26-28) stages of anuran development.

d: dorsal view

v: ventral view

s: lateral view

(From Witschi, 1956)

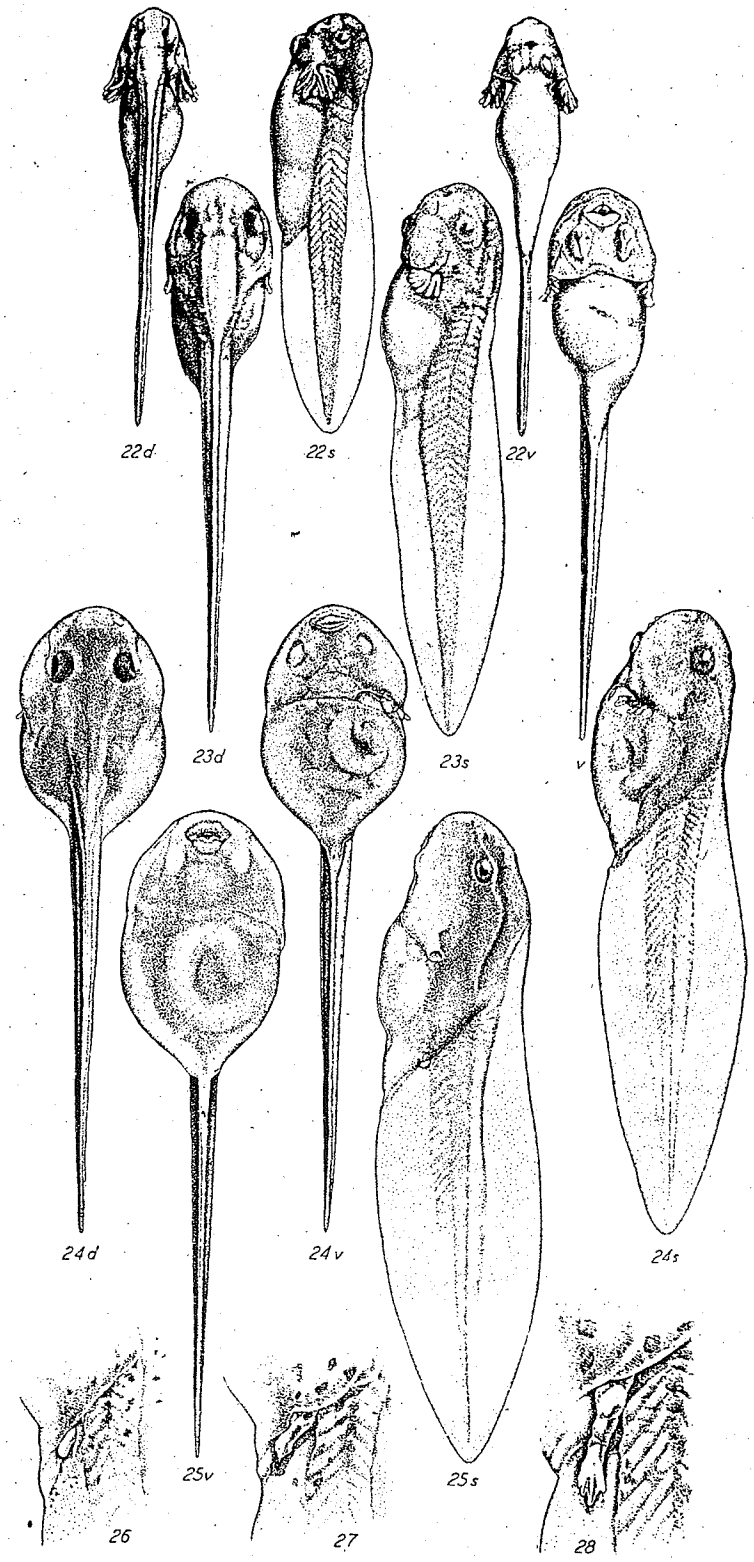

22d
22s
22v
23d
23s
v
24d
24v
24s
25v
25s
26
27
28

Figure 3C Schematic diagrams showing the later metamorphic stages (29-33) of anuran development.

d: dorsal view

v: ventral view

s: lateral view

(From Witschi, 1956)

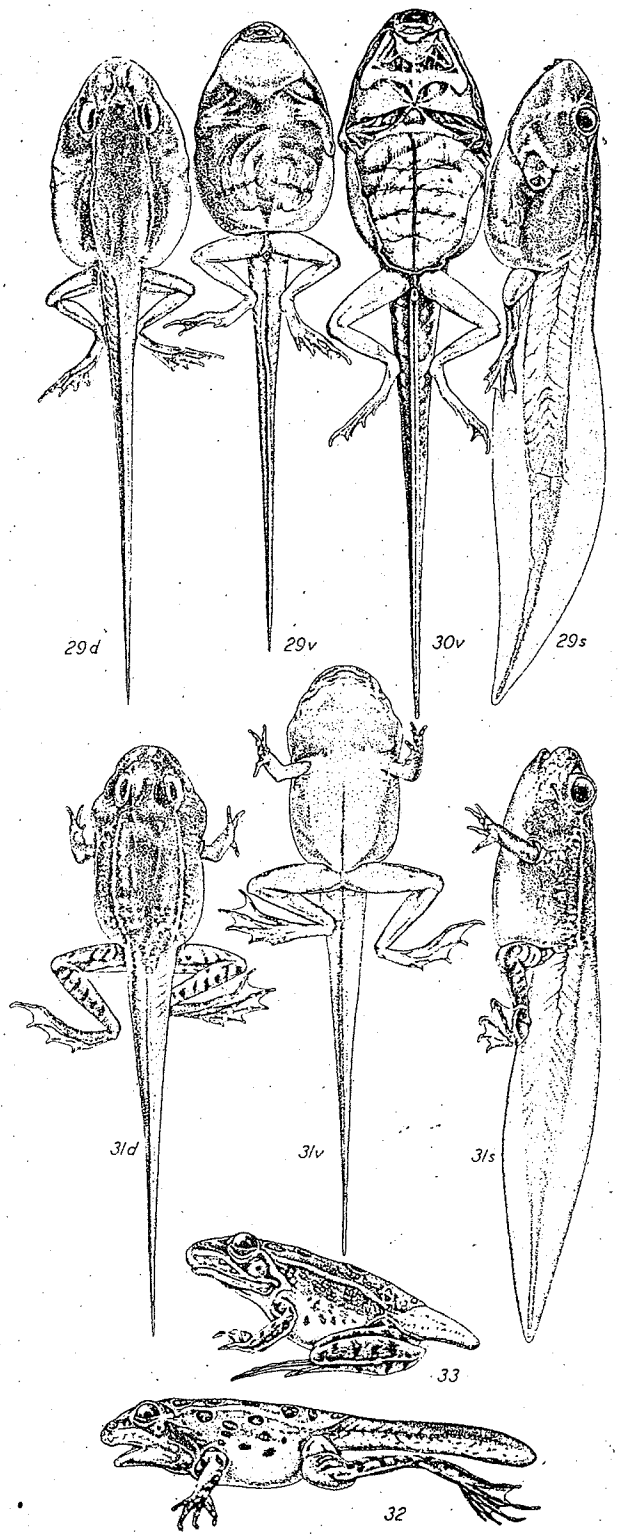
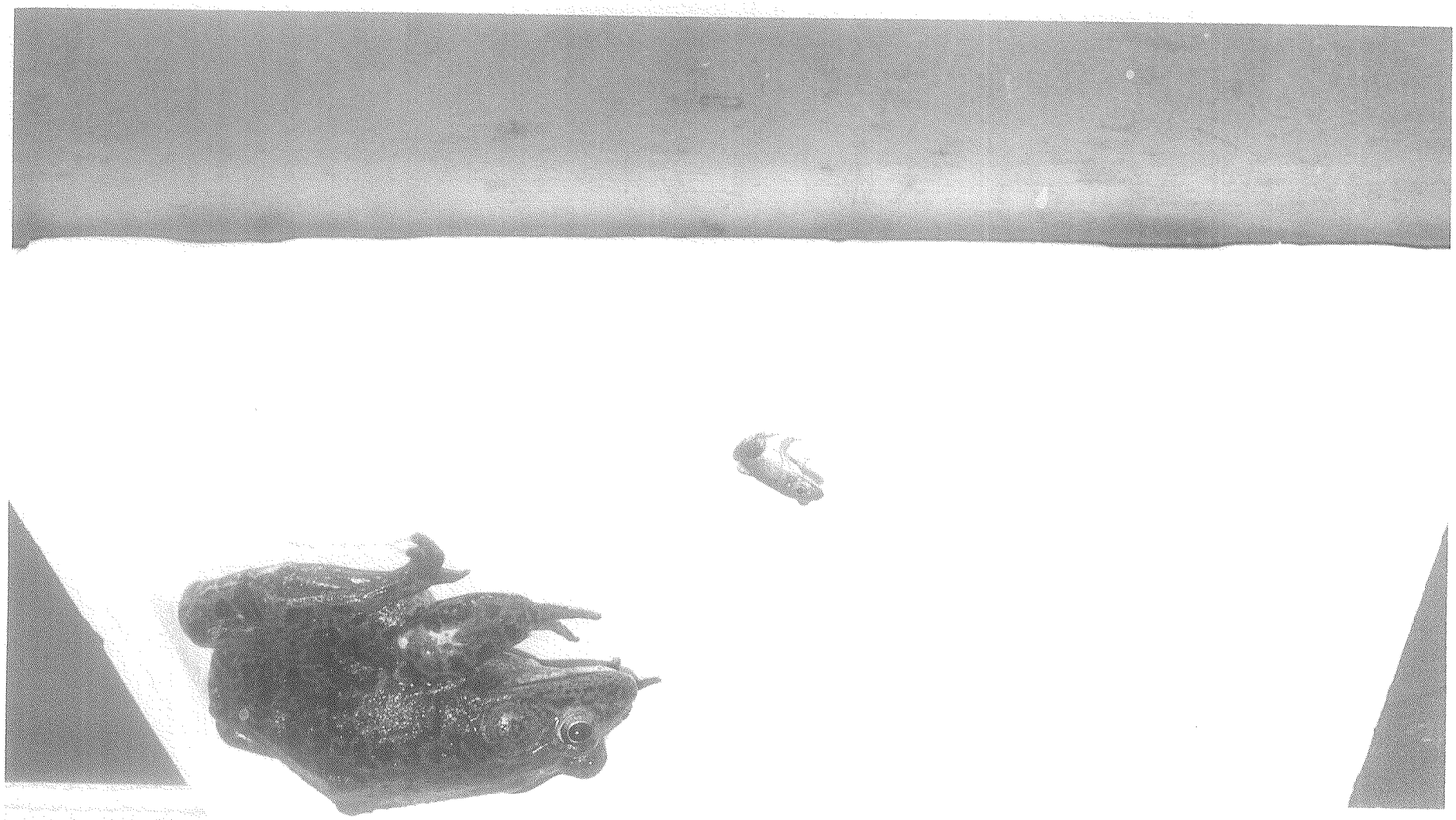

29d
29v
30v
29s
31d
31v
31s
33
32

Figure 4 Post-metamorphic juvenile (left) and adult (right) bullfrogs.



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SECTION II. POST-METAMORPHIC DEVELOPMENT OF THE FREQUENCY SELECTIVITIES AND SENSITIVITIES OF THE PERIPHERAL AUDITORY SYSTEM

INTRODUCTION

Anurans are capable of discriminating conspecific mating calls from those of other species (Martof and Thompson, 1958; Capranica, 1965; Littlejohn and Loftus-Hills, 1968). One of the underlying mechanisms of this discrimination is that the peripheral auditory system acts as a filter that is selectively responsive to the dominant spectral energies present in the call (Frishkopf, et al. 1968).

Comparative studies have shown that an inverse correlation exists between the body size of the anuran and the dominant spectral energies present in the calls, i.e., smaller species of anurans produce higher frequency sounds (Blair, 1963). A similar relationship has also been observed within a given species (Capranica, 1965; Oldham and Gerhardt, 1974; Ryan, 1980). Comparisons of the peripheral auditory selectivities from several anuran species (Sachs, 1964; Frishkopf et al. 1968; Capranica and Moffat, 1974, 1975; Moffat and Capranica, 1974; Feng et al. 1975; Capranica, 1976; Narins and Capranica, 1976; Mudry et al. 1977) as well as central auditory responses (Loftus-Hills and Johnstone, 1970; Capranica et al. 1973; Loftus-Hills, 1973) also show an inverse relationship between body size and high-frequency selectivity (response to frequencies at a constant intensity) and sensitivity (response to intensities at a constant frequency) of the auditory system. Furthermore, mechanical measurements of the vibration of the middle ear in several anuran species reveal that the upper cut-off frequency is lower in large species than in small species (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). Thus, the size of the anuran presumably influences the selectivity of the auditory periphery due to the

differences in the sizes and masses of the peripheral auditory structures found among the various species.

A relationship between auditory selectivity and body size has not been demonstrated ontogenetically within a given species, in spite of the fact that there can be a dramatic increase in body size as well as in the size of the peripheral auditory structures during post-metamorphic development. In maturing bullfrogs, for example, there is a 10-fold increase in the diameter of the tympanum (Fig. 1). The sizes of the middle ear cavity, mouth cavity and columellar bones also increase with age and body size, and thus the acoustic transmission characteristics of these structures are presumably altered. These observations suggest that the frequency selectivity and sensitivity of the bullfrog auditory periphery may undergo some changes during the development following metamorphosis.

Previous single unit studies of the frequency selectivities of adult VIIIth nerve fibres have revealed that three populations of auditory fibres generally exist (see Capranica, 1976 for review): low-frequency selective fibres which show two-tone inhibition, and mid- and high-frequency selective fibres which are non-inhibitable. With this in mind, the purposes of this study were: i) to examine the acoustic response properties of single primary auditory fibres from early post-metamorphic bullfrogs and ii) to compare these response properties to those of adult bullfrogs in order to gain an understanding of the functional development of the anuran auditory periphery. Evidence is presented that a number of the response properties change during post-metamorphic development.

METHODS

Two groups of bullfrogs (Rana catesbeiana) were obtained from Charles Sullivan (Nashville, Tenn.): adults and early post-metamorphic frogs with snout-vent lengths ranging from 152-178 mm and 27-46 mm, respectively. During surgery the animals were anaesthetized by surrounding them in crushed ice (Kaplan, 1969), and the VIIIth nerve was exposed by a dorsal approach (for details, see Feng, 1980). Briefly, the skull overlying the nerve was removed, and the choroid plexus was carefully laid medially to expose the nerve. The dural membranes surrounding the nerve were removed with a sharpened tungsten needle. The dorsal approach has the advantage of recording VIIIth nerve activity with the mouth cavity closed, thus preserving its acoustic property.

The animals were allowed to recover from hypothermia for 2-3 hours and were later immobilized with an intramuscular injection of d-tubocurarine chloride (3 mg/ml) during the recording session. Adults were injected initially with 4 ml/Kg body weight whereas froglets received 2 ml/Kg. Periodic injections were administered to the animal during the recording session to maintain immobilization. Wet gauze was placed over the animal to facilitate cutaneous respiration and prevent evaporative water loss. Blood flow through the vessels of the choroid plexus served as a useful monitor of the physiological condition of the animal.

Animals were placed in a sound proof room (Tracoustics) which was maintained at 20-22°C. Single unit responses were recorded using 3 M NaCl-filled glass micropipettes (10-20 MΩ). The electrodes were advanced by a hydraulic microdrive (Kopf 1207) from outside of the sound proof room. Extracellular action potentials were amplified, filtered from background

noise, and displayed on a storage oscilloscope (Tektronix 5115) as well as audiomonitored. Firing rates were determined by a gated electronic counter (Coulbourn R11-25). Neural responses were recorded on magnetic tape (Akai GX-630D-SS tape recorder) for off-line computer analysis.

Acoustic stimuli were presented through an earphone (Beyer DT48) enclosed in a brass housing, which also held a condenser microphone (Bruel and Kjaer 4134) with a 1/8 inch probe tube attachment. The earphone housing was sealed around the tympanum with non-toxic silicone rubber cement (General Electric RTV-162) to provide a closed acoustical system. The absolute sound pressure level at the tympanum was monitored on a sound level meter (Bruel and Kjaer 2209). The measured sound pressure level was corrected for the frequency response of the probe tube to give the actual sound pressure level at the tympanum in dB SPL with reference to 2×10^{-5} N/m^2. The frequency response of the acoustic system was flat within $\pm$ 5 dB over the range of 100-4500 Hz.

Acoustic stimuli consisted of white noise and pure tones. These stimuli had a duration of 100 ms and a symmetrical rise-fall time of 5 ms. Stimuli were presented at 1-1.2 s intervals. The intensity of the acoustic stimuli was controlled with a Hewlett-Packard 350D attenuator.

White noise at a sound pressure level of 110 dB SPL was used as a search stimulus for exciting auditory fibres of the VIIIth nerve. When an isolated unit responded to the search stimulus, single pure tones of varying frequencies and intensities were presented to determine the tuning curve of the unit and its best excitatory frequency (BEF), i.e., the frequency at which the unit had its lowest threshold of excitation.

RESULTS

Response characteristics of a total of 242 primary auditory fibres from 11 adults and 346 auditory fibres from 22 froglets were studied. All auditory fibres responded tonically to bursts of pure tones for all intensities above threshold levels as shown by the post-stimulus time histograms in Fig. 2. The response of the low frequency auditory fibres was phased-locked to the stimulus in both groups of frogs (Fig. 2). Spontaneous activity in the absence of any acoustic stimulus was noted in most auditory fibres from adults and froglets.

Distribution of Best Excitatory Frequencies

The BEFs of adult auditory fibres fell into three populations (Fig. 3a): a low frequency population with a peak around 100-300 Hz, a mid-frequency population with a peak around 500-600 Hz and a high-frequency population with a peak around 1200-1400 Hz. The high-frequency selective fibres in adults all had BEFs of less than 1700 Hz. As in the adults, three populations of auditory fibres were distinguishable from early post-metamorphic frogs (Fig. 3b). However, the distributions of these populations covered broader frequency ranges than those in adults. For instance, the BEFs of the low-frequency population in post-metamorphic frogs ranged from 100 to 800 Hz. The BEFs of the mid-frequency selective fibres ranged from 900 to 1700 Hz, and those of the high-frequency population ranged from 1800 to 2500 Hz. Note that the distribution of the froglet high-frequency population was outside that of the adults.

Each adult auditory fibre possessed a V-shaped tuning curve with a distinct BEF. Typical tuning curves from adults are shown in Fig. 4a.

It is interesting to note that the high-frequency selective fibre had an upper cut-off frequency at 96 dB SPL of 3125 Hz. In general, the upper cut-off frequencies at about 100 dB SPL were below 3500 Hz and no adult high-frequency selective fibre could be stimulated beyond 4000 Hz at any intensity.

The majority of froglet auditory fibres possessed V-shaped tuning curves, although some high threshold units having broader tuning curves mimicking a U-shape were also found. Typical tuning curves of the three populations from froglets are shown in Fig. 4b. Inspection of the tuning curve for the high-frequency selective fibre (Fig. 4b) showed that the upper cut-off frequency was 5500 Hz at 86 dB SPL. The upper frequency limits of all high frequency selective fibres at about 100 dB SPL was 6000 Hz, which was considerably higher than in adults. The tuning curve of the mid-frequency selective fibre in the froglet (unit F2, Fig. 4b) had a high cut-off frequency of 3500 Hz at 88 dB SPL and resembled that of the high-frequency selective fibre of the adult (unit A3, Fig. 4a). Such a comparison is more clearly shown in Fig. 5. The tuning curve from an adult high-frequency selective fibre (dashed line) and that of a froglet mid-frequency selective fibre (solid line) with similar BEFs and thresholds of excitation were almost identical.

A more quantitative analysis was undertaken to assess the sharpness of the tuning curves in the two groups of frogs. An indicator of the sharpness of tuning is the $Q_{10\ dB}$ value, where $Q_{10dB} = \frac{BEF}{\text{bandwidth at 10dB above threshold}}$ (Kiang et al., 1965). Higher Q_{10dB} values indicate sharper tuning curves. The $Q_{10\ dB}$ value was measured

for each unit whenever possible, and the distributions of these values for 77 adult and 107 froglet auditory units are shown in Fig. 6. Values of $Q_{10\ dB}$ ranged from 0.5 to 3.0 and 0.1 to 2.6 for adults and froglets, respectively. There was no significant differences in the means of $Q_{10\ dB}$ values between the two groups of frogs (ANOVA, $p > 0.05$). Nevertheless, there was a larger number of units with lower $Q_{10\ dB}$ values appearing in froglets than in adults (see outlined box, Fig. 6). These broadly tuned units generally had high thresholds of excitation (units F4-F6, Fig. 3b).

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00

Distributions of Thresholds

Thresholds of excitation at the unit's BEF ranged widely for both adults (22-103 dB SPL, Fig. 7a) and froglets (22-132 dB SPL, Fig. 7b), with some noticeable differences in distribution as shown by the outlined boxes in Fig. 7. It can be seen that while few adult units had thresholds greater than 100 dB SPL, 20% of the low-frequency selective fibres in the froglets, having BEFs less than 800 Hz, had thresholds greater than 100 dB SPL (box 1). These high thresholds cannot be attributed to a decrease in the physiological condition of the frog during the recording session, since units having lower thresholds were often subsequently encountered. High-frequency selective fibres in adults, having BEFs between 1000 to 1700 Hz, had thresholds clustered between 20 and 60 dB SPL. However, thresholds of froglet auditory fibres having BEFs in the same frequency range were widely distributed between 20-105 dB SPL, and 39% of these fibres had thresholds exceeding 60 dB SPL (box 2). Finally, box 3 in Fig. 7 shows the distribution of thresholds for the high-frequency selective fibres that had BEFs between 1700 to 2500 Hz in the froglet. This population

is absent in the adult. Note that the thresholds of the high-frequency population in adults is distributed over a 40 dB range, whereas in the froglets the high-frequency selective fibres had thresholds distributed over a 70 dB range.

Two-Tone Inhibition Properties

The suppression of the auditory response of a primary fibre to an excitatory pure tone by the addition of a second tone has been defined as two-tone inhibition (Sachs and Kiang, 1968). A total of 90 auditory fibres from 6 froglets and 52 fibres from 3 adults were tested for two-tone inhibition (Fig. 3). Units of the low-frequency population were all inhibitable in adults as well as in froglets with a few exceptions. These exceptions were low-frequency selective fibres having high thresholds of excitation. Note that in the froglets, low-frequency selective fibres with BEFs extending to 675 Hz, but mostly below 500 Hz, exhibited two-tone inhibition. Data from the adults showed that generally only units with BEFs below 200 Hz, but a few extending to 600 Hz, exhibited two-tone inhibition. On the other hand, mid- and high-frequency selective units in both groups of frogs, regardless of their thresholds of excitation, did not show two-tone inhibition.

The best inhibitory frequencies of individual units were examined, and data from the adults and froglets showed some differences. The excitatory tone was fixed at 10 dB above threshold at the unit's BEF, and the frequency and intensity of the second tone was varied to find the inhibitory tuning curve and the best inhibitory frequency (BIF), i.e., the frequency with the lowest threshold to reduce the excitatory response by 50%. The BIF was always above the BEF of each unit, and generally

the inhibitory tuning curve was outside of the excitatory tuning curve at the high frequency side. For example, Fig. 8a illustrates excitatory and inhibitory tuning curves of an auditory unit obtained from a 28 mm froglet that had a BEF of 340 Hz and a threshold of 54 dB SPL. The auditory response to this tone at 64 dB SPL (10 dB above threshold) was inhibited with the addition of a second tone. The BIF was 1370 Hz at a threshold of 92 dB SPL, which was 28 dB (ΔI) above the intensity of the 340 Hz tone. The excitatory and inhibitory responses of this unit are shown by the post-stimulus time histograms in Fig. 8. The excitatory response at 340 Hz at 64 dB SPL (10 dB above threshold) was tonic and phase-locked. The addition of a second tone at 1370 Hz at 92 dB SPL suppressed the excitatory response by half and completely inhibited it at 97 dB SPL (Fig. 8). The distributions of BIFs are shown in Fig. 9. The BIFs of the low-frequency selective fibres recorded from froglets ranged from 700 to 1700 Hz, which corresponded to the BEF range of mid-frequency selective fibres in the froglets (and the high-frequency selective fibres in the adults). On the other hand, BIFs in the adults ranged from 485 to 990 Hz, corresponding to the mid-frequency BEF range for adults. The ΔI values for two-tone inhibition ranged from 3 to 40 dB and 8 to 42 dB in froglets and adults, respectively.

DISCUSSION

The results of the present study reveal some of the basic differences and similarities in the response characteristics of primary auditory fibres in adults and early post-metamorphic bullfrogs. In each group studied, three populations of auditory fibres were found. The shapes and sharpness of the tuning curves of these populations as well as the temporal firing

patterns were similar, but the distributions of BEFs differed. The BEF range of mid-frequency selective fibres in the froglets corresponded to that of the adult high-frequency selective fibres. The range of BEFs of the froglet high-frequency selective fibres extended well beyond the range of BEFs of the adult high-frequency population. The upper limit of BEFs was 1700 Hz in adults and 2500 Hz in froglets, and the upper limit of the auditory response at 100 dB SPL was 3500 Hz and 6000 Hz in adults and froglets, respectively. These results clearly indicate that early post-metamorphic bullfrogs respond to higher frequencies than do adults. This is in agreement with trends observed in comparative studies (Loftus-Hills and Johnstone, 1970; Loftus-Hills, 1973), where smaller species were shown to be more responsive to higher frequencies than larger species. In addition, it is interesting to note that the sensitivity of the high-frequency population in froglets (threshold range of 30 to 100 dB SPL) was poorer in comparison to the sensitivity of the high-frequency population in adults (threshold range of 20 to 60 dB SPL). This pattern is consistent with the trends observed from comparative studies, i.e., higher frequency selectivity in smaller species is associated with higher thresholds (Loftus-Hills, 1973; Capranica et al., 1973).

In addition to the differences in high-frequency selectivity observed, there were notable differences in the sensitivity and distribution of the low-frequency population of primary fibres between the two groups of frogs. Although the thresholds of the low-frequency selective fibers were widely distributed in both groups, high threshold units were more commonly observed in froglets. Whereas the BEFs of the great majority of low-frequency selective fibres of the adults fell within a range of 100 to 450 Hz, the low-

frequency population in the froglets had a broader range of 100 to 800 Hz. Only low-frequency selective fibres from the two groups of frogs showed two-tone inhibition. The BIFs of the adults ranged from 485 to 990 Hz and were in close agreement with previous studies on the bullfrog (Frishkopf, et al. 1968; Liff and Goldstein, 1970; Feng et al. 1975), whereas the range of BIFs of the froglets was 700 to 1700 Hz. It is intriguing that in each group, the BIF range corresponded to the range of BEFs of mid-frequency selective fibres.

These results are in contrast to earlier work by Frishkopf et al. (1968) in which no correlation between the body size and frequency selectivity of the bullfrog peripheral auditory system was observed. More recently, Capranica and Moffat (1980) also failed to observe any significant change in the distribution of BEFs from American toads ranging from 10 to 50 g body weight. We have, however, additionally studied the BEFs of 117 single auditory fibres from the VIIIth nerve of 6 intermediate size bullfrogs (62-67 mm) and found that the high-frequency population was centered around 1800 to 2000 Hz (which was intermediate between froglets and full-size adults) with no fibres having BEFs above 2050 Hz. The low- and mid-frequency populations of these intermediate frogs were practically identical to those of the adults. Thus, it appears that the change in the distribution of BEFs during the post-metamorphic growth of the bullfrog is a continuous and gradual process. Therefore, the degree to which the frequency selectivity of the auditory system can be correlated with body size may reflect the degree to which the peripheral structures change with body size. It is noteworthy that Narins and Capranica (1976) have also shown that in the Puerto Rican treefrog,

where a sexual dimorphism in body size exists, the distribution of high-frequency selective fibres differed between adult males and females. Correlations between body size and frequency selectivity in a growing anuran may have gone undetected in previous studies if only subtle differences existed in the peripheral auditory structures among the animals used.

Behavioural studies have advocated the functional significance of the mating call structure in adult bullfrogs (Capranica, 1965). The effect adult calls have on froglets, however, has not been demonstrated. The froglets are not reproductively mature (Howard, 1978), and they obviously would not be participating in mating. However, it could be advantageous for froglets to detect the adult mating calls, since larger adults are potential predators. Interestingly enough, the low- and mid-frequency populations of auditory fibres found in the froglet corresponded to the dominant spectral energies present in the adult mating call (Capranica, 1965). In addition, the BIFs observed in the froglets were in the frequency range of 1000 to 1700 Hz, which corresponded to the dominant high-frequency peak of adult mating calls (Capranica, 1965). Thus, it is possible that the presence of two-tone inhibition in the froglet primary afferent fibers may provide a peripheral basis for predator avoidance. The biological significance of the high-frequency population of auditory fibres in early post-metamorphic bullfrogs is also unclear. It is not known whether froglets produce sounds.

The differences in the frequency selectivities and sensitivities of the peripheral auditory systems of adults and froglets raise questions as to what morphological mechanisms are responsible for these observed changes during post-metamorphic development. A dramatic difference can be seen in the size of the tympanum between the two groups (Fig. 1),

and differences in the sizes of the middle ear cavity and columellar bones are also obvious. These morphological changes presumably alter the transmission of acoustic energy to the inner ear. Unfortunately, the middle ear transfer functions have not been studied in the two groups of bullfrogs. However, mechanical measurements of the tympanum and middle ear displacements as a function of frequency from various anuran species indicate that these structures act as a low-pass filter and dictate the upper cut-off frequency of the peripheral auditory system (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). Furthermore, smaller species are correlated with higher upper cut-off frequencies. Thus, it is likely that the extended frequency range of froglets is in part attributable to a higher upper cut-off frequency in the middle ear response than that found in adults. The increase in the mass of the middle ear with body size would primarily attenuate the transmission of high-frequency sounds.

In addition to the changes in the size of the tympanum and middle ear structures, the volume of the mouth cavity also shows a dramatic increase in size. Recently it has been suggested that the resonance property of the mouth cavity plays an essential role in determining the frequency selectivity of the peripheral auditory system (Chung et al. 1978; Pettigrew et al. 1978). This hypothesis was later refuted by Moffat and Capranica (1978) and by Gerhardt and Mudry (1980). The results from the adult bullfrogs using the dorsal recording approach (with the mouth cavity closed) are in close agreement with those obtained using a ventral recording approach in which the mouth cavity was held opened (Feng et al. 1975). Thus, it is unlikely that the resonance characteristic of the mouth cavity is an important factor in determining the frequency selectivity of the auditory system. Therefore, observed differences between the frequency selectivities of the adult and froglet auditory peripheries cannot be attributed

to the differences in the volumes of the mouth cavities. It is worth noting, however, that the mouth cavity does play an essential role in generating the directional cues of the peripheral auditory system (Chung et al. 1978; A. S. Feng and W. P. Shofner, in preparation).

The inner ear is another possible source for the variations in frequency selectivities and sensitivities observed between adults and froglets. Anurans are unique among the vertebrates in that they possess two auditory organs (Geisler et al. 1964) selective to different frequency ranges. It has been demonstrated in the adult bullfrog that the low- and mid-frequency selective fibres originate from the amphibian papilla, whereas the basilar papilla gives rise to the high-frequency selective fibres (Feng et al. 1975; Lewis et al. 1980). It is probable that the low-frequency population in froglets (100-800 Hz) is derived from the amphibian papilla, since fibres selective to this frequency range also originate in the amphibian papilla in adults. However, the origins of the mid-frequency population of the froglet cannot be directly compared to the adult organization, since the range of froglet mid-frequency selective fibres was the same as that of the adult high-frequency (basilar papilla) population. Nevertheless, the observation that the range of BIFs corresponded to the range of mid-frequency selective fibres in both adults and froglets suggests that the froglet mid-frequency population is probably derived from the amphibian papilla. Therefore, the froglet high-frequency population presumably originates from the basilar papilla. If these suppositions were correct, however, then the adult basilar papilla, which is believed to act as a simple resonating structure (Capranica and Moffat, 1977), would have different resonance characteristics from those of froglets. Thus, some morphological changes in the basilar papilla must

occur during post-metamorphic development to account for the varying resonance characteristics. Interestingly, Li and Lewis (1974) have shown that the size of the basilar papilla is smaller in tadpoles than in adults. It is possible that this relationship also holds true for early post-metamorphic and adult bullfrogs but further investigations are necessary to extend these findings to froglets and to clarify the basis for the changes in frequency selectivities and sensitivities observed between the two groups of frogs.

Fig. 1. The diameter of the tympanum as a function of body size for the bullfrog. Measurements were taken using a caliper. Females (O); males (●); sex not determined (☆).

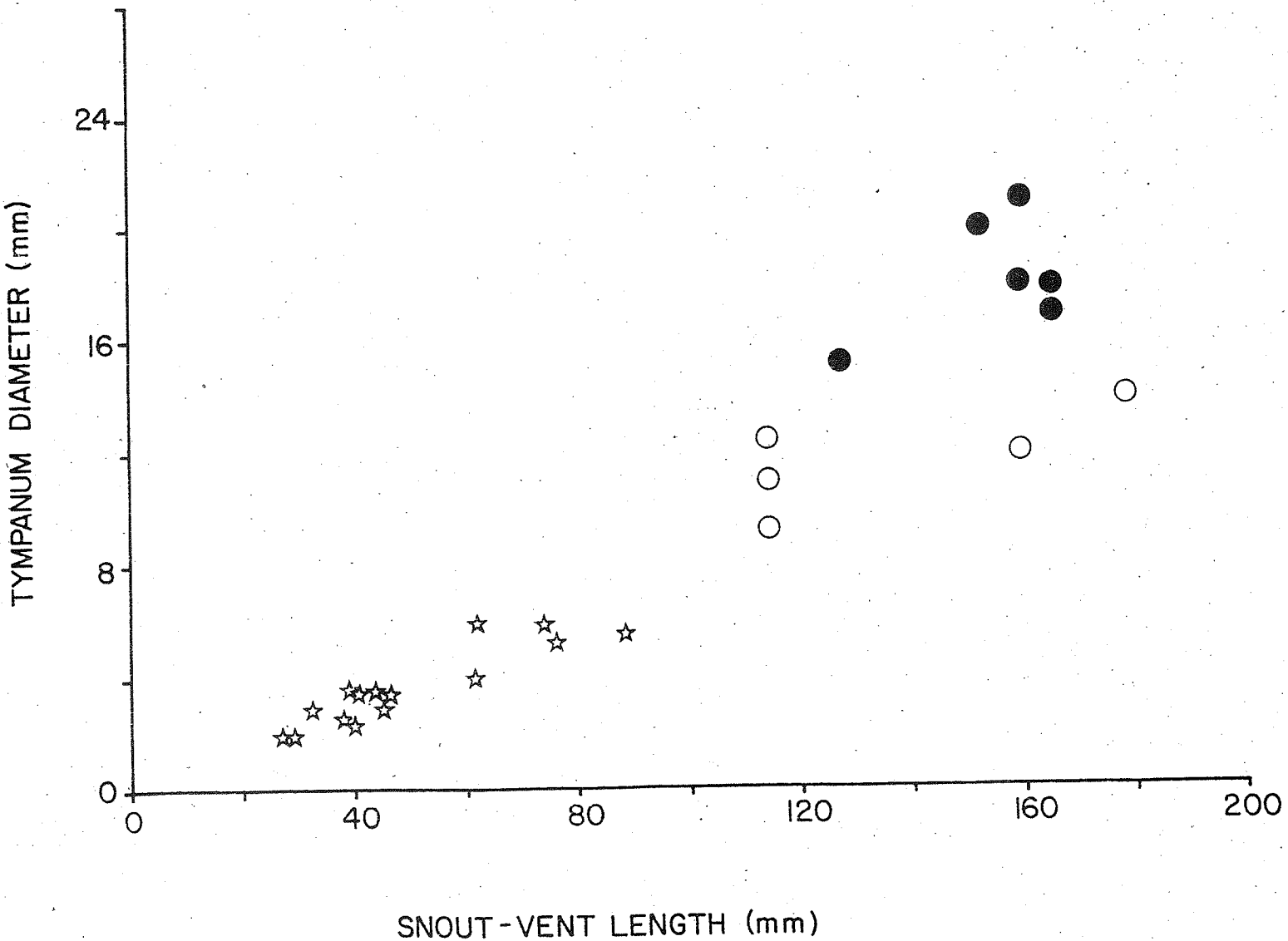

TYMPANUM DIAMETER (mm)
24
16
8
0
0
40
80
120
160
200
SNOUT-VENT LENGTH (mm)

Fig. 2. Post-stimulus time histograms (PSTHs) of primary auditory fibres showing the temporal response characteristics of the fibres to 100 ms tone bursts at each unit's best excitatory frequency at 10 dB above threshold for 20 presentations.

a. Tonic and phase-locked responses of an adult low-frequency selective fibre. (BEF=210 Hz, threshold=96 dB SPL).

b. Tonic and phase-locked responses of a froglet low-frequency selective fibre. (BEF=250 Hz, threshold=82 dB SPL).

c. Tonic responses of an adult high-frequency selective fibre. (BEF=1375 Hz, threshold=47 dB SPL).

d. Tonic responses of a froglet high-frequency selective fibre. (BEF=2210 Hz, threshold=57 dB SPL).

Bin width=0.5 ms.

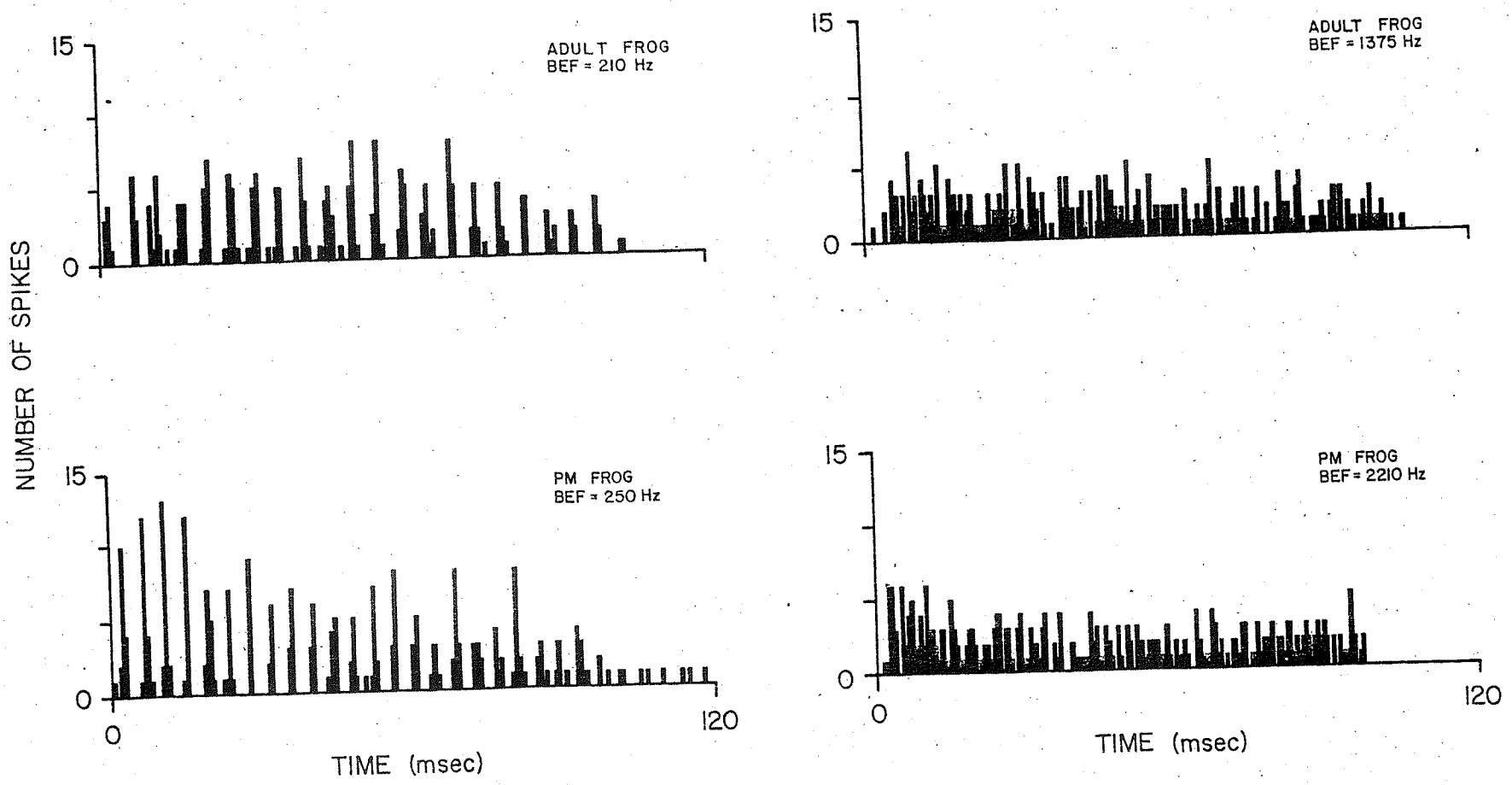

NUMBER OF SPIKES
15
0
ADULT FROG
BEF = 210 Hz
15
0
ADULT FROG
BEF = 1375 Hz
15
0
PM FROG
BEF = 250 Hz
0
120
TIME (msec)
15
0
PM FROG
BEF = 2210 Hz
0
120
TIME (msec)

Fig. 3. Histograms of the distributions of best excitatory frequencies. Bin width=100 Hz.

a. BEF distribution of 242 adult primary units. Low-frequency selective fibres with BEFs ranging from 100 to 400 Hz generally showed two-tone inhibition; mid-frequency selective fibres with BEFs ranging from 500 to 900 Hz generally were non-inhibitable; high-frequency selective fibres with BEFs ranging from 1000 to 1700 Hz were non-inhibitable.

b. BEF distribution of 346 froglet fibres. Low-frequency selective fibres with BEFs ranging from 100 to 800 Hz generally showed two-tone inhibition; mid-frequency selective fibres with BEFs ranging from 1000-1700 Hz were non-inhibitable; high-frequency selective fibers with BEFs ranging from 1800 to 2500 Hz were non-inhibitable.

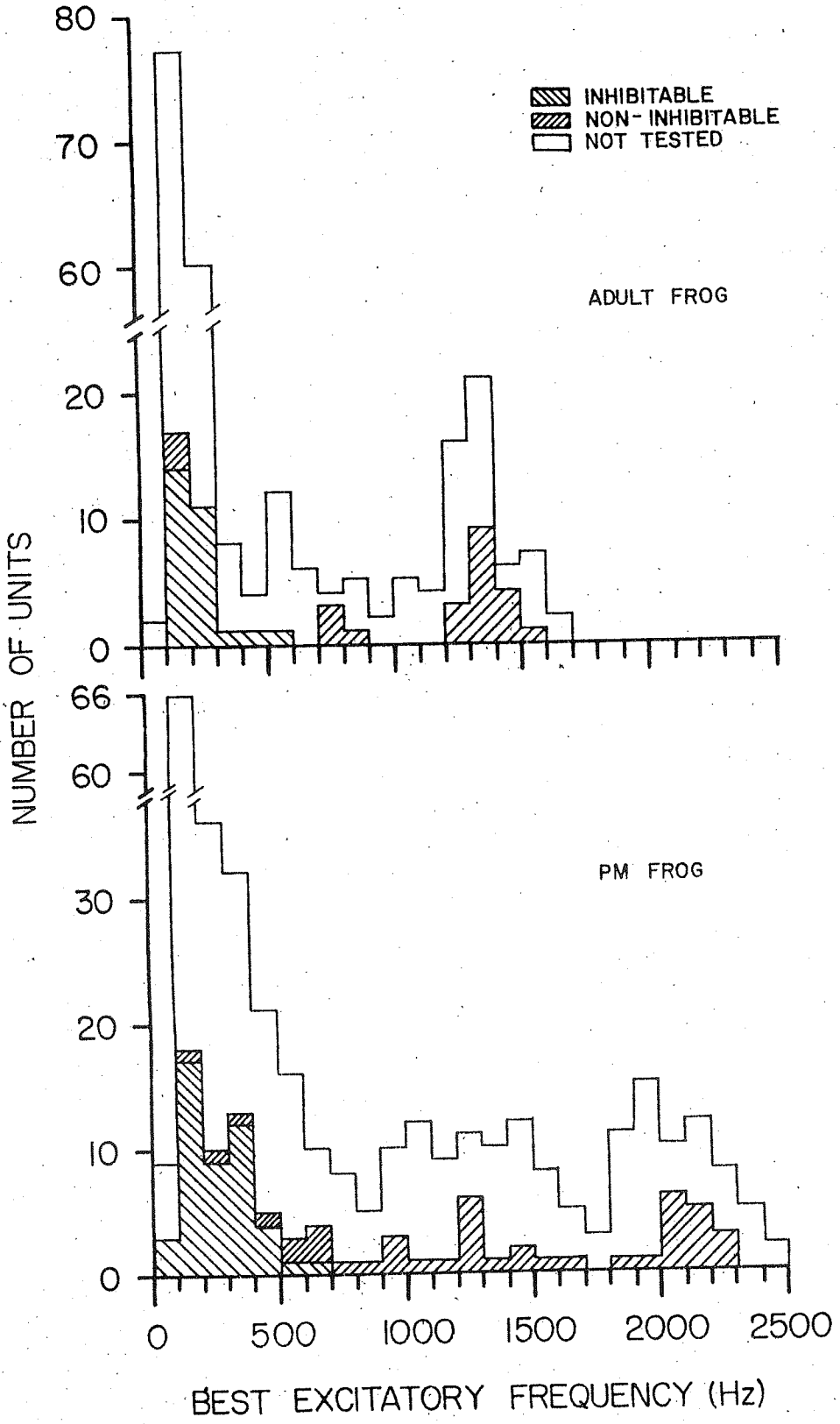

INHIBITABLE
NON-INHIBITABLE
NOT TESTED
ADULT FROG
PM FROG
NUMBER OF UNITS
80
70
60
20
10
0
66
60
30
20
10
0
0
500
1000
1500
2000
2500
BEST EXCITATORY FREQUENCY (Hz)

Fig. 4. Representative tuning curves from the three populations of auditory fibres in adults and froglets.

a. Tuning curves obtained from adults. Units A1, A2 and A3 had BEFs of 295 Hz, 800 Hz and 1350 Hz and thresholds of 30 dB SPL, 48 dB SPL and 40 dB SPL, respectively.

b. Tuning curves obtained from froglets. Units F1, F2 and F3 had BEFs of 324 Hz, 1250 Hz and 2175 Hz and thresholds of 40 dB SPL, 33 dB SPL and 46 dB SPL, respectively. Units F4, F5 and F6 represent broadly tuned, high threshold units and had BEFs of 390 Hz, 1000 Hz and 2010 Hz with thresholds of 98 dB SPL, 103 dB SPL and 92 dB SPL, respectively.

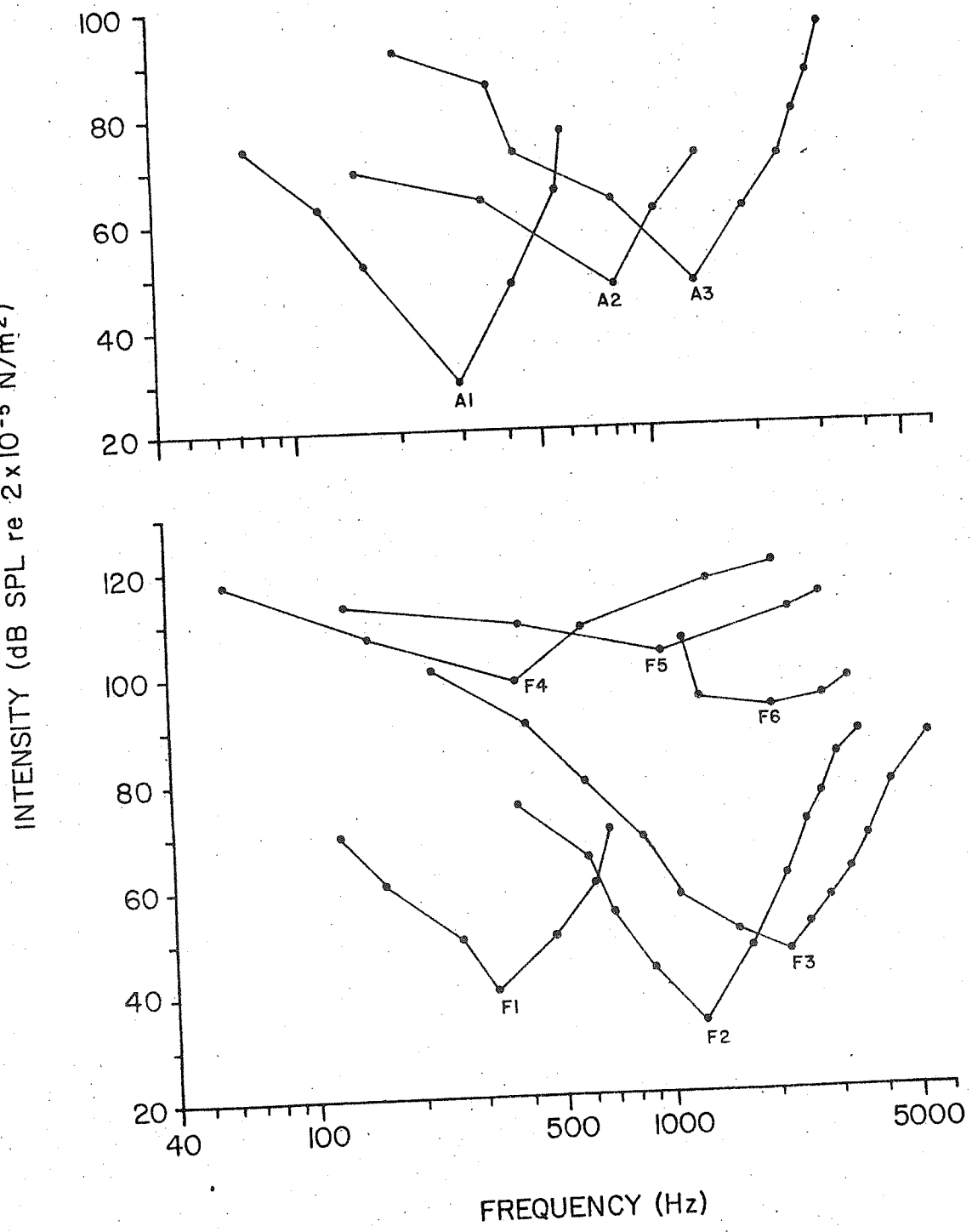

100
80
60
40
20
A1
A2
A3
120
100
80
60
40
20
F1
F2
F3
F4
F5
F6
40
100
500
1000
5000
INTENSITY (dB SPL re 2x10⁻⁵ N/m²)
FREQUENCY (Hz)

Fig. 5. Similarity in the tuning curves of an adult high-frequency selective fibre (dashed line) and a froglet mid-frequency selective fibre (solid line). The adult fibre had a BEF of 1350 Hz and a threshold of 41 dB SPL, and the froglet fibre had a BEF of 1345 Hz and a threshold of 39 dB SPL.

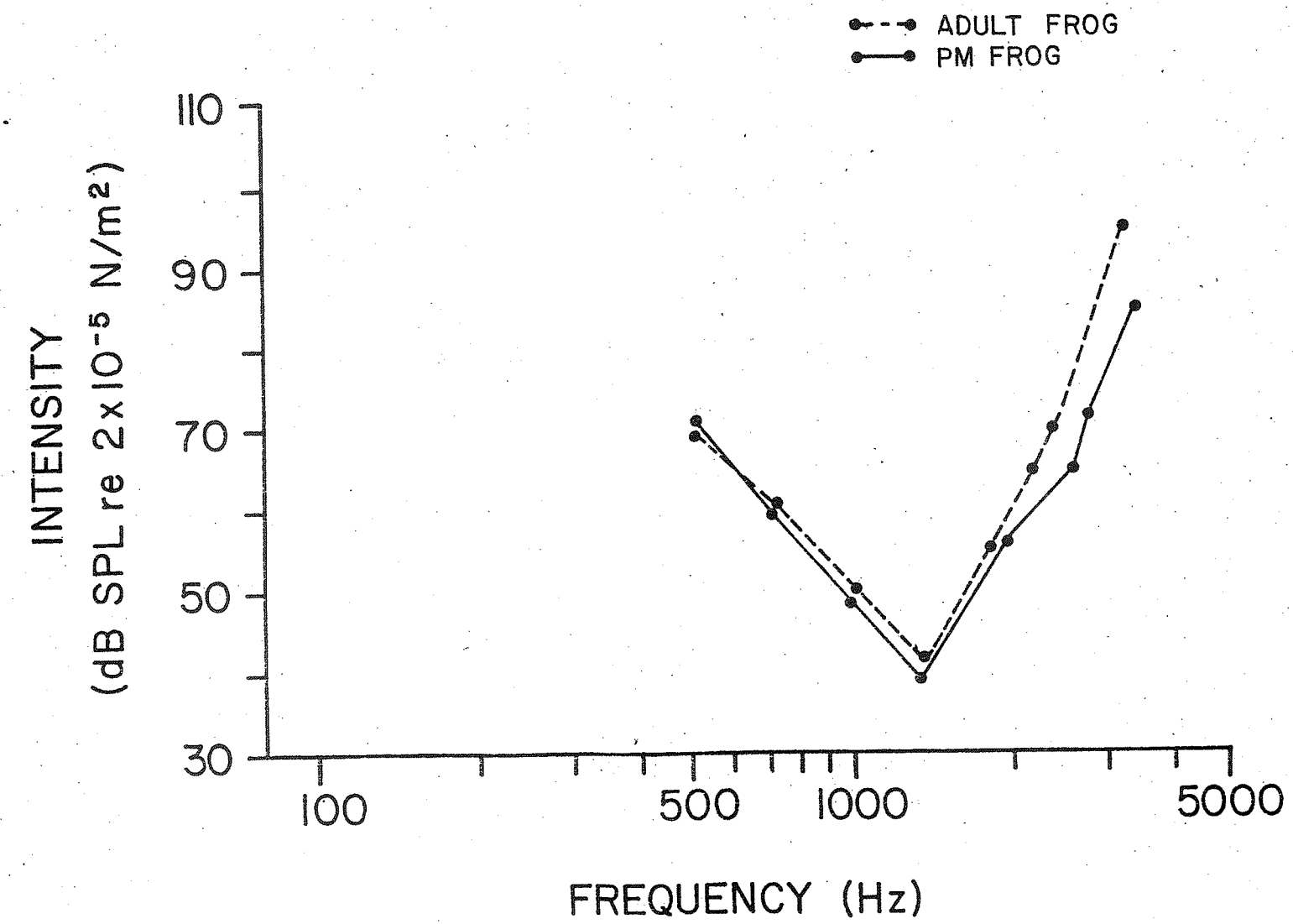

ADULT FROG
PM FROG
INTENSITY
(dB SPL re 2x10⁻⁵ N/m²)
110
90
70
50
30
100
500
1000
5000
FREQUENCY (Hz)

Fig. 6. Scatter diagrams of $Q_{10\ dB}$ values in adults and froglets as a function of unit's BEF.

a. Distribution of adult values (N=77) with a range of 0.5 to 3.0. The outlined box indicates the absence of units with $Q_{10\ dB}$ values less than 0.5.

b. Distribution of froglet values (N=107) with a range of 0.1 to 2.6. The outlined box indicates that units with $Q_{10\ dB}$ values less than 0.5 were found.

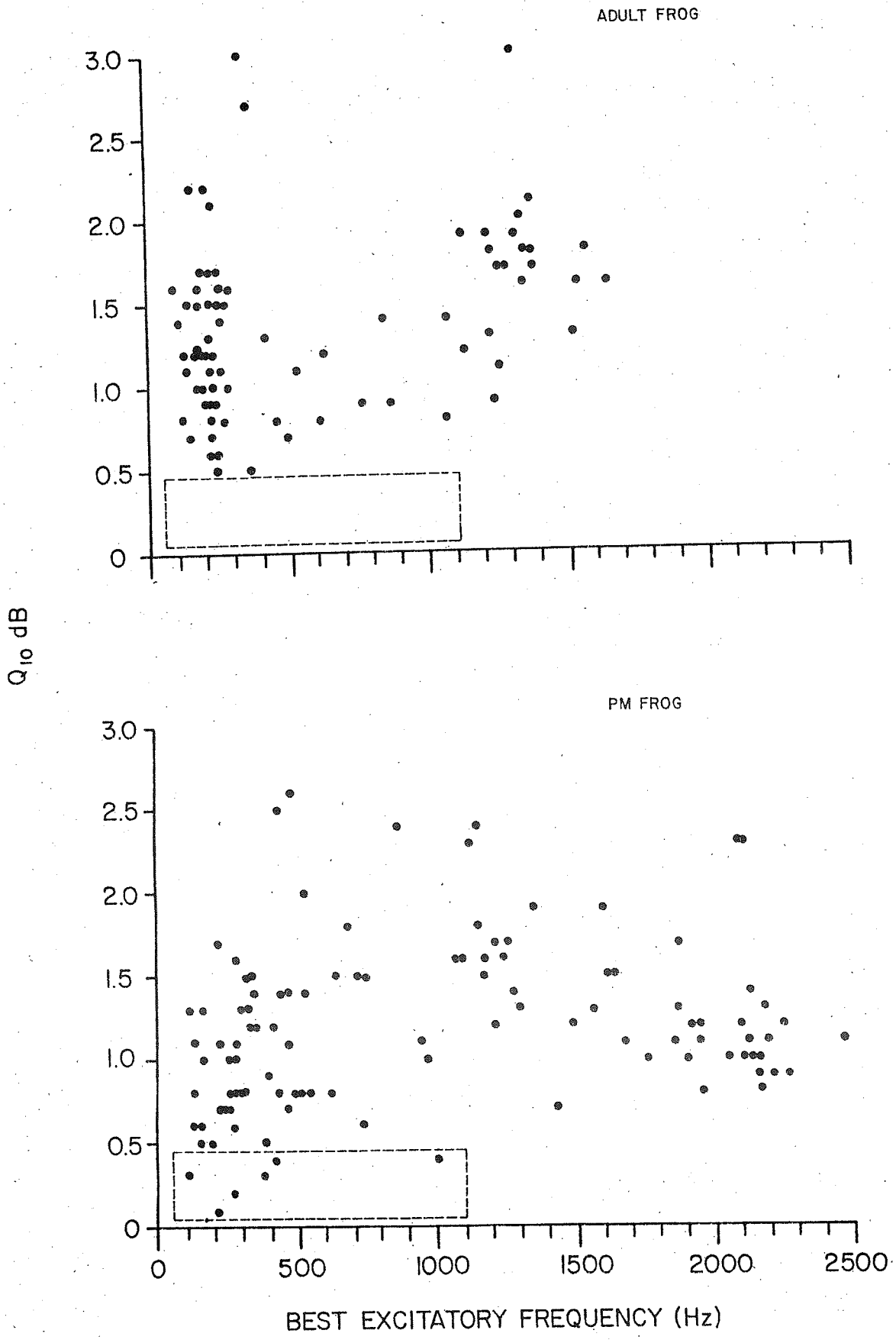

ADULT FROG
3.0
2.5
2.0
1.5
1.0
0.5
0
Q_{10} dB
PM FROG
3.0
2.5
2.0
1.5
1.0
0.5
0
0
500
1000
1500
2000
2500
BEST EXCITATORY FREQUENCY (Hz)

Fig. 7. Scatter diagrams of the distributions of thresholds in adults and froglets.

a. Thresholds of adult fibres (N=242) ranged from 22 to 103 dB SPL. Box 1 indicates that few units having BEFs between 100 and 800 Hz with thresholds above 100 dB SPL were found. Box 2 indicates that few units having BEFs from 1000 to 1700 Hz with thresholds above 60 dB SPL were found. Box 3 indicates that no units with BEFs above 1700 Hz were found.

b. Thresholds of froglet fibres (N=346) ranged from 22 to 132 dB SPL. Box 1 indicates that units with BEFs from 100 to 800 Hz having thresholds above 100 dB SPL were commonly found. Box 2 indicates that units with BEFs between 1000 and 1700 Hz having thresholds above 60 dB SPL were often found. Box 3 shows the distribution of thresholds of units having BEFs from 1700 to 2500 Hz.

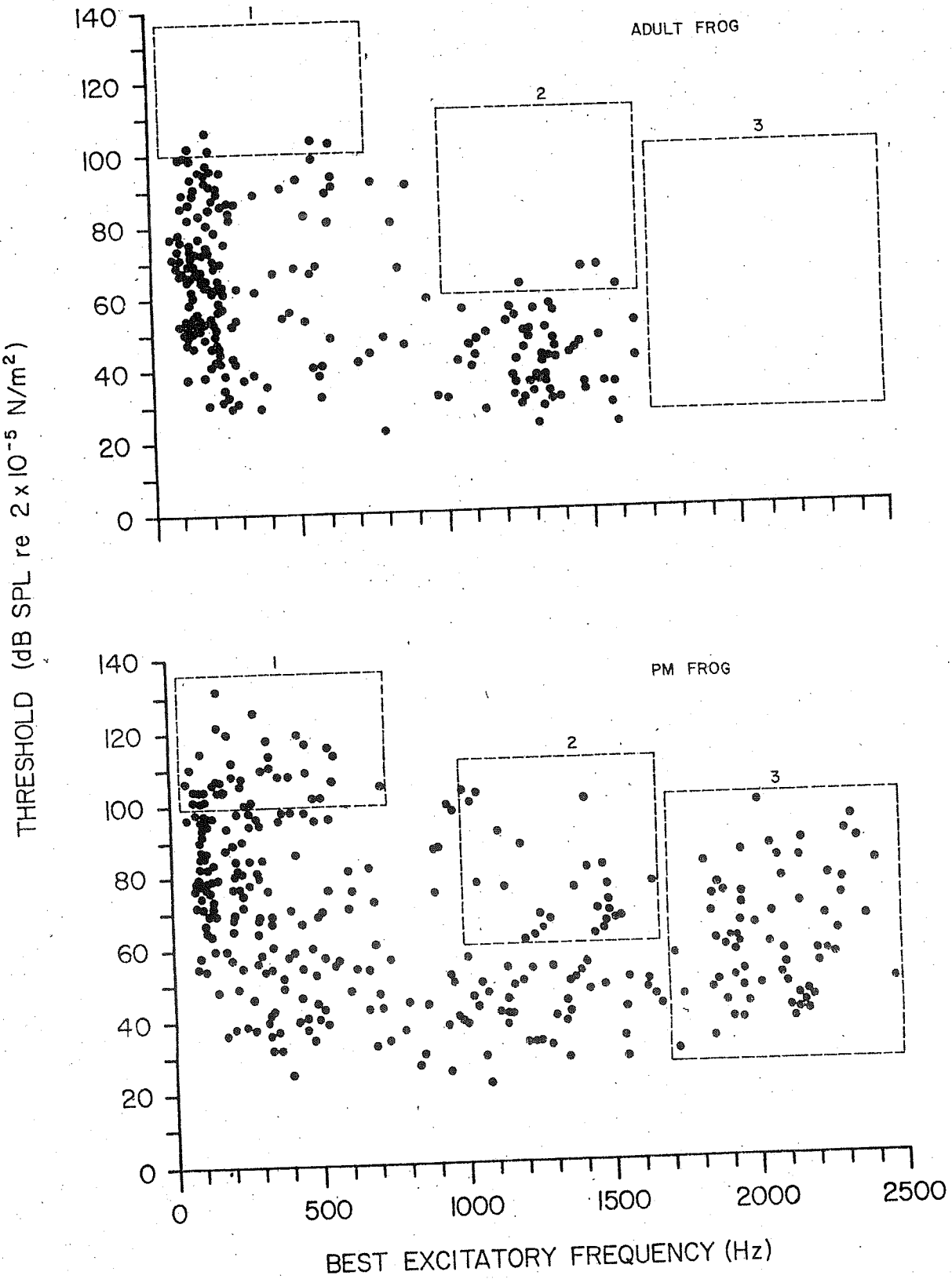

ADULT FROG
PM FROG
1
2
3
140
120
100
80
60
40
20
0
0
500
1000
1500
2000
2500
THRESHOLD (dB SPL re 2 x 10⁻⁵ N/m²)
BEST EXCITATORY FREQUENCY (Hz)

Fig. 8. Two-tone inhibitory response characteristics from a low-frequency selective fibre of a post-metamorphic frog.

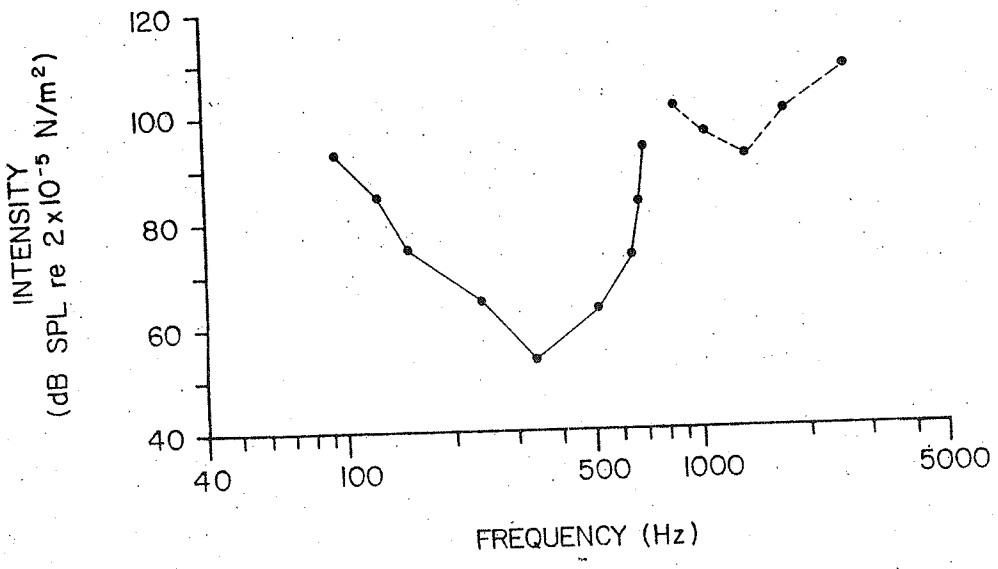
a. Excitatory and inhibitory tuning curves are shown by the solid line and dashed line, respectively. BEF=340 Hz at an excitatory threshold of 54 dB SPL. BIF=1370 Hz at an inhibitory threshold of 92 dB SPL.

b. PSTH of the unit's excitatory response to a 340 Hz tone at 64 dB SPL (10 dB above threshold).

c. PSTH of the unit's inhibitory response to a combination of a 340 Hz tone at 64 dB SPL and a 1370 Hz tone at 92 dB SPL (threshold of inhibition).

d. PSTH of the unit's inhibitory response to a combination of a 340 Hz tone at 64 dB SPL and a 1370 Hz tone at 97 dB SPL (5 dB above the threshold of inhibition).

All PSTHs shown are for 20 presentations of a 100 ms tone burst. Bin width=0.5 ms.


INTENSITY
(dB SPL re 2×10^{-5} N/m^2)
120
100
80
60
40
40
100
500
1000
5000
FREQUENCY (Hz)

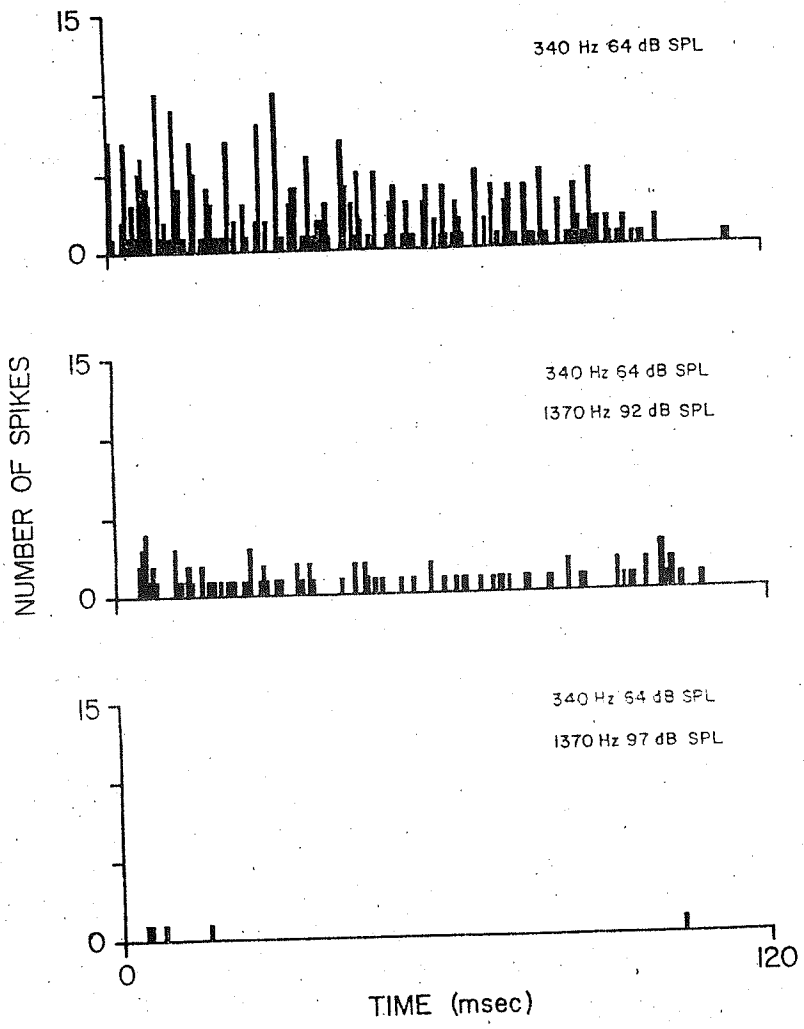
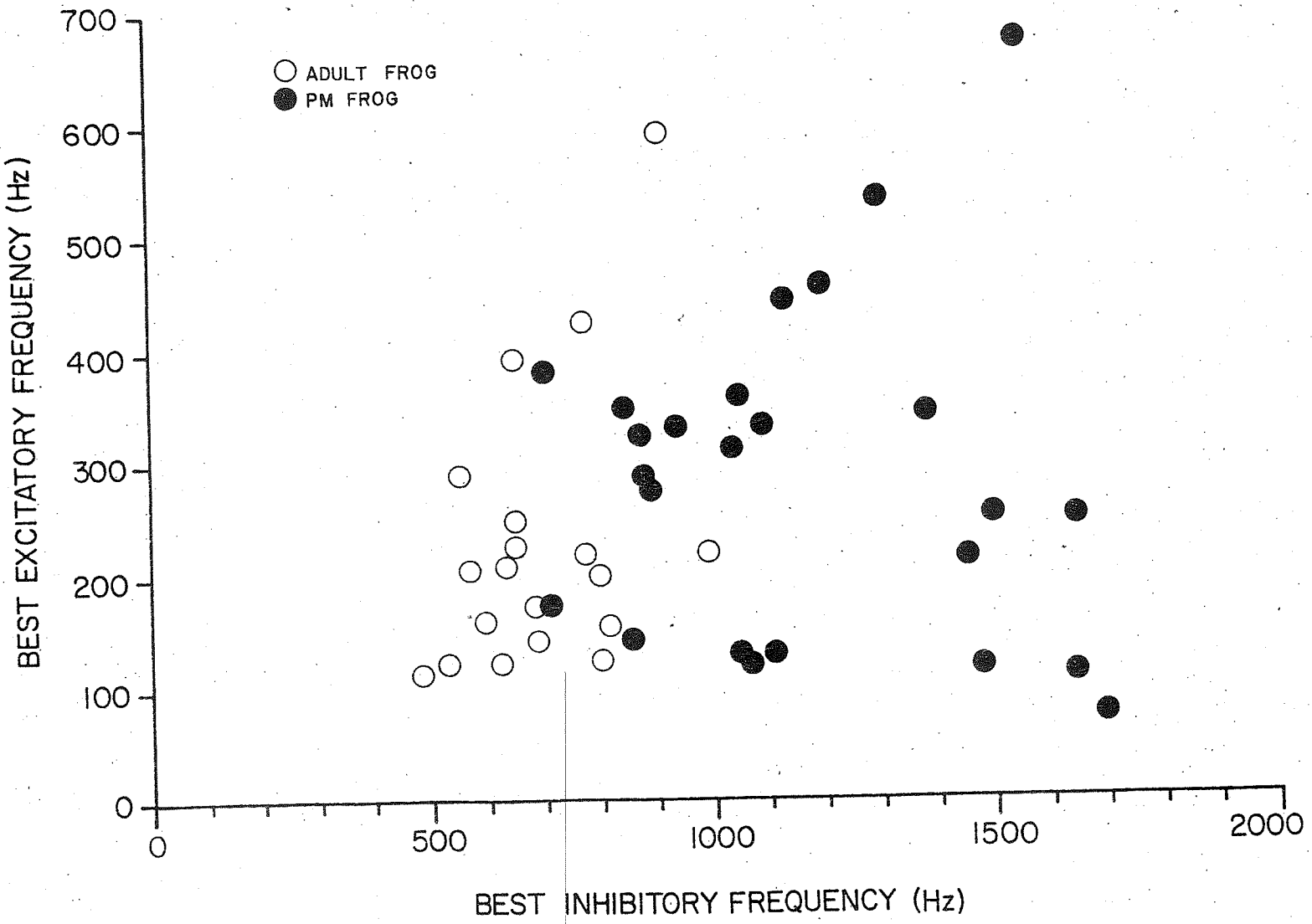

15
0
340 Hz 64 dB SPL
NUMBER OF SPIKES
15
0
340 Hz 64 dB SPL
1370 Hz 92 dB SPL
15
0
340 Hz 64 dB SPL
1370 Hz 97 dB SPL
0
120
TIME (msec)

Fig. 9. Scatter diagram of the distributions of best inhibitory frequencies for adults (0) and froglets (●). BIFs ranged from 485 to 990 Hz in adults and 700 and 1700 Hz in froglets. N=19 for adults. N=25 for froglets.


ADULT FROG
PM FROG
BEST EXCITATORY FREQUENCY (Hz)
700
600
500
400
300
200
100
0
0
500
1000
1500
2000
BEST INHIBITORY FREQUENCY (Hz)

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SECTION III. QUANTITATIVE LIGHT MICROSCOPY OF THE AMPHIBIAN PAPILLA TECTORIAL MEMBRANE: CORRELATION WITH THE TONOTOPIC ORGANIZATION OF ADULTS

INTRODUCTION

In all terrestrial vertebrates, a frequency map exists along the length of the sensory epithelium of the peripheral auditory organ (1,3,10,14-16,20,22,25, 27). The basis of this tonotopic map in the mammalian cochlea and avian basilar papilla is primarily due to the spatial gradation in the width of the basilar membrane (2,7,21,24,26). The change in width gives rise to a graded compliance and a graded variation of the natural frequency of vibration of the basilar membrane along the length of the auditory organ (1). In some reptilian species, however, the tonotopic organization is not related to the spatial gradations in the width of the basilar membrane (17,19) but rather to the variation in the height of stereocilia of the hair cells along the basilar papilla (17,25).

In anurans, which possess two distinct auditory organs (amphibian and basilar papillae), a tonotopic map has been shown to be present in the amphibian papilla, but not in the basilar papilla (14,15). However, the basis of the tonotopic organization in the amphibian papilla is unclear. This organ lacks a basilar membrane (6,28), and the variation in the height of stereocilia of the hair cells cannot be related with the tonotopic map, since the gradations in stereocilia height occur along the medial-lateral axis (11,12), while the change in frequency selectivity is along the rostral-caudal axis (14,15). Lewis (13-15) has proposed that the tonotopy in this organ may be due to the spatial gradation in the thickness (and therefore mass) of the tectorial membrane (TM). While the morphology of the amphibian papilla and its TM has been previously described (6,28), it has not been quantitatively analysed. In this paper, we describe results of quantitative measurements of the TM and their relation to the tonotopic organization of the papilla.

MATERIALS AND METHODS

Three adult bullfrogs (Rana catesbeiana) ranging from 13-16 cm in snout-vent length were used in this study. The animals were anesthetized with pentobarbitol (65 mg/Kg) and decapitated. A ventral opening was immediately made into the otic capsule, and a small incision was made into the saccule with a sharpened tungsten needle. The inner ear was then perfused with 4% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. One otic capsule from each animal was excised and stored in buffered fixative at 4°C. Following fixation, the otic capsules were washed in phosphate buffer and decalcified in 0.5 M EDTA in phosphate buffer. The decalcified otic capsules were further washed in buffer, dehydrated in an ethanol series, cleared in benzene, and infiltrated and embedded in paraffin (melting point 56-57°C). Serial sections (15μm) were cut on a rotary microtome in the frontal plane. This plane cuts through the amphibian papilla along its rostral-caudal axis. The sections were stained in hematoxylin and eosin and viewed on an Aus Jena Flouval and Olympus BH-2 microscopes.

Camera lucida drawings of every section of the amphibian papilla TM were digitized and analysed on a PDP-11/23 computer to obtain measurements of their heights, widths, and cross sectional areas. The width measurements were taken at the base of the TM immediately opposing the sensory epithelium, whereas the maximum heights perpendicular to the base were used as the height measurements. The cross sectional areas were actual measurements from the drawings and were not products of the height and width measurements. No correction was made for tissue shrinkage which occurred during histological processing. Thus, these measurements are underestimates of their true values. Since the TM appears to be a homogenous gelatinous structure it is reasonable to assume that the

shrinkage will be uniform throughout the length of the organ and should not affect the relative dimensions of the TM.

RESULTS

The amphibian papilla is located in the medial wall of the saccule. The sensory epithelium can be clearly visualized in a scanning electron micrograph prepared for a separate study (Shofner and Feng, in preparation) and it consists of two distinct patches: a rostral triangular patch and an S-shaped caudal patch (Fig. 1). The amphibian papilla lacks a basilar membrane, and the sensory hair cells are anchored to the dorsal cartilaginous limbic wall (Fig. 2). The TM lies ventrally to the sensory epithelium as shown in Fig. 2. The total length of the TM ranged from 960-1185 μm with an average of 1060 ± 114 μm.

The TM associated with the rostral patch is not attached to the ventral limbic wall (Fig. 2A). In the most rostral sections as illustrated in Fig. 2A, the TM has more or less a rectangular shape. Proceeding caudally, both the rostral and caudal patches of epithelium can be seen in individual sections (Figs. 2B-E). In this bimodal region the two patches are initially contiguous (Figs. 2B-C) and are disjoined further caudally (Figs. 2D-E). The TM in this bimodal region takes on a V-shaped appearance making it possible to separate the membrane associated with each individual patch. We have arbitrarilly bisected the membrane as a line of separation in order to quantify TM measurements. As shown in Fig. 2, each portion of the TM becomes narrower proceeding caudally. In Figure 2E, only that portion of TM associated with the caudal patch is visible.

Caudal to the bimodal region, all sections show only the caudal patch of sensory epithelium (Figs. 2F-K). At approximately the mid-point of this region, the TM becomes broader and forms the tectorial curtain (sensing membrane of Wever), which anchors the TM to the ventral limbic wall (Fig. 2G). Caudal to the tectorial curtain (Figs. 2I-K), the TM appears as a small structure and is

no longer attached to the ventral limbic wall. Caudally from the tectorial curtain, the lumen of the organ becomes enclosed by the limbic walls (Fig. 2J). At the extreme caudal end, only a thin contact membrane is present separating the endolymph from the perilymph (Fig. 2K).

The maximum height of the TM varies systematically along the length of the papilla, and its distribution is similar in individual organs (Figs. 3A-C) despite slight deviations in the sectioning plane. The maximum height of the TM associated with the rostral triangular patch is approximately constant following the initial increase in the most rostral end and has an average value of 150 μm (Fig. 4A). From the bimodal region to the caudal end of the papilla, the TM associated with the caudal patch increases in height to an average maximum of 270 μm at the tectorial curtain, and then decreases to an approximately constant level with an average of 80 μm (Fig. 4A). In one of the papillae, however, there is a distinct increase in the height at the caudal extreme of the organ (Fig. 3C). This is attributed to the specific angle of the sectioning plane used in this specimen being deviated slightly from the frontal plane.

The width of the TM also varies along the length of the papilla, and while its pattern is strikingly similar among individuals (Figs. 3D-F), it differs from that of the height (Figs. 3A-C). The TM width associated with the rostral patch is relatively constant with an average value of 230 μm (Fig. 4B). In contrast, the TM associated with the caudal patch is more variable in width than that portion associated with the rostral patch. Proceeding caudally, the width first decreases to an average minimum of 55 μm (Fig. 4B) at the caudal end of the bimodal region (Fig. 2F). The width then increases in the region of the tectorial curtain (Fig. 2H) to reach an average peak value of 130 μm (Fig. 4B).

Caudal to the tectorial curtain, the width once again decreases to a relatively constant value (average of 70 μm) and it gradually increases at the caudal end to an average of 100 μm (Fig. 4B).

The cross sectional area of the TM associated with the rostral patch has an approximately constant value in each individual specimen (Figs. 3G-I) with an average of 3.0×10^4 μm^2 (Fig. 4C). On the other hand, the TM area fluctuates along the length of the caudal patch (Figs. 3G-I). The area decreases throughout the bimodal region reaching a local minimum with an average of 0.9×10^4 μm^2 in the caudal end of this region (Figs. 2F,4C), and increases again to reach an average of 1.3×10^4 μm^2 at the tectorial curtain (Figs. 2G-H, 4C). Depending on the specific angle of the sectioning plane, this local minimum can be quite pronounced (Figs. 3G-I) or somewhat less distinct (Fig. 3I). The average values of the area in this region appear less variable (Fig. 4C). Caudal to the tectorial curtain, the area decreases to a minimum average value of 0.4×10^4 μm^2 and increases again in the caudal end of the organ to an average of 1.0×10^4 μm^2 (Fig. 4C).

Comparison of the distribution in the cross sectional area of the TM along the length of the papilla to those of the height and width (Figs. 3, 4) shows that the area distribution closely follows that of the width more so than that of the height. The contrast is most evident from the bimodal region to the tectorial curtain where minimum cross sectional area is associated with minimum width and maximum height, and maximum area is associated with maximum width and minimum height.

DISCUSSION

The anuran auditory system is unique among vertebrates in that anurans possess two distinct auditory organs, namely the basilar and amphibian papillae. The basilar papilla gives rise to high frequency selective auditory fibers (5,14) and appears to act as a tuned mechanical resonator (4). The amphibian papilla is innervated by low and intermediate frequency auditory fibers (5,14,15) and has a tonotopic organization along its sensory epithelium with low frequencies represented rostrally and intermediate frequencies represented caudally (14,15). While both auditory organs lack a basilar membrane, they do possess separate tectorial membranes.

The present quantitative study of the morphology of amphibian papilla TM in bullfrogs shows that the height, width and cross sectional area do not appear to be linearly graded along the length of the organ. Since all sections were cut at a uniform thickness, the cross sectional area provides a measure of the volume, and thus the mass of the TM. The TM associated with the rostral patch has a more or less uniform height and width, and hence an approximately constant area. The TM is most massive in this region due to the large values in the height and width. From the bimodal region to the tectorial curtain, the height and width of TM undergo oppposite variations along the length of the epithelium resulting in a relatively constant area (or mass). The TM is least massive caudal to the tectorial curtain due to the minimum width and height. Therefore, the area (or mass) of the TM seems to be spatially graded in a more or less step-wise fashion.

It has been proposed that the spatial gradations in the mass of the TM along the sensory epithelium may give rise to the tonotopic organization in the amphibian papilla (13-15). The results of the present quantitative study support this hypothesis. Direct comparison between our normalized data (Fig. 4) and the composite tonotopic map of the amphibian papilla (Fig. 4 of ref. 15) shows that the most massive TM in the rostral region of the epithelium is also the region innvervated by auditory fibers having the lowest best excitatory frequencies, i.e., BEFs (< 300 Hz). Furthermore, auditory fibers which possess the highest best excitatory frequencies (500-1000 Hz) innervate the caudal most region of the epithelium where TM is least massive. From the bimodal region to the tectorial curtain, the TM has intermediate mass, and the fibers innervating this portion of the epithelium also have intermediate values of BEFs (300-500 Hz).

In addition to this general trend, Lewis et al. (15) noted that in the rostral triangular patch there apears to be a tendency for BEFs to decrease as one moves caudally. While our data shows that the TM area (or mass) is relatively constant in this region, there is nevertheless a tendency for the area (or mass) to increase moving caudally. In the S-segment, Lewis et al. (15) observed a trend of increasing BEF as one proceeds caudally, but deviations from this trend are also observed in the region of the tectorial curtain. It is interesting to note that our area measurements show a general decreasing trend as one moves caudally with some deviations around the tectorial curtain. Thus, our quantitative data provides strong support to the hypothesis that TM is the primary contributing factor for the frequency map. Further support of this

hypothesis comes from studies in the post-metamorphic juvenile bullfrog where the frequency selectivity of the amphibian papilla is higher than that of the adult (23). Comparison of the amphibian papilla TM between juveniles and adults suggests that the absolute mass of the TM in each locus is a primary factor in determining frequency selectivity of the region (Shofner and Feng, in preparation).

It is unclear, however, exactly how the spatially graded mass of the TM is related functionally to the tonotopic map in the amphibian papilla. From acoustic emission studies in the frog, Palmer and Wilson (18) provided evidence that a travelling wave may occur in the TM of amphibian papilla, which lacks a basilar membrane. The presence of the graded mass suggests that the TM could support a travelling wave much like the basilar membrane in the mammalian cochlea. Given the different polarization patterns of hair cells along the sensory epithelium in the amphibian papilla (11,12) however, if indeed a travelling does occur, then the axis of vibration of TM must also vary along the epithelium to maximally excite the hair cells (8,9). Whether or not the TM does indeed support a travelling wave requires further physiological studies.

Figure 1 A. Scanning electron micrograph of the bullfrog amphibian papilla. The tectorial membrane has been excised to observe the sensory epithelium.

B. Schematic drawing of the sensory epithelium illustrating the positions of the frontal sections (a-k) in Figure 2.

R: rostral

L: lateral

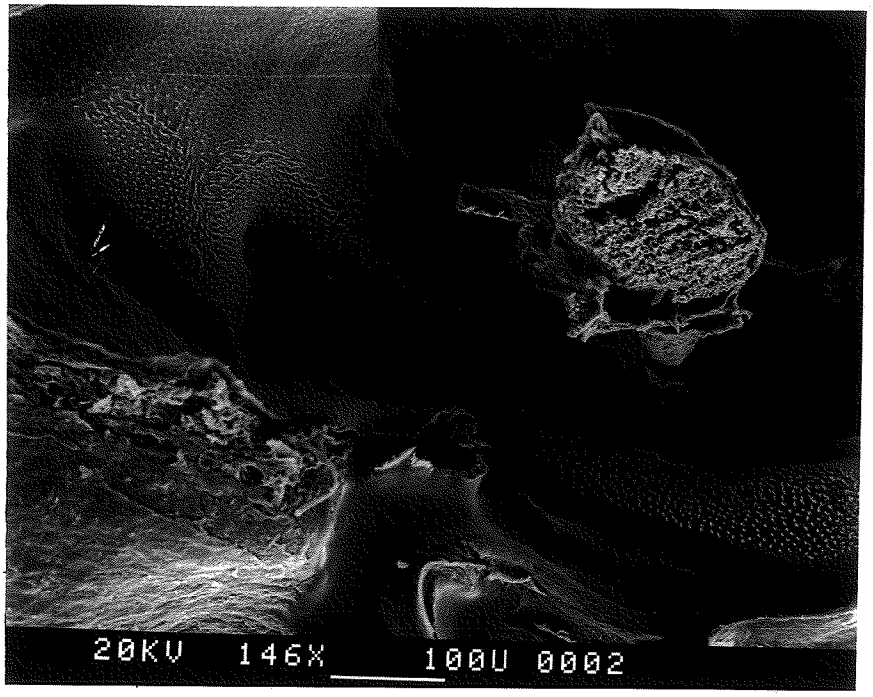
RSE: Rostral triangular patch of sensory epithelium

CSE: Caudal S-segment of sensory epithelium

VIIIAP: Branch of VIIIth cranial nerve which innervates the sensory epithelium

Calibration mark: 100 µm

A



B

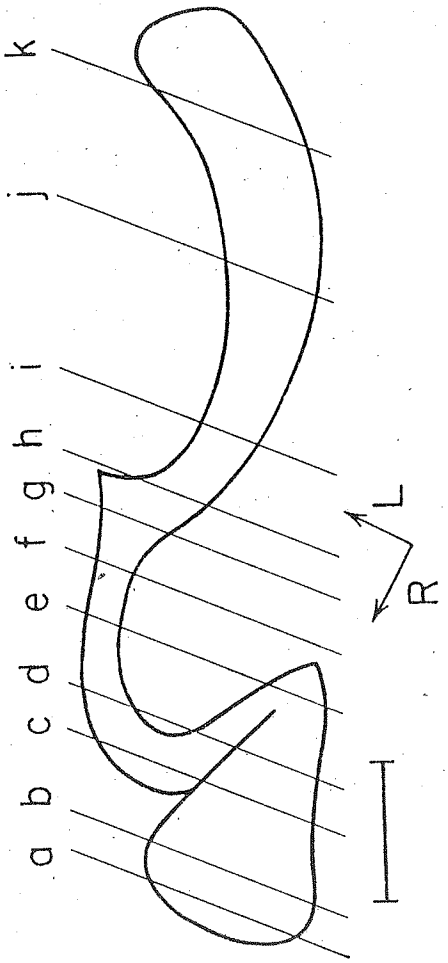


Figure 2. A-K. Representative frontal sections from an individual bullfrog amphibian papilla along its length. See text for description.

RSE: Rostral triangular patch of sensory epithelium

CSE: Caudal S-segment of sensory epithelium

TM: Tectorial membrane

TC: Tectorial curtain of TM

DW: Dorsal limbic wall

VW: Ventral limbic wall

CM: Contact membrane

VIIIAP: Branch of VIIIth cranial nerve which innervates the sensory epithelium.

Calibration mark: 250 μm

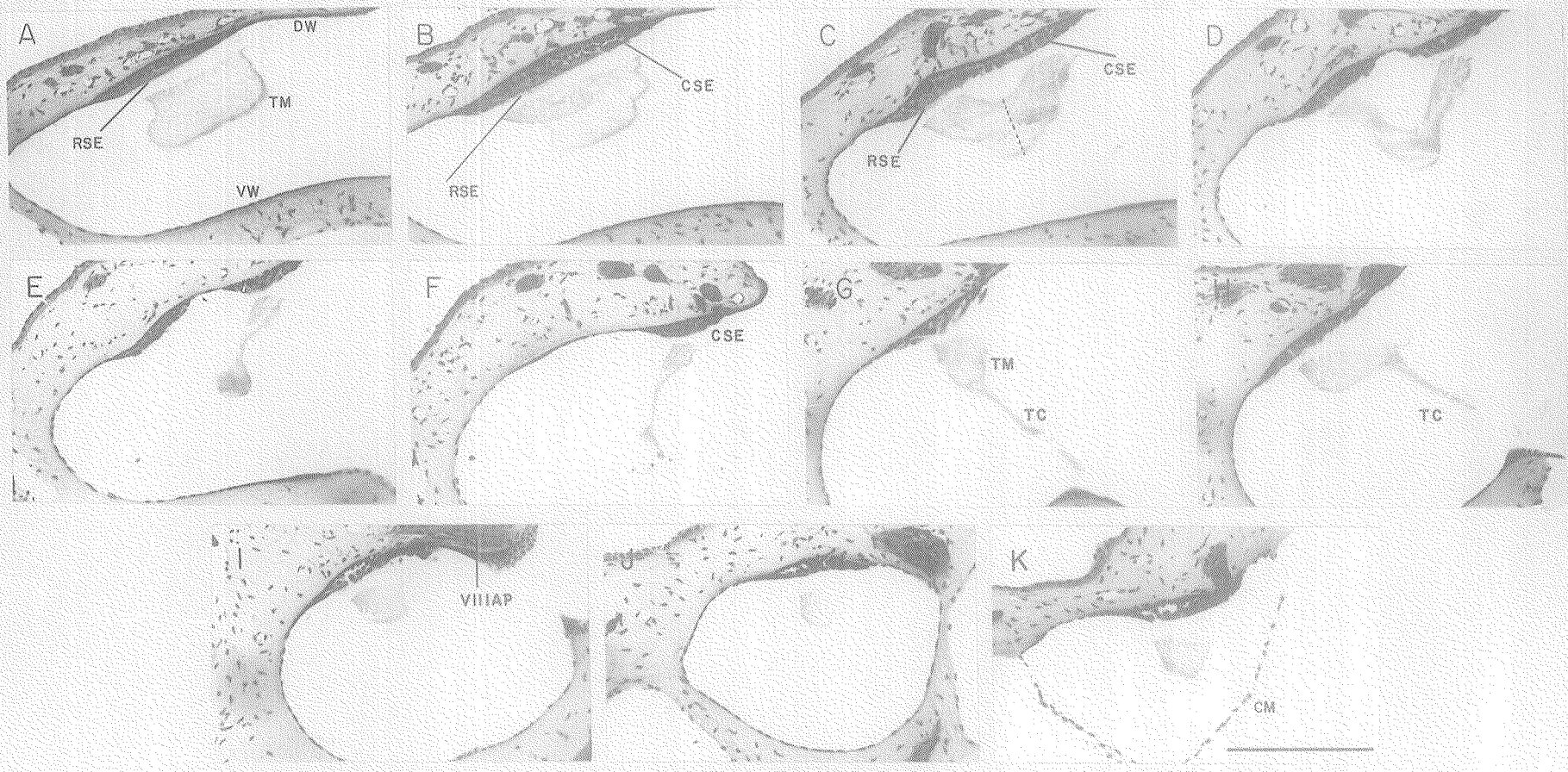

A
DW
TM
RSE
VW
B
CSE
RSE
C
CSE
RSE
D
E
F
CSE
G
TM
TC
H
TC
I
VIIIAP
J
K
CM

Figure 3. Distribution of the height, width and cross sectional area of the tectorial membrane from frontal serial sections of the amphibian papilla of three individuals. Filled circles are measurements taken from the tectorium associated with the rostral triangular patch of sensory epithelium, while measurements from TM associated with the caudal S-segment are shown as open circles. Note that the ordinates are logarithmic scales.

A-C. Maximum height of the tectorial membrane measured perpendicular to its base. Representative frontal sections shown in Fig. 1 are marked correspondingly a-k for that individual in A.

D-F. Width of the tectorial membrane measured at its base. Frontal sections in Fig. 1 are marked a-k in D.

G-I. Cross sectional areas of the tectorial membrane. Measurements are the actual cross sectional areas as determined from digitized camera lucida drawings and are not the products of the height and width data. Frontal sections in Fig. 1 are marked a-k in G.

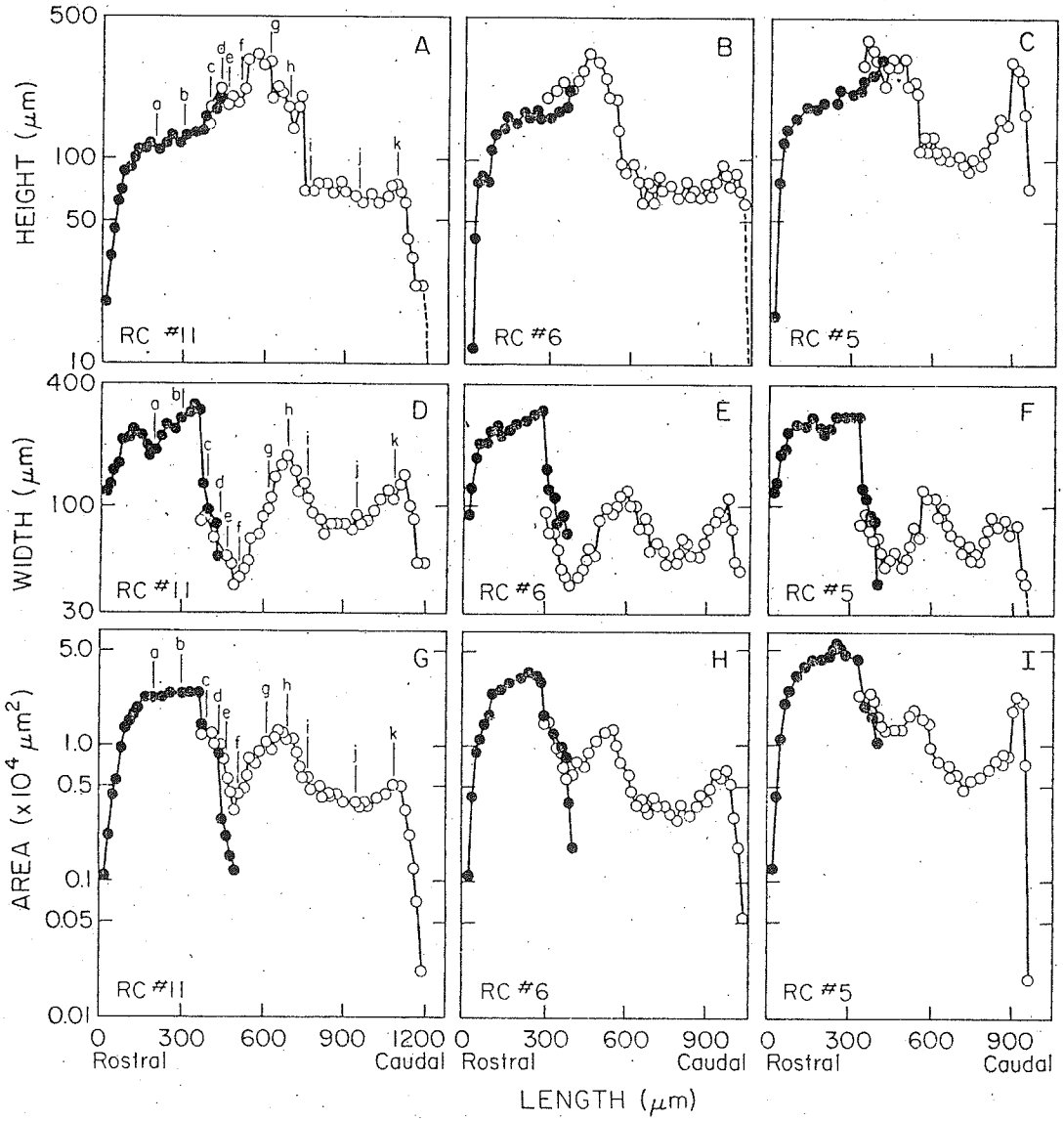
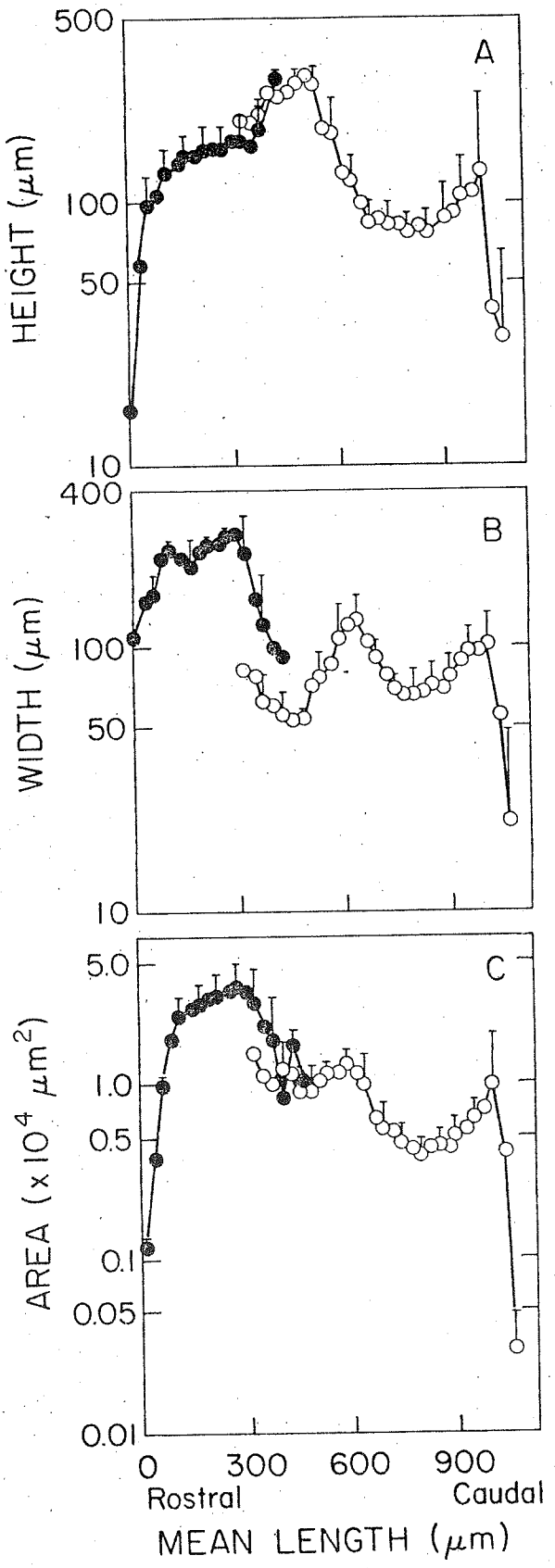

HEIGHT (μm)
500
100
50
10
A
B
C
RC #11
RC #6
RC #5
a
b
c
d
e
f
g
h
i
j
k
WIDTH (μm)
400
100
30
D
E
F
AREA (x10⁴ μm²)
5.0
1.0
0.5
0.1
0.05
0.01
G
H
I
0
300
600
900
1200
Rostral
Caudal
LENGTH (μm)

Figure 4. Normalized distributions of the height (A), width (B) and cross sectional area (C) along the epithelium length. The length of individual tectorial membranes were converted to percentages of the total length where 0% and 100% represent the extreme rostral and caudal sections, respectively. Values of height, width and cross sectional area were determined every 3-5% from each individuals, and the values for a given percent length were averaged. The 100% length of the tectorium was converted to an average of 1060 μm, and the average values were then graphed with respect to this normalized length. Vertical bars represent standard deviations of average values. Filled and open circles are averaged values taken from tectorial membrane associated with the rostral triangular patch and the caudal S-segment of the sensory epithelium, respectively. Note that the oridinates are logarthimic scales.


A
B
C
HEIGHT (μm)
WIDTH (μm)
AREA (x10⁴ μm²)
500
100
50
10
400
100
50
10
5.0
1.0
0.5
0.1
0.05
0.01
0
300
600
900
Rostral
Caudal
MEAN LENGTH (μm)

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SECTION IV. QUANTITATIVE LIGHT AND SCANNING ELECTRON MICROSCOPY OF THE AMPHIBIAN AND BASILAR PAPILLAE DURING POST-METAMORPHIC DEVELOPMENT

INTRODUCTION

During the development of the bullfrog following metamorphosis, we have previously shown that there is a downward shift in the frequency selectivity of the auditory periphery (Shofner and Feng, 1981). While three populations of primary auditory fibers are found in both adult and post-metamorphic juvenile bullfrogs, the ranges of best excitatory frequencies (BEFs) in the two groups of frogs differ: low frequency selective fibers in adults and juveniles range from 100-400 Hz and 100-800 Hz, respectively; intermediate frequency fibers in adults range from 500-1000 Hz and 900-1700 Hz in juveniles; high frequency selective auditory fibers range from 1000-1700 Hz and 1800-2500 Hz in adults and juveniles, respectively. It has been demonstrated in the adult bullfrog that the low and intermediate frequency selective auditory fibers innervate the amphibian papilla tonotopically (Lewis et al., 1982a; 1982b), whereas the basilar papilla acting as a tuned mechanical resonator (Capranica and Moffat, 1977) gives rise to the high frequency auditory fibers (Feng et al., 1975; Lewis et al., 1982a). While this has not yet been directly demonstrated in the juvenile, indirect evidence suggests the same situation occurs (see discussion and in Shofner and Feng, 1981).

The downward shift in frequency selectivity during post-metamorphic development is presumably due in part to the increase in the sizes of the tympanic membrane, middle ear cavity and middle ear bones. The middle ear acts as a low pass filter and primarily determines the upper cutoff frequency of the auditory periphery (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). The changes in middle ear structures can, therefore, explain the decrease in the upper limit of hearing observed with age (Shofner and Feng, 1981). However, the more refined tuning of individual auditory fibers are primarily determined by

the physical properties of the auditory organs, and hence the observed shifts in the frequency selectivities of the three populations of auditory fibers from the two organs cannot be attributed to changes in middle ear structures alone. Morphological changes in the auditory organs may also occur during post-metamorphic development and contribute to the physiological changes.

While the anatomy of the two auditory organs in bullfrogs have been previously described (van Bergeijk and Witschi, 1957; Geisler et al., 1964; Frishkopf and Flock, 1974; Lewis and Li, 1975; Lewis, 1976; 1977a; 1977b; 1978), they have not been quantitatively analysed nor have the post-metamorphic changes been observed. The purpose of the present study is to investigate and quantitatively analyse the post-metamorphic development of the bullfrog amphibian and basilar papillae under light and scanning electron microscopy and to discuss the functional implications of the observed morphological changes.

MATERIALS AND METHODS

Adult and post-metamorphic juvenile bullfrogs of both sexes (snout-vent lengths of 13-17 cm and 3.5-4.9 cm, respectively) were obtained from Charles Sullivan (Nashville, TN). Animals were anesthetized under pentobarbitol (65 mg/kg), decapitated, and the otic capsules were opened ventrally to expose the membranous labryrinth. An incision was made in the saccule with a sharpened tungsten needle and the inner ear was then perfused with 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Whole heads were stored in the same buffered fixative at 4°C.

Light Microscopy

Three adult and three juvenile otic capsules were excised from the fixed heads under phosphate buffer, decalcified in 0.5 M sodium (tetra) ethylene diamine tetraacetate in 0.1 M phosphate buffer (pH 7.4), washed in buffer, and dehydrated through an increasing ethanol series. Dehydrated otic capsules were then cleared in benzene, infiltrated with melted Paraplast (melting point 56-57°C) and embedded in Paraplast. Serial sections (15μm) were transversely cut on a rotary microtome and every section was mounted on gelatin subbed slides. The frontal plane was most useful in studying the auditory organs, since it involved cutting transversely through the basilar papilla and along the rostral-caudal axis of the amphibian papilla. This sectioning plane can clearly depict the relation between the sensory epithelium and tectorial membrane for both organs. The mounted sections were deparaffinized in xylene, re-hydrated through a decreasing ethanol series and stained in Mayer's hematoxylin and counterstained in 1% aqueous eosin. Sections were then dehydrated, cleared and coverslipped.

The basilar and amphibian papillae were observed on an Aus Jena Fluoval or Olympus BH-2 microscope. The sensory epithelium length was determined as the product of section thickness and the total number of sections in which the epithelium was observed. The area of the contact membrane in individual sections was calculated as the product of contact membrane width and section thickness. The total area was obtained by summing all the individual cross sectional areas. Cross sectional areas of the lumen and tectorial membrane were measured from camera lucida drawings digitized on a Houston Instruments Hi-Pad and analysed on a PDP 11/23 computer. Cross sectional volumes of individual sections were determined from the product of the cross sectional areas and section thickness. Total volume was obtained by summing all the cross sectional volumes.

Scanning Electron Microscopy

A total of six adult and seven juvenile basilar papillae, and four adult and eight juvenile amphibian papillae were observed. Following fixation, the basilar and amphibian papillae were dissected out of the inner ear under phosphate buffer, washed in buffer, dehydrated through an increasing ethanol series and dried in a Tousimis Samdri-790 critical point drier using liquid carbon dioxide as the transitional fluid. Amphibian papillae were processed as described above with the exception that specimens were post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4) following their removal from the otic capsule. The dried auditory organs were mounted on aluminum stubs using colloidal graphite in isopropanol and coated with gold/palladium in an SPI sputter coater. Specimens were observed in a Cambridge Stereoscan or an

International Scientific Instruments DS-130 scanning electron microscope at 20 KV.

All quantitative measurements were obtained from stereopair scanning electron micrographs using the formula (Boyde and Howell, 1977)

$$Z_L = \frac{\cos \Theta \, X_L - X_R}{\sin \Theta} \qquad (1)$$

where Θ is the angle of tilt, X_L and X_R are the distances between the same two points in the left and right micrographs, respectively, and Z_L is the height of the photographic point (FIGURE 1). The true distance (T) between the two points measured is then determined using the Pythagorean theorem,

$$T = (X_L^2 + Z_L^2)^{1/2} \qquad (2)$$

In order to determine the area of the basilar papilla lumen, the specimen was first oriented such that the direction of tilt was along the length of the papilla and a stereopair micrograph was made as illustrated in Figure 2. The specimen was then rotated 90° such that the direction of tilt was along the width of the organ and another stereopair was taken (FIGURE 2). From the two sets of stereopairs, the true width and true length were determined and the area was calculated using the formula for an ellipse. Where possible, the height of the tallest stereocilia of the amphibian papilla hair cells were determined from stereopair scanning electron micrographs in which the direction of tilt was along the length of the stereocilia bundle and where the tallest stereocilia could clearly be observed. The heights of the stereocilia of the basilar papilla were not determined since the entire basilar papilla was observed and it

was not possible to orient the specimen to clearly observe the tallest stereocilia.

No correction was made for tissue shrinkage in all of the light and scanning electron microscopic measurements. The inner ear is complex in its histology being comprised of cartilaginous, epithelial, neural and gelatinous structures. There will be differential shrinkage among the various types of tissue within the inner ear, i.e., cartilaginous structures will shrink less than gelatinous structures such as the tectorial membrane. However, since there are no fundamental histological differences for each tissue type in the auditory organs of the two groups of frogs, it is reasonable to assume that differential shrinkage of a given type of tissue between juveniles and adults will be minimal. Thus, while the reported values may be underestimates of the true values, there should be little effect on the relative dimensions.

For all quantitative measurements, the means and standard deviations were determined. A one-tailed t-test was used to establish statistical differences in mean values between the two groups of animals.

RESULTS

Basilar papilla

The basilar papilla is a tubular evagination of the ventro-caudal wall of the saccule and has a lumen that is typically elliptical in shape (FIGURES 3 and 4). The organ lacks a basilar membrane, and the hair cells rest on the cartilaginous tissue forming a sensory epithelium which is semicircular in shape. Overlying the sensory epithelium is a gelatinous tectorial membrane (TM) which is attached to the lumen walls. A thin contact membrane is located medially to the sensory epithelium separating the perilymphatic and endolymphatic systems (FIGURE 5).

During the post-metamorphic development of the bullfrog, there is an overall increase in the size of the basilar papilla (FIGURES 3 and 4). From stereopair scanning electron micrographs (FIGURE 6), a 1.6 fold increase in lumen area was observed (FIGURE 7): the mean value is 3.71×10^4 μm^2 in juvenile bullfrogs and 6.29×10^4 μm^2 in adults ($p<0.0005$). From serial reconstruction of the papilla, we found that the mean lumen volume (FIGURE 8) is 8.54×10^6 μm^3 in juveniles and 2.39×10^7 μm^3 in adults ($p<0.0005$), which is a 2.8 fold increase with age.

Concurrent with the increase in lumen volume, the mean TM volume (FIGURE 8) increases by 2.6 fold. The average TM volumes are 1.22×10^5 μm^3 and 3.22×10^5 μm^3 in juveniles and adults, respectively ($p<0.005$). There is also a 1.9 fold enlargement of the area of the contact membrane during post-metamorphic development with mean values of $1.40 \pm 0.21 \times 10^4$ μm^2 in juveniles and $2.65 \pm 0.54 \times 10^4$ μm^2 in adults ($p<0.01$).

The change in TM volume was accompanied by a slight increase in the length of the sensory epithelium with means of 95±9 μm in juveniles and 120±15 μm in adults ($p<0.05$). The total number of hair cells also increases with post-metamorphic growth with means of 106±12 and 122±17 in juveniles and adults, respectively ($p<0.05$).

Three types of hair cells are found in both juvenile and adult basilar papillae (FIGURE 9) using hair cell classification criteria of Lewis and Li (1975). Type A hair cells are characterized by short bundles of stereocilia and a long unbulbed kinocilium, and they are located in the lateral edges of the epithelium in both juveniles and adults. Type F hair cells are found in the central regions of the epithelium in both groups of animals, and they are characterized by long graded bundles of stereocilia and a long unbulbed kinocilium. Type D hair cells possess short graded bundles of stereocilia and a short bulbed kinocilium, and they are found in the medial regions of the epithelium in both groups. Interestingly, in the juveniles along the lateral edge of the sensory epithelium an occassional immature looking hair cell was observed having short stereocilia and no kinocilium (FIGURE 9F). In both juvenile and adult organs, the kinocilia of all hair cells are oriented medio-laterally.

Amphibian papilla

The amphibian papilla is located on the medial wall of the saccule. Like the basilar papilla, this organ lacks a basilar membrane, and the hair cells are anchored to the dorsal wall of the papilla (FIGURE 10). A tectorial membrane (TM) lies ventrally from the sensory epithelium, and its size and shape change along the length of the papilla (FIGURE 10). The tectorial curtain (Figure

10E-F) attaches the TM to the ventral wall of the organ, and it is only in this region where the TM is attached to the ventral wall. The sensory epithelium has an S-shaped structure (FIGURE 11) consisting of a rostral triangular patch and a caudal S-segment. Frontal sections through the amphibian papilla at the level of the bimodal region (Figure 10C-D) show both patches of epithelium and a V-shaped TM.

During post-metamorphic development, there is an increase in the size of the amphibian papilla (FIGURE 10). There is a parallel increase in the length of the tectorial membrane (FIGURE 12) with means of 725±48 μm and 1060±114 μm in juveniles and adults, respectively ($p<0.005$). Quantitative analysis of the TM shows that its cross sectional area is spatially graded in a step-wise fashion along the length of the papilla in both juveniles and adults, and that the absolute cross sectional area increases with post-metamorphic development (FIGURE 12). In order to facilitate comparison between the two groups of animals, the data are normalized as percent mean length in Figure 13. The TM areas associated with the rostral triangular patch of epithelium (filled circles in Fig. 12) have more or less constant values of 1.0×10^4 μm^2 and 3.0×10^4 μm^2 in juvenile and adult bullfrogs, respectively. From the bimodal region to the tectorial curtain (open circles left of the arrow in Fig. 13) the TM areas have lower values and flucuate slightly along the length of the epithelium around values 5.0×10^3 μm^2 in juveniles and 1.1×10^4 μm^2 in adults. Caudal to the tectorial curtain (open circles right of the arrow in Fig. 13), the cross sectional areas have the lowest values: around 2.8×10^3 μm^2 and 5.5×10^3 μm^2 in juveniles and adults, respectively. The increase

in TM area in the extreme caudal end is an artefact resulting from sectioning the curved papilla at the selected angle.

From the low power scanning electron micrographs (FIGURE 11), there does not appear to be a dramatic difference in the length of the amphibian papilla between the two groups of frogs. However, a quantitative analysis from serial sections of the organ shows that the length of the sensory epithelium increases significantly with age with means of 720±84 μm in juveniles and 1020±79 μm in adults ($p<0.01$). Since most amphibian papillae used for scanning electron microscopy had parts of the sensory epithelium either obscured by TM debris or had areas where stereocilia were pulled out during the removal of the TM, it was not possible to obtain accurate hair cell counts from a large number of specimens. However, observation from the cleanest juvenile and adult papillae shows that they had approximately 1050 and 1420 hair cells, respectively.

The orientation pattern of hair cells was similar in both juveniles and adults. Kinocilia of hair cells are oriented rostral-caudally in the rostral-triangular patch. In the S-segment, however, kinocilia are tangentially oriented with respect to the epithelium rostral to the tectorial curtain, and medio-laterally oriented caudal to the tectorial curtain.

The sensory epithelium of both juvenile and adult bullfrogs also contains three types of hair cells (FIGURE 14). Type A and Type D hair cells, similar to those found in the basilar papilla, are located along the lateral and the medial regions of the epithelium, respectively, in both groups of frogs. In the central regions of both groups, type E hair cells are found, and they are characterized by long graded stereocilia and a long bulbed kinocilium. In the juvenile, intermediate type hair cells are occassionally observed between type A

and Type E hair cells (FIGURE 15). These hair cells show most of the morphological characteristics of type A hair cells (i.e., short bundles of stereocilia and a long kinocilium) with the exception of having a bulbed kinocilium (one of the features of type E hair cells).

The maximum height of the stereocilia of the predominant hair cell type (type D) in the amphibian papilla can be determined from stereopair scanning electron micrographs, since their stereociliary bundles are typically straight following histological processing for scanning electron microscopy (FIGURE 16). The curvature of stereocilia of other hair cell types make stereopair mesurements impractical to carry out. In the adult, the maximum height of the stereocilia of type D hair cells appears to be uniform along the rostral-caudal axis of the sensory epithelium (FIGURE 17). On the other hand, there is a noticeable difference in the maximum height of the stereocilia along the length of the juvenile amphibian papilla, i.e., there is a progressive decrease in the mean height as one moves caudally (FIGURE 17). Comparison of stereocilia heights between juveniles and adults shows that in the rostral triangular patch of epithelium the mean heights are similar between the two groups, while in the caudal tail (region C, FIGURE 17) the mean height increases with age having a mean of 3.8 μm in juveniles and 6.6 μm in adults ($p<0.005$).

DISCUSSION

The results of the present study show that the morphology of the basilar papilla changes during post-metamorphic development. The TM volume, lumen volume and contact membrane area increase dramatically during this period of growth, and these increases will alter the functional properties of the organ contributing to the observed physiological changes (Shofner and Feng, 1981). Assuming that the density of the TM does not change appreciably during development, the increase in TM volume indicates an increase in TM mass. The increase in TM mass increases the load on the stereocilia of those hair cells associated with an overlying tectorial membrane resulting in a decrease in their natural frequency of vibration. Additionally, the enlargement of the lumen volume with age increases the acoustic compliance resulting in a decrease in the resonant frequency of the organ. This factor would alter the frequency selectivity of the hair cells, particularly those hair cells which may be free standing (Lewis, 1977a).

The basilar papilla is known to give rise to the high frequency population of auditory fibers in the adult bullfrog (Feng et al., 1975; Lewis et al., 1982a). Assuming that the basilar papilla is a tuned mechanical resonator as suggested by Capranica and Moffat (1977), measurements of lumen volume and contact membrane area allow us to evaluate the resonance properties and acoustic characteristics of this organ. The resonant frequency (f_o) of a Helmholtz resonator having acoustic inertance of M and acoustic compliance of C is given by:

$$f_o = \frac{1}{2\pi} \left(\frac{1}{MC}\right)^{1/2} \qquad (3)$$

Under conditions where viscous forces are negligable and inertial forces are dominant, M and C are

$$M = \frac{\rho \ell'}{S} \tag{4}$$

and

$$C = \frac{V}{\rho c^2} \tag{5}$$

where ρ is the density of the medium, c is the velocity of sound, S is the area of the resonator opening, V is the resonator volume, and ℓ' is the effective length of the resonator neck. For a Helmholtz resonator without a neck, as is the case for the basilar papilla, ℓ' is given as $16a/3\pi$, where a is the radius of the resonator opening (Kinsler and Frey, 1962). For the basilar papilla, the radius of the contact membrane is taken as a. Assuming that the density and velocity of sound in endolymph are those for sea water at 25°C, namely, 1026 Kg/m^3 and 1531 m/sec, respectively, substitution of the reported values of contact membrane area for S and lumen volume for V into equations 3-5 for adult and juvenile basilar papillae, results in f_o of 6.5×10^5 Hz and 9.2×10^5 Hz, respectively (Table 1). These calculated values are several orders of magnitude larger than the BEF ranges of the high frequency selective auditory fibers in both groups of frogs (Shofner and Feng, 1981) suggesting that for this system the viscous forces probably cannot be ignored.

If we now assume that the viscous forces of this system are dominant and

inertial forces are negligable, different M and C values can be determined. Acoustic inertance (M) is defined as the ratio of the effective mass of the resonator opening to the square of its area (Kinsler and Frey, 1962). Under the assumed conditions, the effective mass is given as $\eta S/c$ where η is the coefficient of viscosity. Thus,

$$M = \frac{\eta S}{cS^2} = \frac{\eta}{cS} \tag{6}$$

Acoustic compliance (C) is defined as the ratio of resonator volume to applied pressure (Kinsler and Frey, 1962). For conditions where viscous forces dominate, pressure is $\eta^2/\rho S$ and thus,

$$C = \frac{V}{\eta^2/\rho S} = \frac{\rho S V}{\eta^2} \tag{7}$$

Using the viscosity of sea water for endolymph (η = 0.001 Newton sec/m^2), the calculated f_o from equations 3, 6 and 7 are 1259 Hz and 2128 Hz for adult and juvenile basilar papillae, respectively (Table 2). These values match closely to the mean BEFs of the high frequency populations of primary afferent fibers which are 1379±145 Hz in adults and 2122±169 Hz in juveniles (Shofner and Feng, 1981). These results indicate that (1) the basilar papilla indeed behaves as a tuned Helmholtz resonator but under the condition where viscous forces are dominant, and (2) the high frequency population of auditory fibers of juvenile bullfrogs also originates from the basilar papilla, since the match can be extended to this group of frogs. Therefore, the remaining low and intermediate frequency selective fibers in the juvenile bullfrog are presumably derived from the amphibian papilla much like the situation in adults.

The present study shows that the morphology of the amphibian papilla also changes during post-metamorphic development. The cross sectional area (or volume), and thus cross sectional mass of the TM is spatially graded in a step-wise manner along the length of the papilla in both juvenile and adult bullfrogs, but the absolute values are smaller in juveniles. Since the amphibian papilla in adult bullfrogs is tonotopically organized along its length (Lewis et al., 1982a; 1982b) and since this tonotopy is well correlated with the spatial gradations in TM mass (Lewis, 1981; Shofner and Feng, submitted), it is therefore conceivable that a tonotopic organization may also be present in the juvenile amphibian papilla. Namely, low frequencies are represented rostrally where the TM is most massive and higher (intermediate) frequencies are represented caudally where the TM is less massive. More definitive physiological experiments, however, will be required to demonstrate the presence of such an organization in the juvenile.

The increase in TM mass during post-metamorphic development will alter the functional properties of the organ. The enlargement of the TM mass with age increases the load on the stereocilia resulting in a decrease in their natural frequency of vibration. Physiologically, the range of BEFs of the intermediate frequency population in the juvenile bullfrog is beyond the frequency range of the adult amphibian papilla fibers (Shofner and Feng, 1981). It is interesting to note that the TM in the caudal tail of the juvenile amphibian papilla is clearly less massive than that of the adult counterpart (FIGURE 13), and that this region of the epithelium is associated with the intermediate frequency population (Lewis et al., 1982a,b). Additionally the distribution of BEFs of the juvenile low frequency population extends into the range of the adult intermediate frequency population (Shofner and Feng, 1981), and our present anatomical data show that the TM mass associated with the rostral portion of the

S-segment in the juvenile papilla has approximately the same value as that of the TM of the caudal tail in the adult (FIGURE 13). Thus, there appears to be a close correlation between the absolute TM mass and the frequency selectivity of individual loci along the length of the organ.

In addition to the change in TM mass with age, there is also an increase in the height of the stereocilia in the caudal tail of the organ. The height of the stereocilia of type D hair cell, the predominant hair cell type, is uniform along the length of the adult amphibian papilla. On the other hand, there is a systematic decrease in the stereocilia height as one moves caudally in the juvenile organ. The shorter stereocilia possesses a greater stiffness which results in a higher natural frequency of vibration. Thus, the extended BEF range of the juvenile low and intermediate frequency populations is presumably the result of a combination of less massive TM and shorter stereocilia.

Comparison of the morphology of the juvenile and adult amphibian papillae with data from VIIIth nerve recordings (Shofner and Feng, 1981) may provide some insight into a possible mechanism for two tone inhibition (suppression) in low frequency auditory fibers of Ranid anurans. Physiologically, the range of best inhibitory frequencies (BIFs) of low frequency selective fibers in both juvenile and adult bullfrogs coincides with the range of intermediate frequency fibers in both groups of animals. Anatomical data show that hair cells in the rostral triangular patch are oriented along the rostrocaudal axis, while those in the caudal tail are oriented along the medio-lateral axis in both groups of frogs. Since the maximum hair cell response occurs if it is stimulated along the axis of stereocilia orientation (Hudspeth and Jacobs, 1979; Hudspeth, 1982) then in order for different tones to maximally excite the hair cells in different

patches of the organ, the TM must vibrate along different axes. Namely, intermediate frequency tones vibrate the caudal TM mediolaterally and low frequency tones vibrate the rostral TM rostrocaudally. If this is the case, the presence of intense mechnical energy due to the suppressor tone at intermediate frequencies (which vibrates the caudal TM mediolaterally) may interfere with the rostrocaudal vibration pattern of the rostral TM to excitatory low frequency tones. Such mechanical interference can decrease the neural response to the excitor tone. While this hypothesis has its attractiveness, it does not explain why intermediate frequency auditory fibers, which innervate the caudal epithelium, cannot be inhibited by low frequency tones which excite the rostral patch associated with the most massive TM. Nevertheless, it is possible that the presence of the tectorial curtain may determine the direction of mechanical interaction between the rostral and caudal TM. This scheme is in contrast to that of the mammalian cochlea where two tone inhibition is thought to arise from the nonlinear properties of the basilar membrane motion (Rhode and Robles, 1974; Rhode, 1977). In the spadefoot toad, two tone inhibition of low frequency fibers occurs (Capranica and Moffat, 1975) but the amphibian papilla of this species lacks a caudal extention of sensory epithelium (Lewis, 1977b). The mechanism of two tone inhibition in this case is unknown, but the phenomenon may be similar to that of reptiles (Holton and Weiss, 1978) where only hair cells associated with the TM show two tone inhibition.

The above discussion suggests that the morphology of the auditory organs plays a critical role in determining the frequency selectivity of the auditory periphery. While there are increases in the diameter of the tympanic membrane

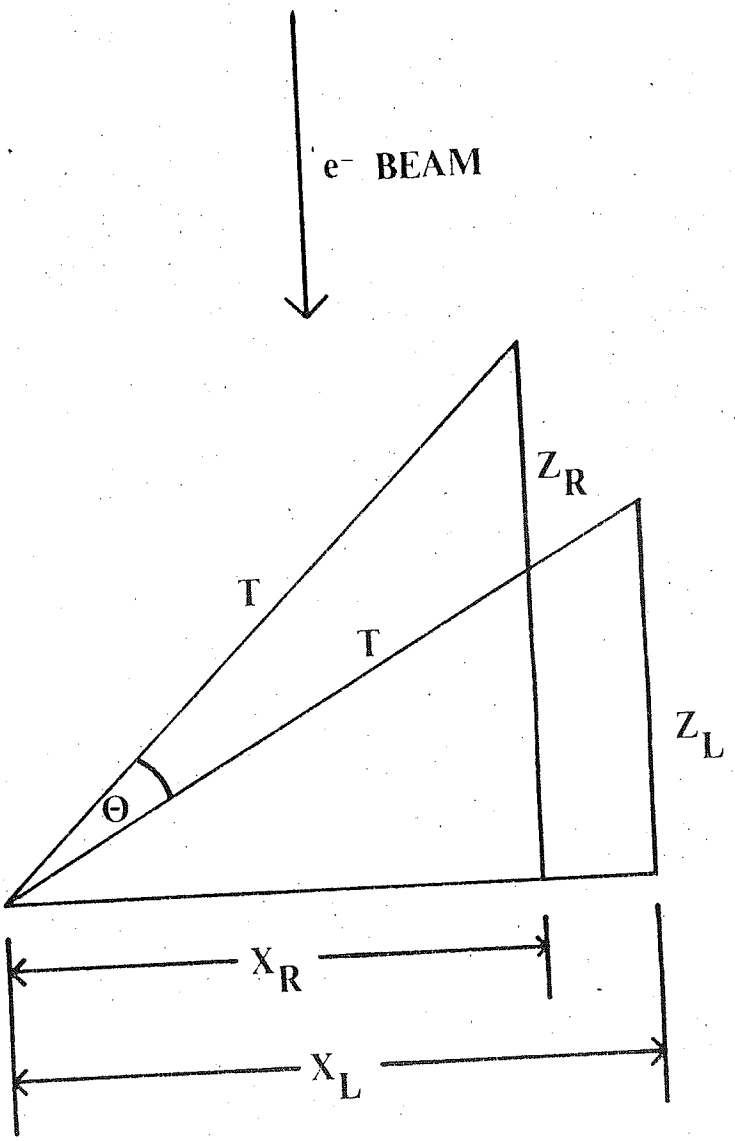
and middle ear structures during post-metamorphic development (Shofner and Feng, 1981), these changes will primarily alter the upper frequency limit of the system. Comparative studies have shown that the anuran middle ear acts as a low pass filter and that the upper cut off frequency is inversely related to the body size and tympanic membrane size of the frog (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). Thus, a downward shift in the upper cut off frquency presumably occurs during the post-metamorphic growth of the bullfrog. However, our data suggest that the morphological characteristics of the amphibian and basilar papillae are the major elements which shape the distribution of BEFs recorded from the VIIIth nerve.

Beyond the changes in frequency selectivity which occur during post-metamorphic development, changes in the sensitivity of the various populations of auditory fibers occur with age (Shofner and Feng, 1981). It is unclear what the morphological basis of these sensitivity changes are. We observe an increase in the lengths of the sensory epithelia as well as in the number of hair cells of the auditory organs during post-metamorphic growth. If the ratio of auditory fibers to hair cells increases, then there may be an increase in sensitivity as observed in sharks (Corwin, personal communication). Maturation of the innervation pattern of the hair cells appears to be important for the increase in auditory sensitivity during development in mammals (Pujol, et al., 1980; Lenoir et al., 1980).

The growth of the sensory epithelia in the saccule, basilar and amphibian papillae in larval anurans (Lewis and Li, 1973; Li and Lewis, 1974) as well as macula neglecta in sharks (Corwin, 1981) appears to occur at the lateral edges of the epithelium. Our study in post-metamorphic frogs supports this finding.

It is only along the lateral edges of the basilar papilla in juvenile bullfrogs that immature hair cells are occasionally observed. In the amphibian papilla, it is along the lateral edges where intermediate type hair cells are observed . The morphological characteristics of the stereociliary bundles of the intermediate hair cells further suggest that a transformation from type A to type E hair cells may occur. This observation lends further support to the hypothesis that type A hair cells are morphogenetic precursor cells (Lewis and Li, 1973; Li and Lewis, 1974). If this apparent differentiation of hair cells is accompanied by changes in their innervation, then thresholds of excitation may be modified during post-metamorphic development. The morphological basis of threshold changes during post-metamorphic development, however, requires further investigation.

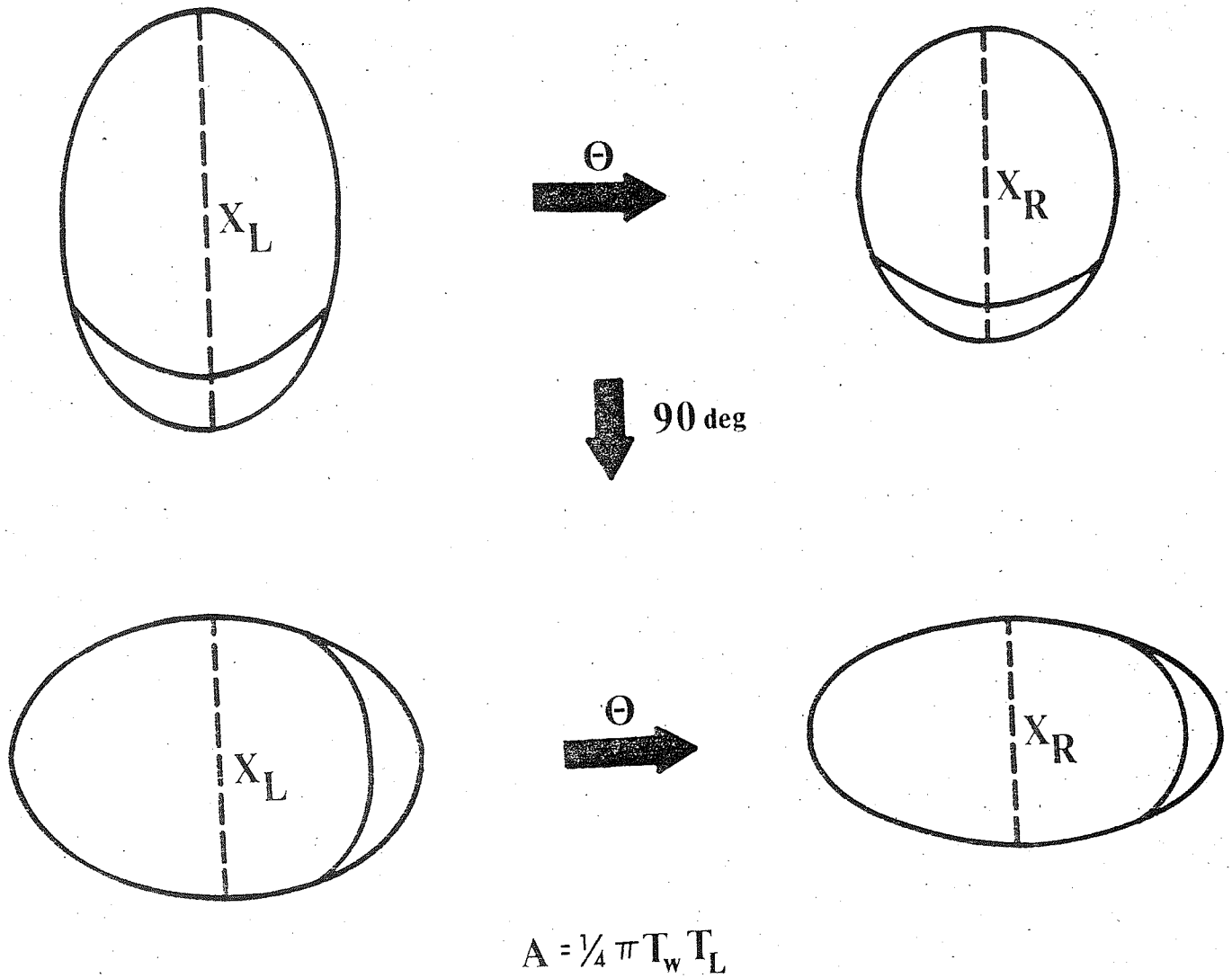
Figure 1. Schematic diagram illustrating how quantitative measurements of the true dimension (T) are calculated from stereopair scanning electron micrographs. T is first projected as length X_L in the left micrograph of the stereopair. The specimen is then tilted by Θ degrees and T is projected as X_R from the right micrograph of the stereopair. The height of the photographic point (Z_L) can be calculated as described in the text, and T can then be determined from the Pythagorean theorm.



$$Z_L = \frac{X_L \cos\Theta - X_R}{\sin\Theta}$$

$$T = \sqrt{X_L^2 + Z_L^2}$$

Figure 2. Schematic diagram illustrating how the lumen area of the basilar papilla is determined from scanning electron micrographs. A stereopair was first taken with the direction of tilt along the height of the organ, and the true length (T_L) was determined. The specimen was then rotated 90°. A second stereopair was taken with the direction of tilt along the width of the organ, and the true width (T_W) was calculated. The area was calculated using the formula for the area of an ellipse.



$$A = \tfrac{1}{4}\pi T_w T_L$$

Figure 3. Scanning electron micrographs of adult (A) and juvenile (B) basilar papillae.

L: lumen

TM: tectorial membrane

SE: sensory epithelium

Calibration mark = 100 µm

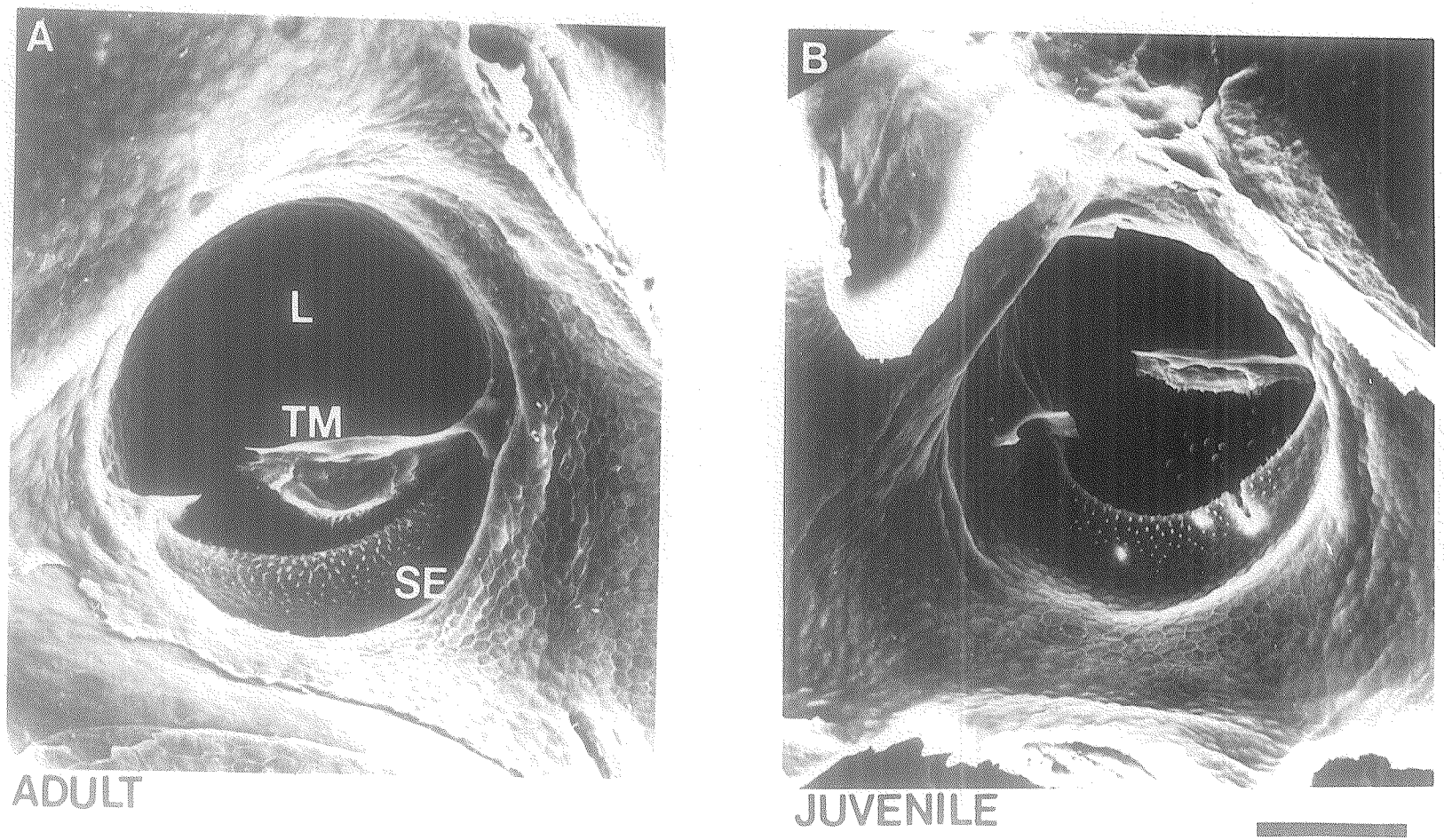

A
L
TM
SE
ADULT
B
JUVENILE

Figure 4. Light micrographs of frontal sections through juvenile (A) and adult (B) basilar papillae.

L: lumen

TM: tectorial membrane

SE: sensory epithelium

VIIIBP: branch of VIIIth nerve which innervates the basilar papilla

Calibration mark = 100 µm

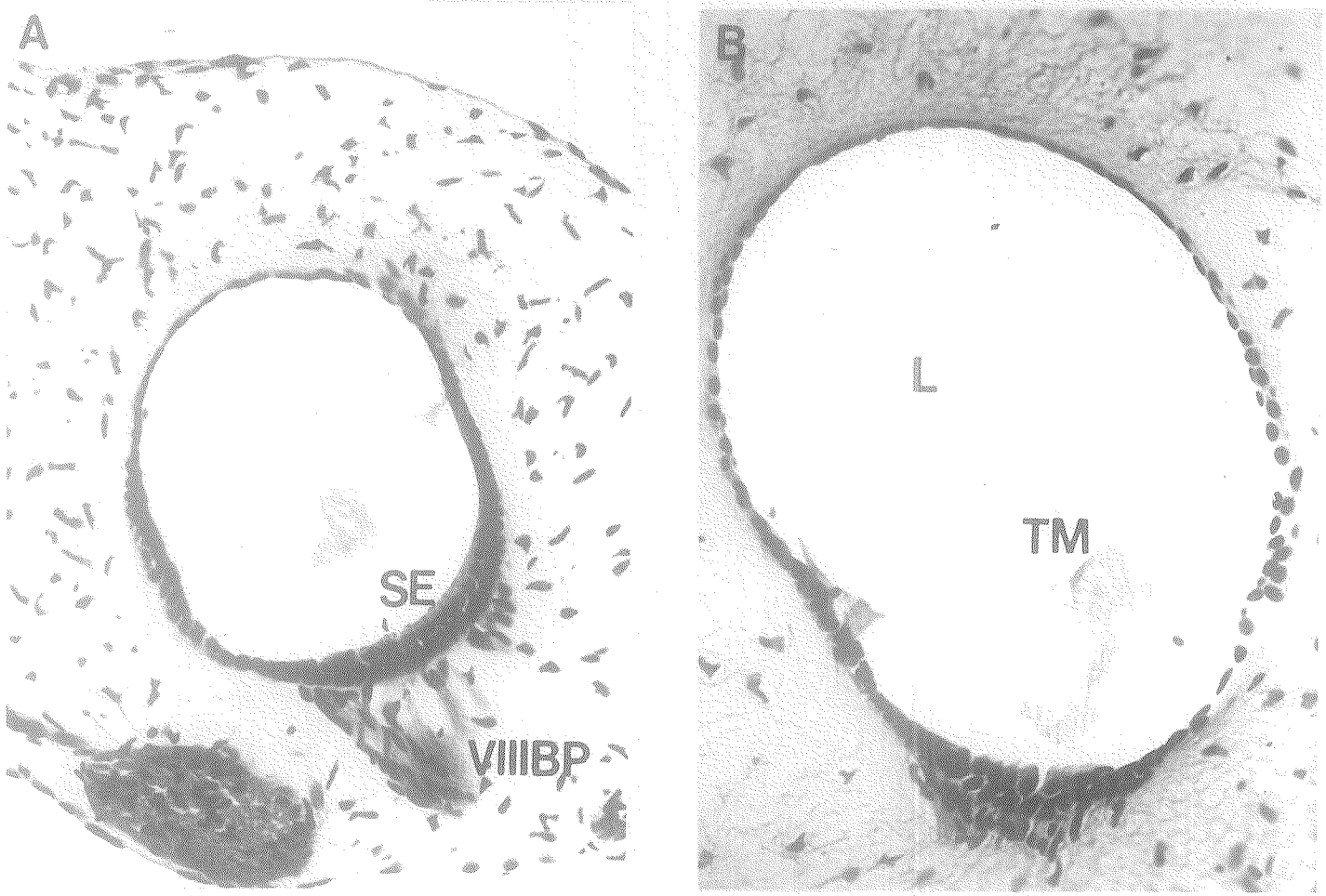

A
SE
VIIIBP
JUVENILE
B
L
TM
ADULT

Figure 5. Light micrographs of frontal (A) and horizontal (B) sections through the basilar papilla showing the contact membrane.

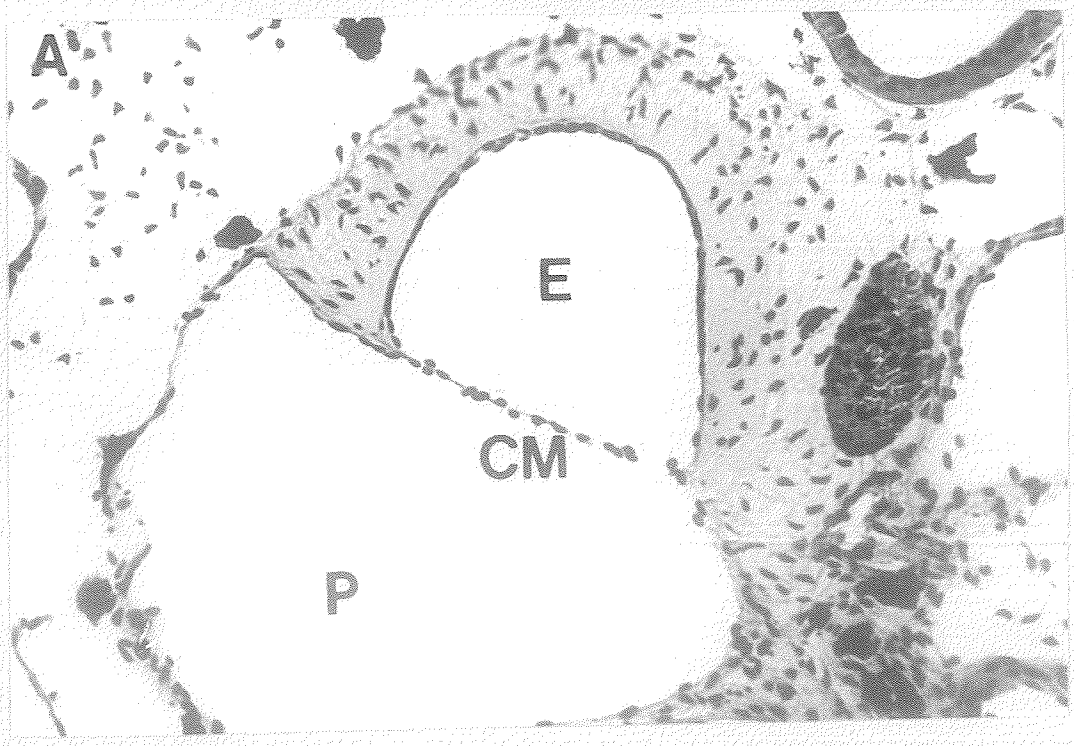
CM: contact membrane

TM: tectorial membrane

E: endolymph

P: perilymph

Calibration mark = 100 µm


A
E
CM
P

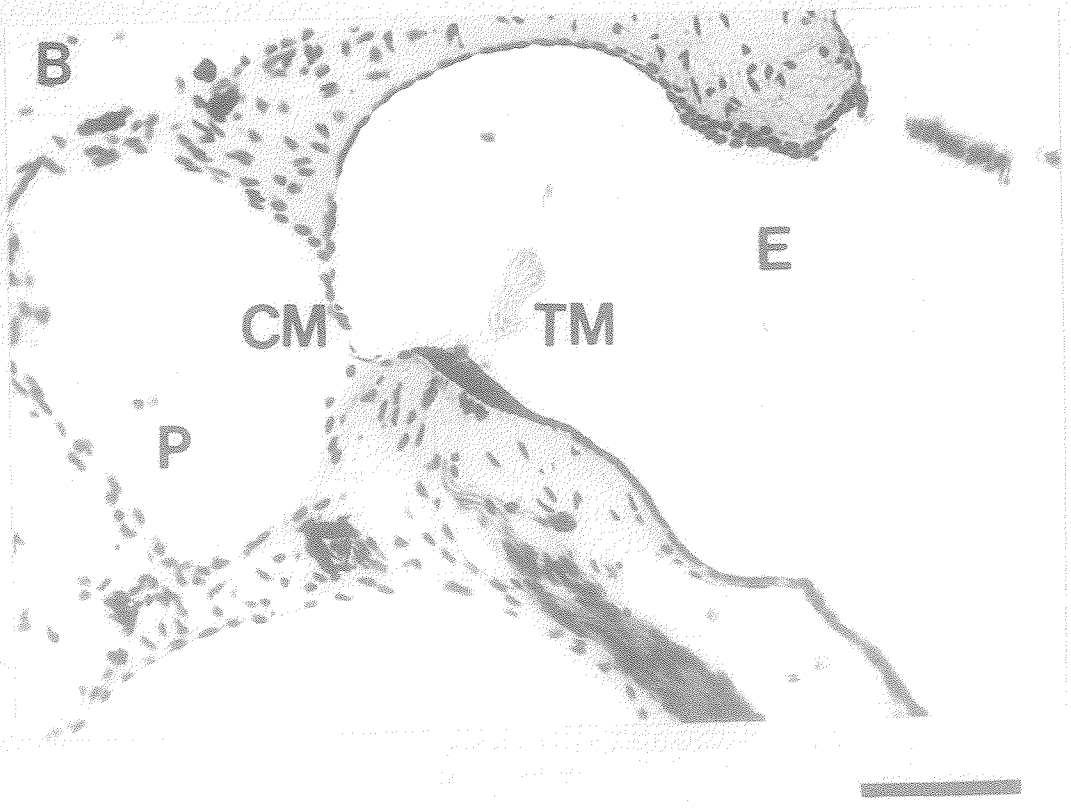

B
E
CM
TM
P

Figure 6. Stereopair scanning electron micrograph of the basilar papila. Width of entire micrograph is 465 µm.

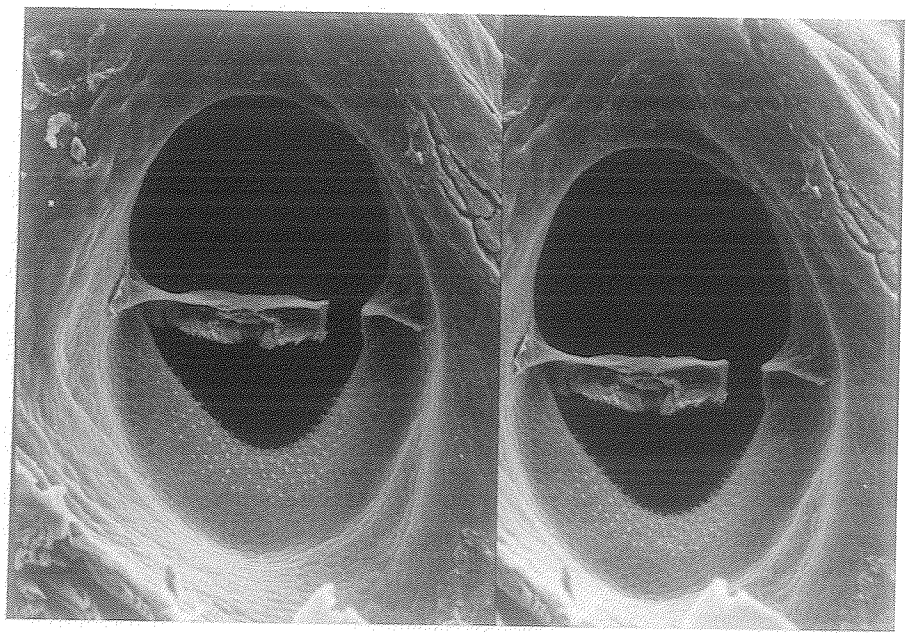


Figure 7. Histogram of the mean lumen area of juvenile (J) and adult (A) basilar papillae as determined from stereopair scanning electron micrographs. Vertical lines show standard derivations.

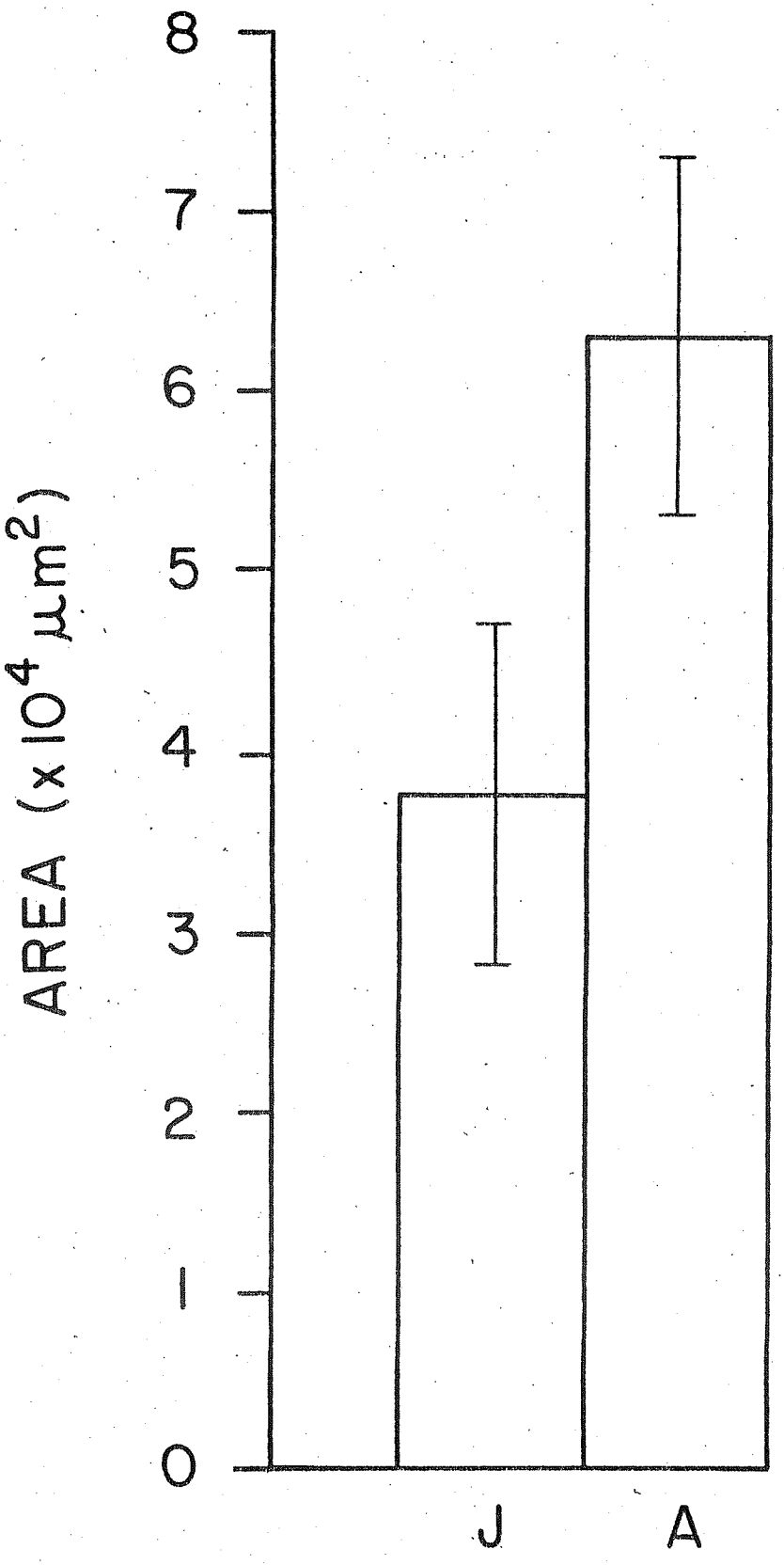

AREA (x 10⁴ μm²)
8
7
6
5
4
3
2
1
0
J
A

Figure 8. Histograms of the mean lumen volume and mean tectorial membrane volume for juvenile (J) and adult (A) basilar papillae as determined from serial sectioned otic capsules. Vertical lines show shandard deviations.

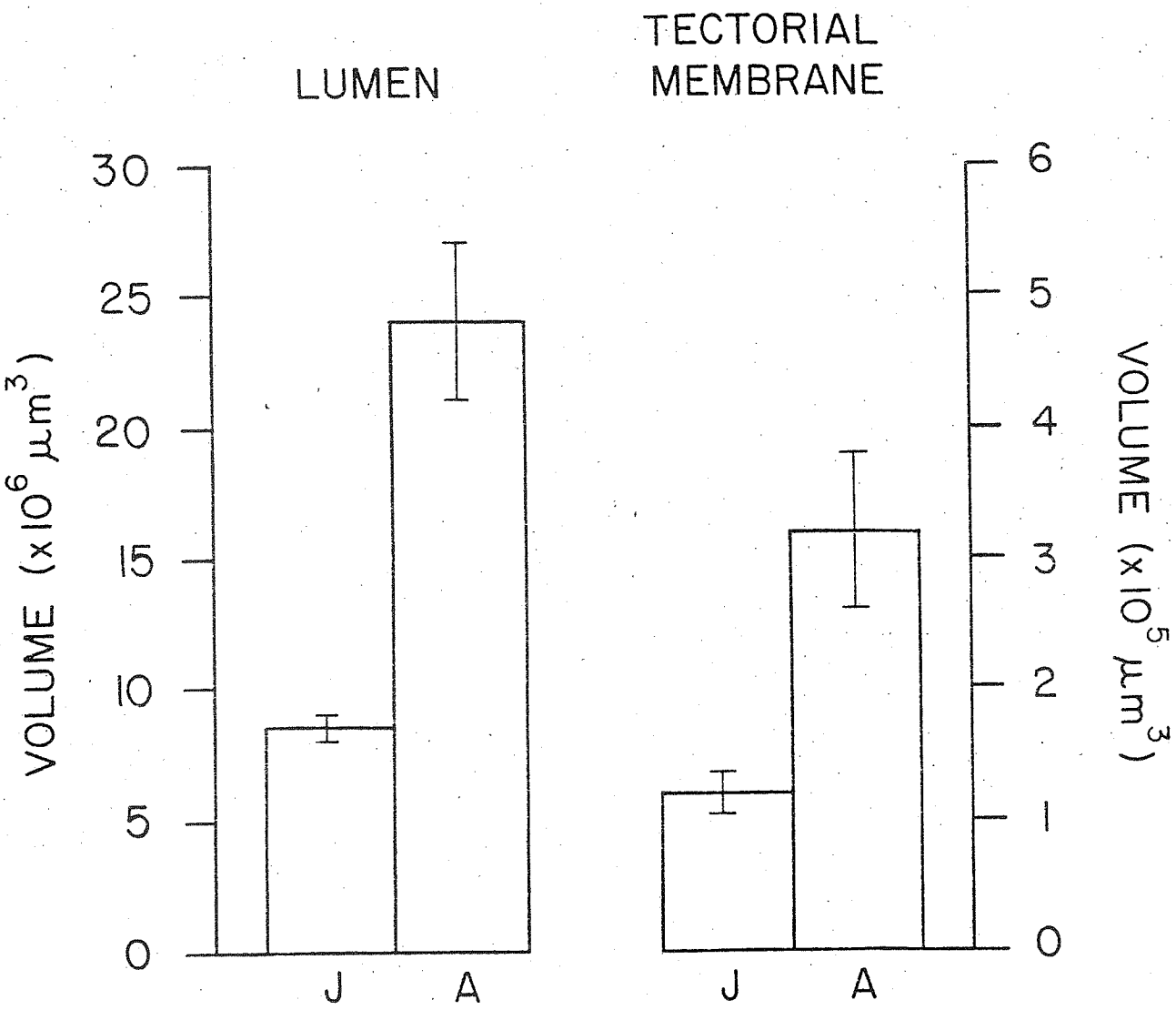
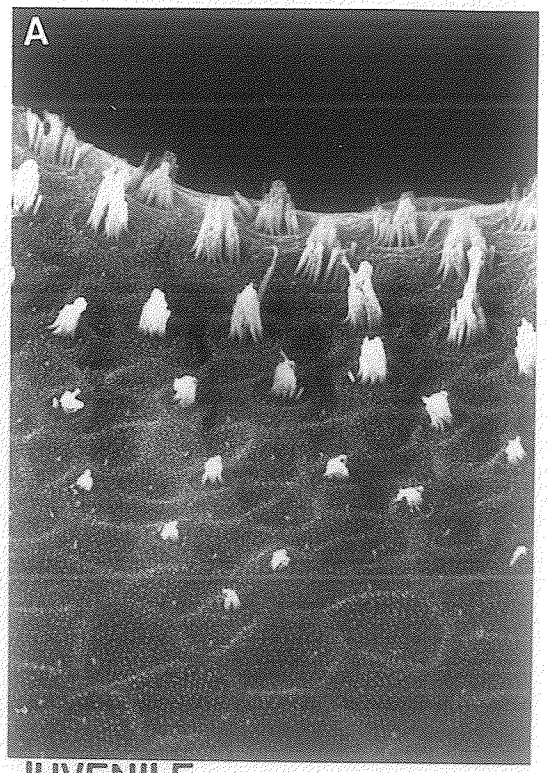
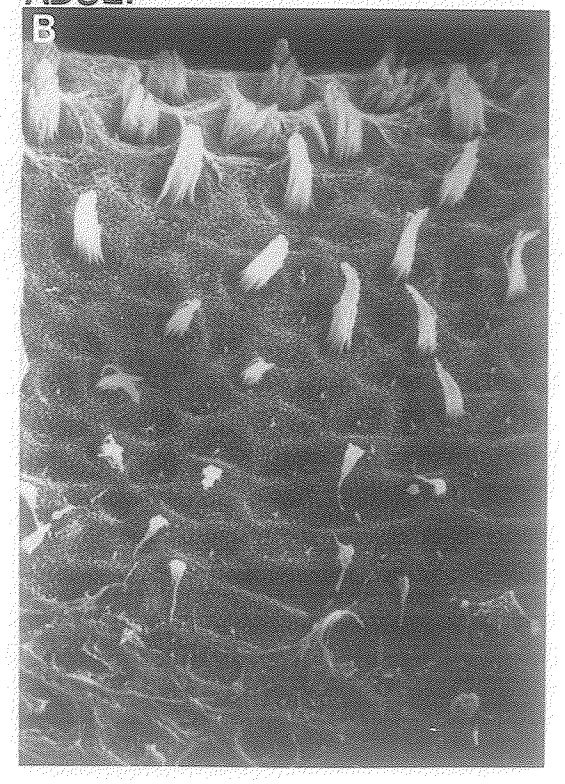

LUMEN
TECTORIAL MEMBRANE
VOLUME (x10^6 µm^3)
VOLUME (x10^5 µm^3)
30
25
20
15
10
5
0
6
5
4
3
2
1
0
J
A
J
A

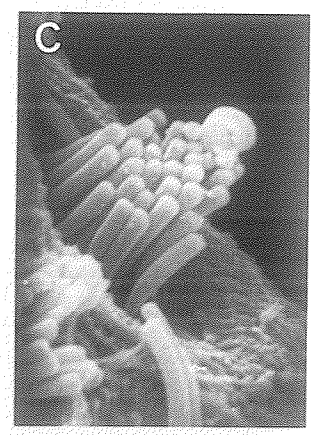
Figure 9. A-B. Scanning electron micrographs showing the gradation of the three hair cell types along the mediolateral axis in juvenile (A) and adult (B) basilar papillae. Calibration mark = 100 µm.
C-F. Scanning electron micrographs of the three types of hair cells found in juvenile and adult basilar papillae. See text for description. (C) Type D; (D) Type F; (E) Type A; (F) Immature hair cell. Calibration mark = 1 µm

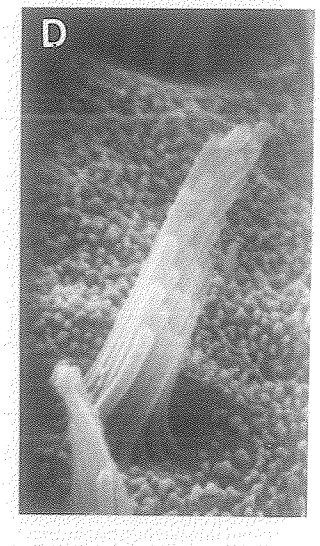


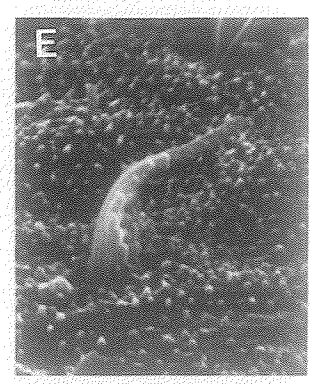
JUVENILE

ADULT









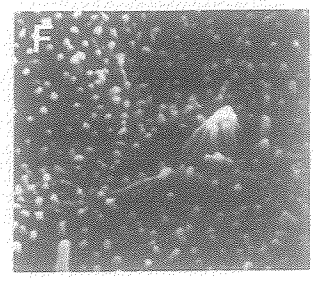


Figure 10. Light micrographs of frontal sections taken along the length of the juvenile and adult amphibian papillae.

A-B. Frontal sections through the rostral region of the papilla.

C-D. Frontal sections through the bimodal region of the papilla showing both patches of epithelium.

E-F. Frontal sections through the region of the tectorial curtain.

G-H. Frontal sections through the caudal tail of the papillae.

RSE: rostral triangular patch of sensory epithelium.

CSE: caudal S-segment of sensory epithelium.

TM: tectorial membrane

TC: tectorial curtain

VIIIAP: branch of VIIIth nerve which innervates the amphibian papilla.

Calibration mark = 200 µm

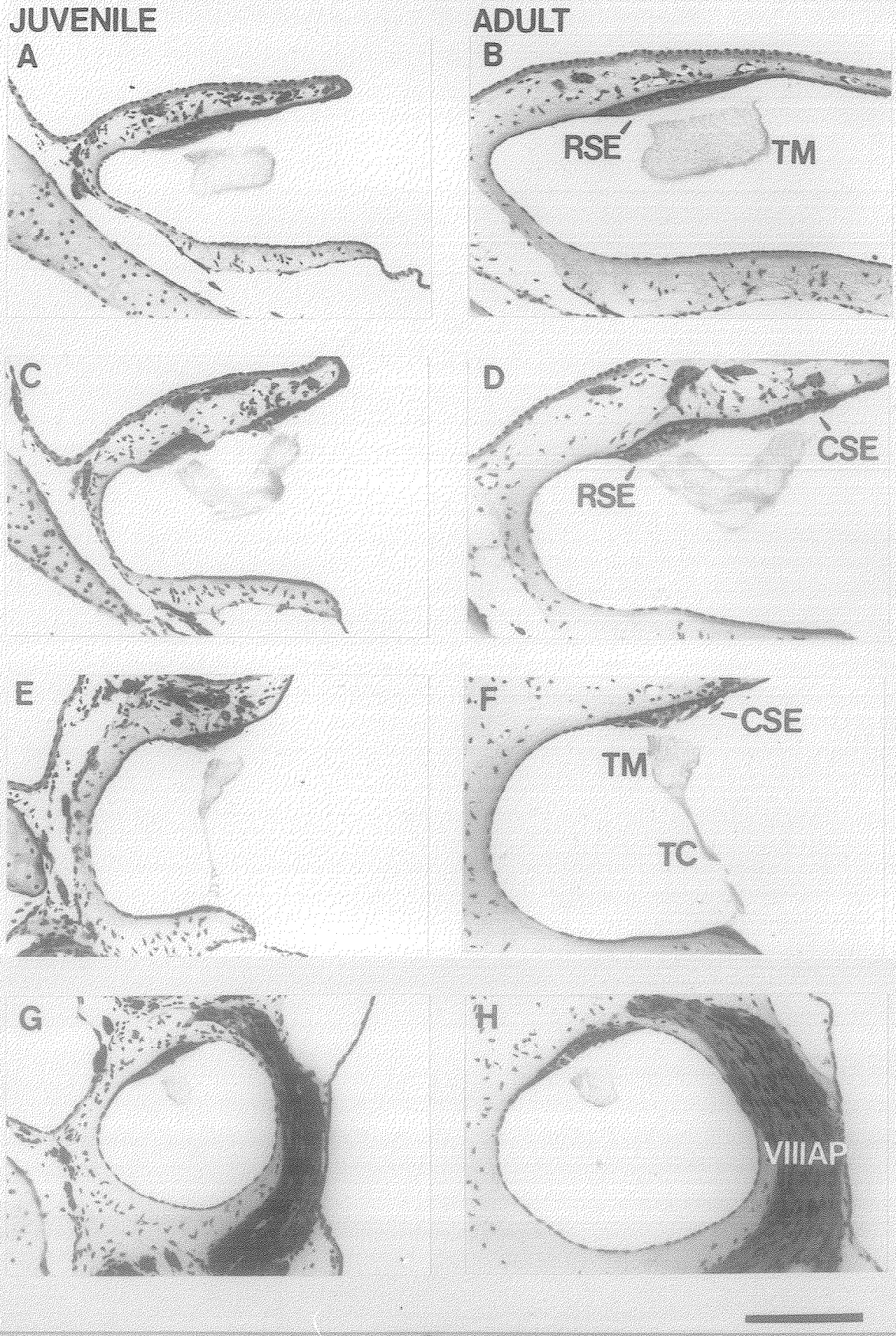

JUVENILE
ADULT
A
B
RSE
TM
C
D
CSE
RSE
E
F
CSE
TM
TC
G
H
VIIIAP

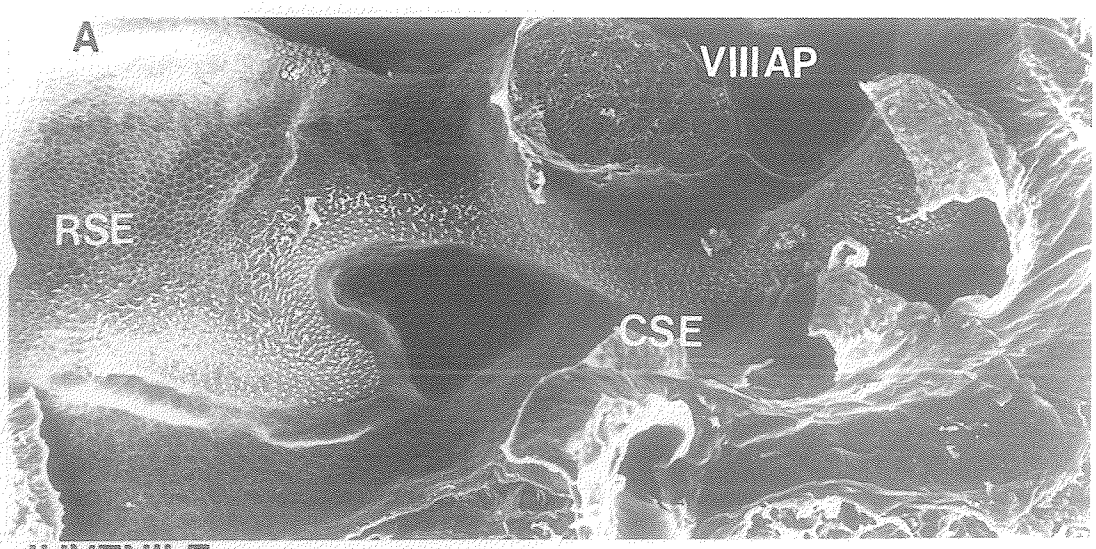
Figure 11. Scanning electron micrographs of the sensory epithelia of juvenile (A) and adult (B) amphibian papillae.

RSE: rostral triangular patch of sensory epithelium.

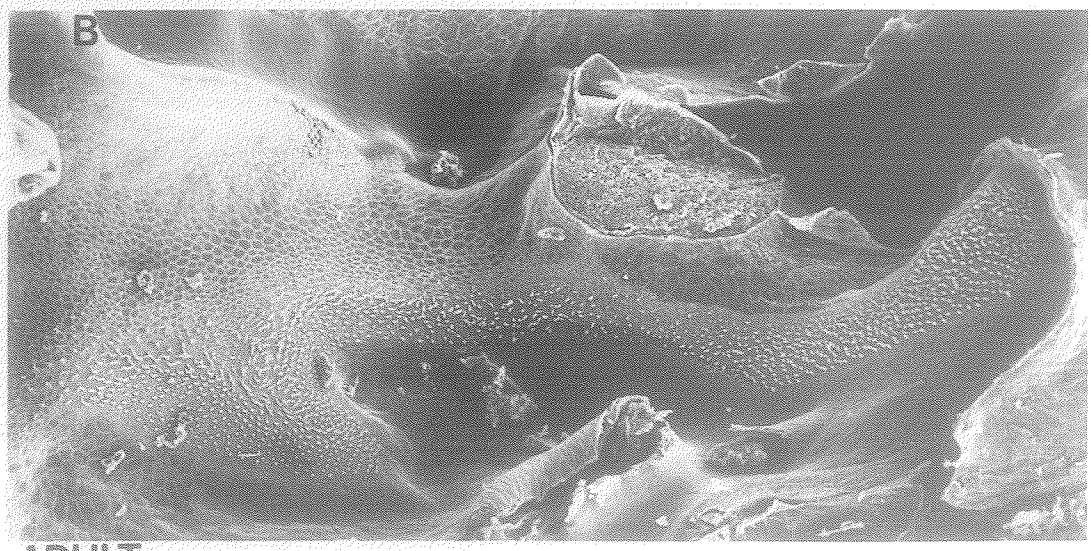
CSE: caudal S-segment of sensory epithelium.

VIIIAP: branch of VIIIth nerve which innervates the amphibian papilla.

Calibration mark = 100 µm.



JUVENILE



ADULT

Figure 12. Mean cross sectional areas of the tectorial membrane (TM) as a function of mean length for juvenile (broken line) and adult (solid line) amphibian papillae. Filled and open circles are values of the TM associated with the rostral triangular patch and caudal S-segment of epithelium, respectively.

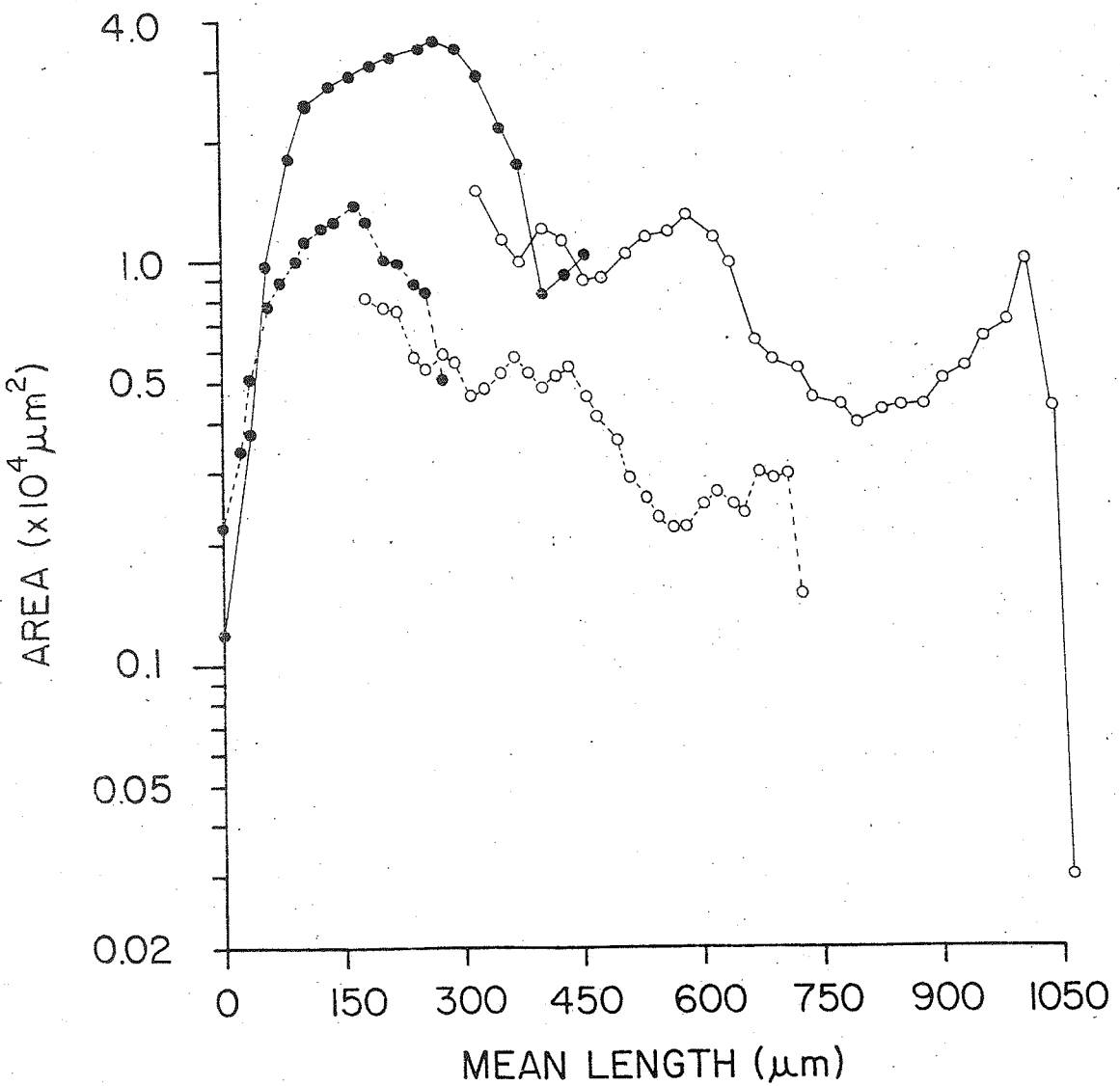

4.0
1.0
0.5
0.1
0.05
0.02
AREA (x10⁴μm²)
0
150
300
450
600
750
900
1050
MEAN LENGTH (μm)

Figure 13. Mean cross sectional areas of TM normalized as a function of percent mean length for juvenile (broken line) and adult (solid line) amphibian papillae. Filled and open circles are values of TM associated with the rostral triangular patch and caudal S-segment of epithelium, respectively. The arrow indicates the location of the tectorial curtain. Vertical lines show standard deviations.

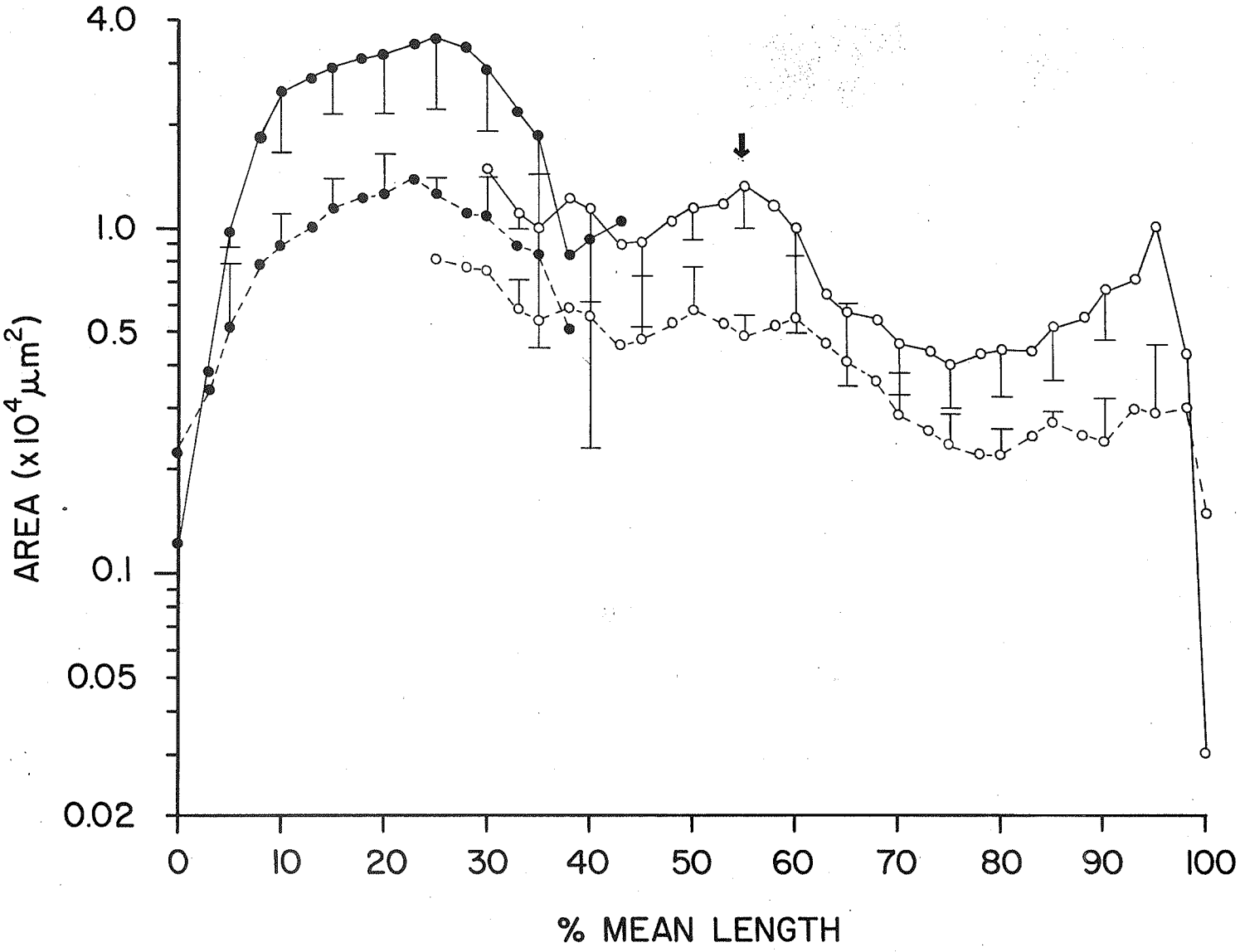
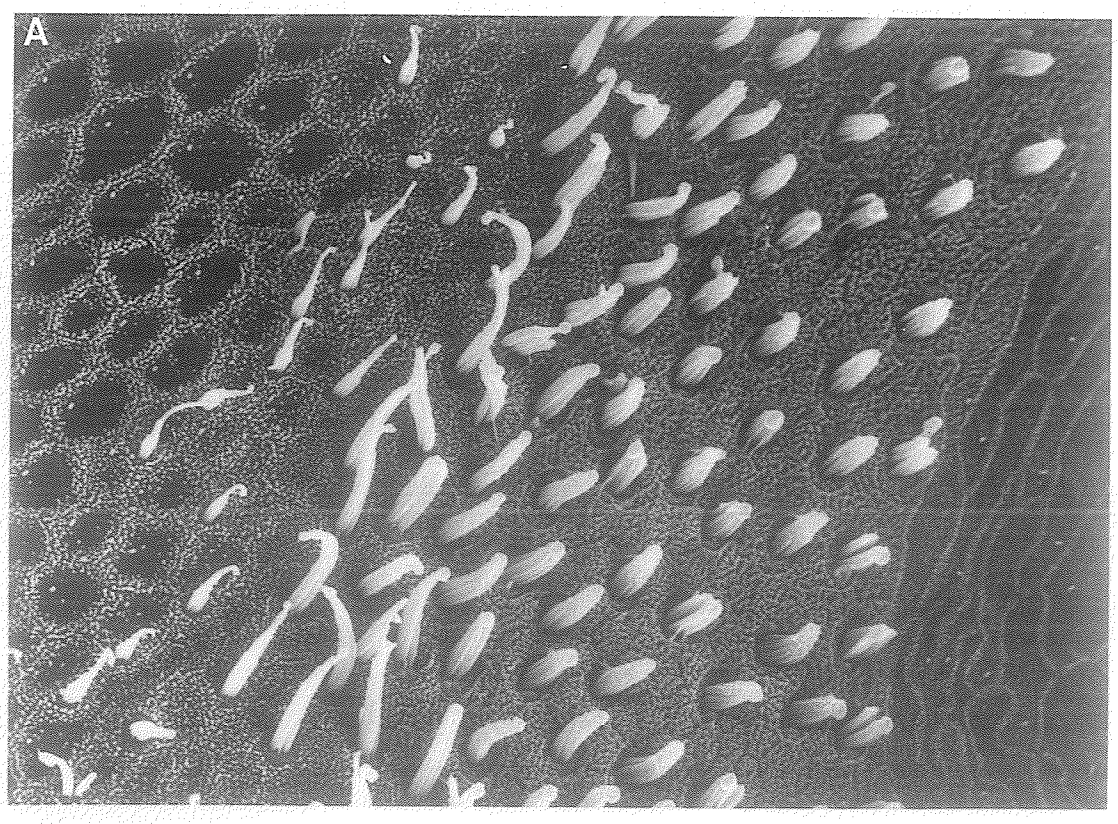
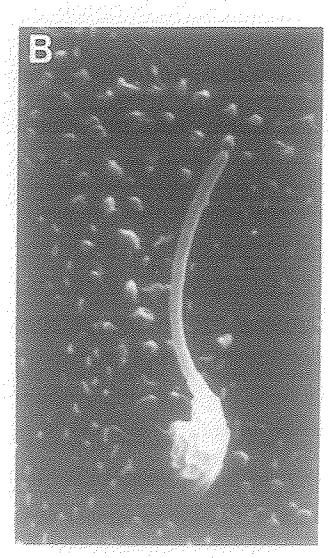
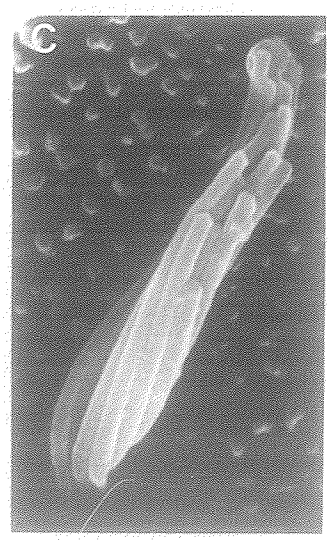

4.0
1.0
0.5
0.1
0.05
0.02
AREA (x10⁴μm²)
0
10
20
30
40
50
60
70
80
90
100
% MEAN LENGTH

Figure 14. A. Scanning electron micrograph showing the gradation of the three hair cell types along the mediolateral axis observed in the juvenile amphibian papilla. Calibration mark = 10 µm.

B-D. Scanning electron micrographs of the three types of hair cells found in the amphibian papilla. See text for description. (B) Type A; (C) Type E; (D) Type D. Calibration mark = 1 µm.


A


B


C

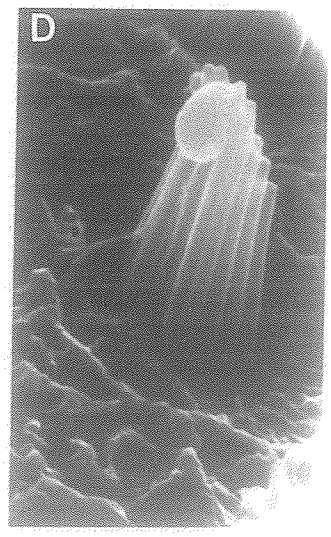
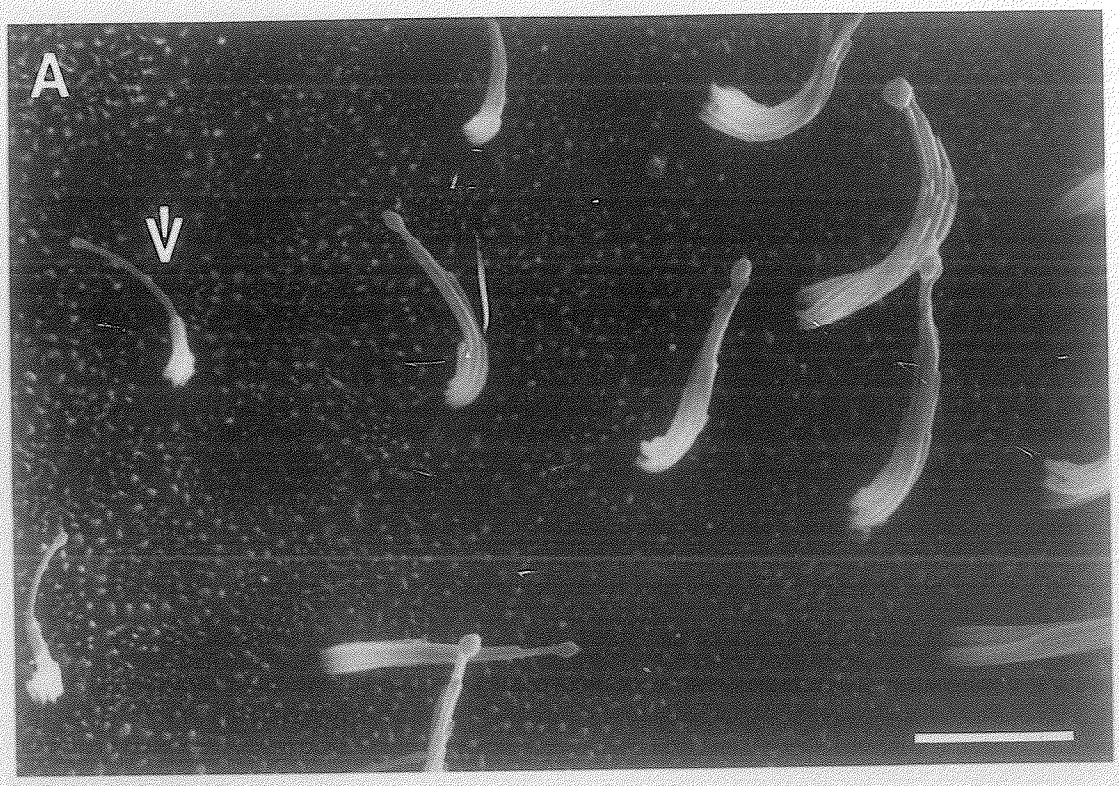

D

Figure 15. A. Scanning electron micrograph showing the lateral edge of a juvenile amphibian papilla. Calibration mark = 5 μm.

B. High magnification of the most lateral hair cell on the above figure (indicated by the arrow). Note the bulbed kinocilium. Calibration mark = 1 μm.


A

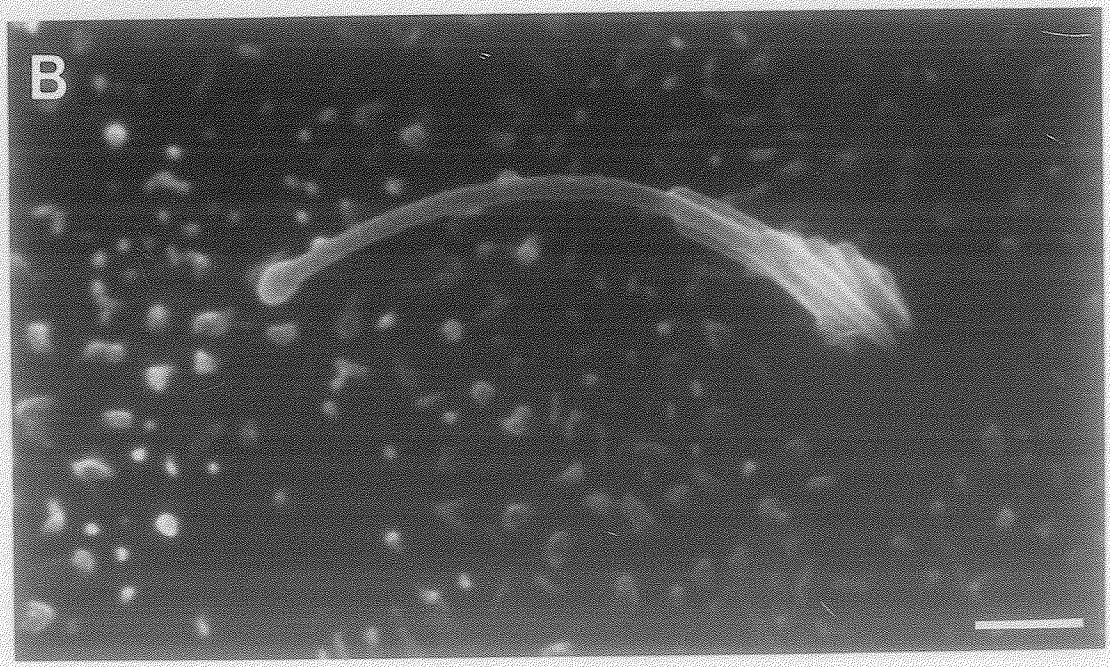

B

Figure 16. Stereopair of a type D hair cell from the amphibian papilla. Width of the entire micrograph is 11 μm.

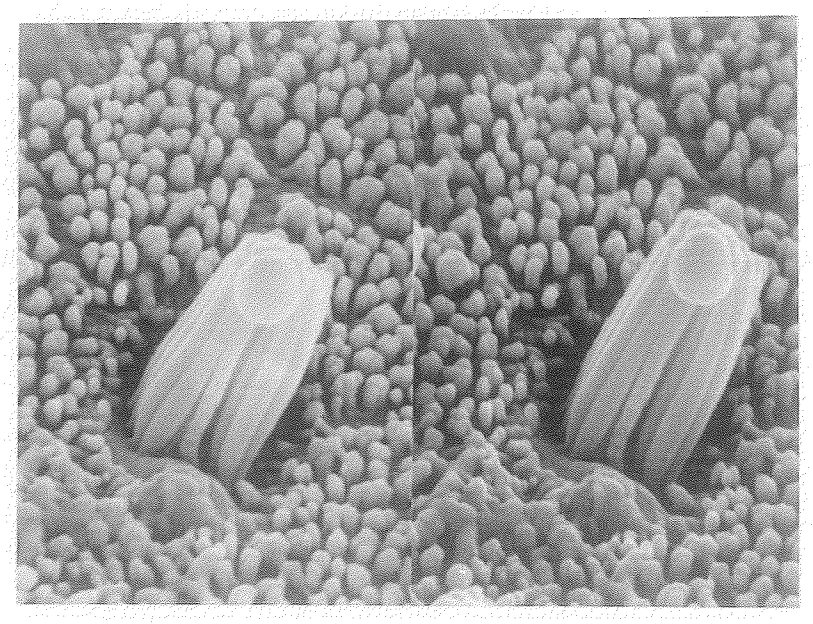


Figure 17. Histograms of the mean height of the tallest stereocilia of type D hair cells along the sensory epithelia of a juvenile and an adult amphibian papilla.

a. Mean height from rostral triangular patch.

b. Mean height from the region around the tectorial curtain.

c. Mean height from the caudal tail.

Schematic at the top of figure illustrates regions along the epithelium where measurements were made.

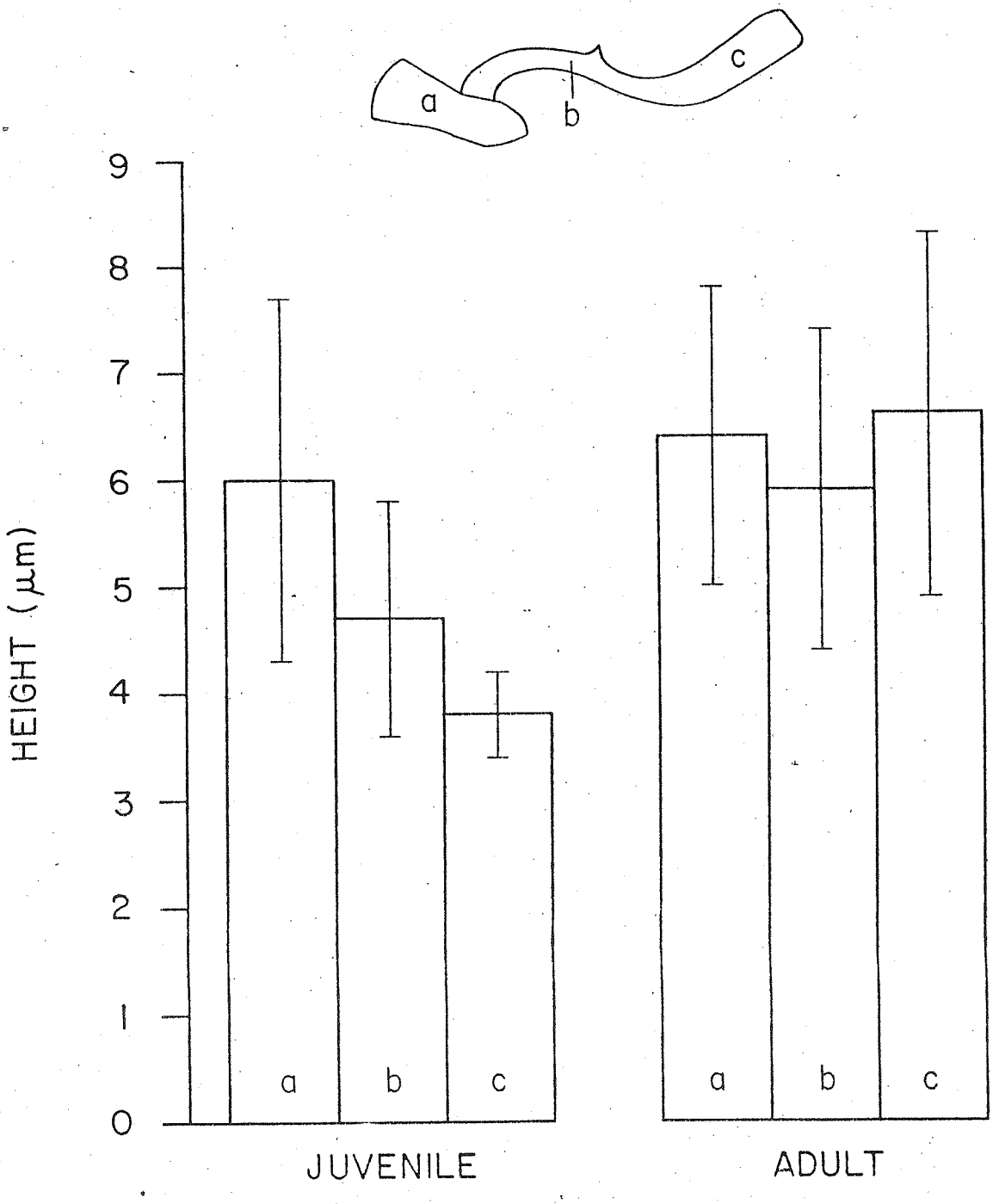

a
b
c
HEIGHT (μm)
0
1
2
3
4
5
6
7
8
9
a
b
c
JUVENILE
a
b
c
ADULT

TABLE 1

	M	C	f_o
ADULT	6.0×10^6 Kg/m^4	9.9×10^{-21} $\frac{m^4 sec^2}{Kg}$	6.5×10^5 Hz
JUVENILE	8.3×10^6 Kg/m^4	3.6×10^{-21} $\frac{m^4 sec^2}{Kg}$	9.2×10^5 Hz

Table 1. Calculated values of acoustic inertance (M), acoustic compliance (C), and resonant frequency (f_o) for adult and juvenile basilar papillae assuming viscous forces are negligable.

TABLE 2

	M	C	f_o
ADULT	24.6 Kg/m^4	$6.5 \times 10^{-10} \frac{m^4 sec^2}{Kg}$	1259 Hz
JUVENILE	46.6 Kg/m^4	$1.2 \times 10^{-10} \frac{m^4 sec2}{Kg}$	2128 Hz

Table 2. Calculated values of acoustic inertance (M), acoustic compliance (C), and resonant frequency (f_o) for adult and juvenile basilar papillae assuming viscous forces are dominant.

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SECTION V. PHASED-LOCKED RESPONSES OF AMPHIBIAN PAPILLA AUDITORY FIBERS TO LOW FREQUENCY TONES

INTRODUCTION

The amphibian papilla of the bullfrog is tonotopically organized along its length with low frequencies represented rostrally and higher frequencies represented caudally (Lewis etal., 1982a; 1982b). The tonotopy observed in the amphibian papilla appears to be due in part to the spatial gradations in the mass of the tectorial membrane (Lewis, 1981; Lewis and Leverenz, in press; Sections III and IV of this thesis). It has been suggested that these spatial gradations in mass may give rise to a traveling wave in the tectorial membrane of the amphibian papilla (Palmer and Wilson, 1981; Lewis etal., 1982b) analogous to that found in the basilar membrane of the mammalian cochlea where there exists spatial gradations in the compliance of the membrane (Bekesy, 1960). The phase locked neural discharge of mammalian VIIIth nerve fibers (Pfeiffer and Molnar, 1970; Anderson etal., 1971; Geisler etal., 1974) and anteroventral cochlear nucleus neurons (Gibson etal., 1977; Brugge etal., 1978) to the stimulus cycle has been used to determine the travel time of the mechanical disturbance along the basilar membrane. This travel time is proportional to the propagation velocity of the traveling wave (Gibson etal., 1977). The purpose of the present study is to investigate the phased locked responses of auditory fibers derived from the amphibian papilla of the bullfrog in order to gain some insight into whether a traveling wave may occur in the tectorial membrane.

METHODS AND MATERIALS

Intermediate sized bullfrogs ranging from 6.2-6.7 cm in snout-vent lengths were obtained from Charles Sullivan (Nashville, TN). Animals were anesthetized with pentobarbitol (65 mg/Kg), and the VIIIth nerve was exposed dorsally as described in Section II. Methods used for single unit recording and acoustic stimulation have been described in Section II.

When an auditory fiber was isolated, its best excitatory frequency (BEF) was first determined. After the BEF was characterized, the phase locked neural responses to the stimulus cycle of different low frequency tones were studied. Action potentials were generated in response to tone bursts of 100 msec duration having frequencies from 50-400 Hz. The frequency of the tone was increased in 50-100 Hz increments while the intensity was maintained at a constant level. Neural responses and acoustic stimuli were receorded on magnetic tape (TEAC A-2340SX tape recorder) for off line computer analysis.

Cycle histograms having 50 usec binwidths were generated and stored on a PDP-11/23 computer. The zero crossing of the negative slope of the sine wave was used as a phase reference of 0π in each cycle histogram. The degree of synchronization and mean phase angle were calculated according to Goldberg etal (1969) for each cycle histogram. The mean phase angle can vary from 0 to 2π, and the degree of synchronization can range from 0 to 1 where 0 implies no phase locking of the neural response to the stimulus and 1 implies perfect phase locking (Goldberg etal., 1969).

RESULTS

A total of 56 single auditory fibers was isolated from 3 intermediate sized bullfrogs, and the phased locked responses to the stimulus cycle were obtained for 18 fibers.

Figure 1 shows the cycle histograms obtained from an auditory fiber having a BEF of 705 Hz in response to frequencies ranging from 100-400 Hz at an intensity of 100 dB SPL. It can be observed from the cycle histograms that as the stimulus frequency is increased, there is a shift in the mean phase angle of the neural response. The cumulative shift in mean phase angle as

a function of stimulus frequency is shown in Figure 2 for six fibers studied from one animal. Each phase-frequency plot has been fit with a regression line. The slopes of these linear phase-frequency plots appear to be independent of the BEF and are similar for all fibers having values of approximately 0.018 rad/Hz. The basilar papilla fiber has a BEF of 1530 Hz and a slope of 0.024 rad/Hz.

DISCUSSION

The results of the present study suggest that for auditory fibers innervating the amphibian papilla, the cumulative phase-frequency function is independent of the BEF. In addition, the slopes of these phase-frequency plots are similar in a given animal. The slope of the linear phase-frequency function is an estimate of the time it takes the mechanical disturbance at the oval window to reach a given location along the auditory organ (Anderson etal., 1971; Gibson etal., 1977). Since the slopes of the phase-frequency plots appear to be similar and independent of BEF for amphibian papilla fibers, it suggests that the travel time of the mechanical disturbance may be the same for all loci along the tectorial membrane.

The results obtained from the VIIIth nerve fibers in the present study are in contrast to those obtained from the mammalian VIIIth nerve (Pfeiffer and Molnar, 1970; Anderson etal., 1971; Geisler etal., 1974) and the antero-ventral cochlear nucleus (Gibson etal., 1977; Brugge etal., 1978). In mammals the slope of the cumulative phase-frequency function is dependent on BEF such that there is a systematic decrease in the slope with increasing BEF. The relationship between the slope and BEF in mammals indicates that the time delay for high frequencies is less than that for low frequencies and is thought to

reflect the propagation velocity of the traveling wave (Gibson etal., 1977). If this is the case, then the results of the present study suggest that a traveling wave may not occur in the tectorial membrane of the amphibian papilla. This conclusion is consistent with the hypothesis that the input acoustic energy is distributed over the surface of the entire tectorial membrane and the local resonant characteristics of the tectorial membrane determine the frequency at a given locus (Lewis and Leverenz, in press). However, the results of the present study are preliminary, and further experiments are required before a definite conclusion can be reached.

Figure 1. Representative cycle histograms obtained from an amphibian papilla auditory fiber having a BEF of 705 Hz. The phased-locked responses shown are for stimulating frequencies of 100 Hz, 200 Hz and 400 Hz at 100 dB SPL. The cycle histograms have been normalized to a 50 bin histogram. Thus, each bin is 0.04 π.
Mean phase and synchronization (r) are as follows:
100 Hz: mean phase 0.55 π ; r = .819
200 Hz: mean phase 1.72 π ; r = .821
400 Hz: mean phase 0.34 π ; r = .741

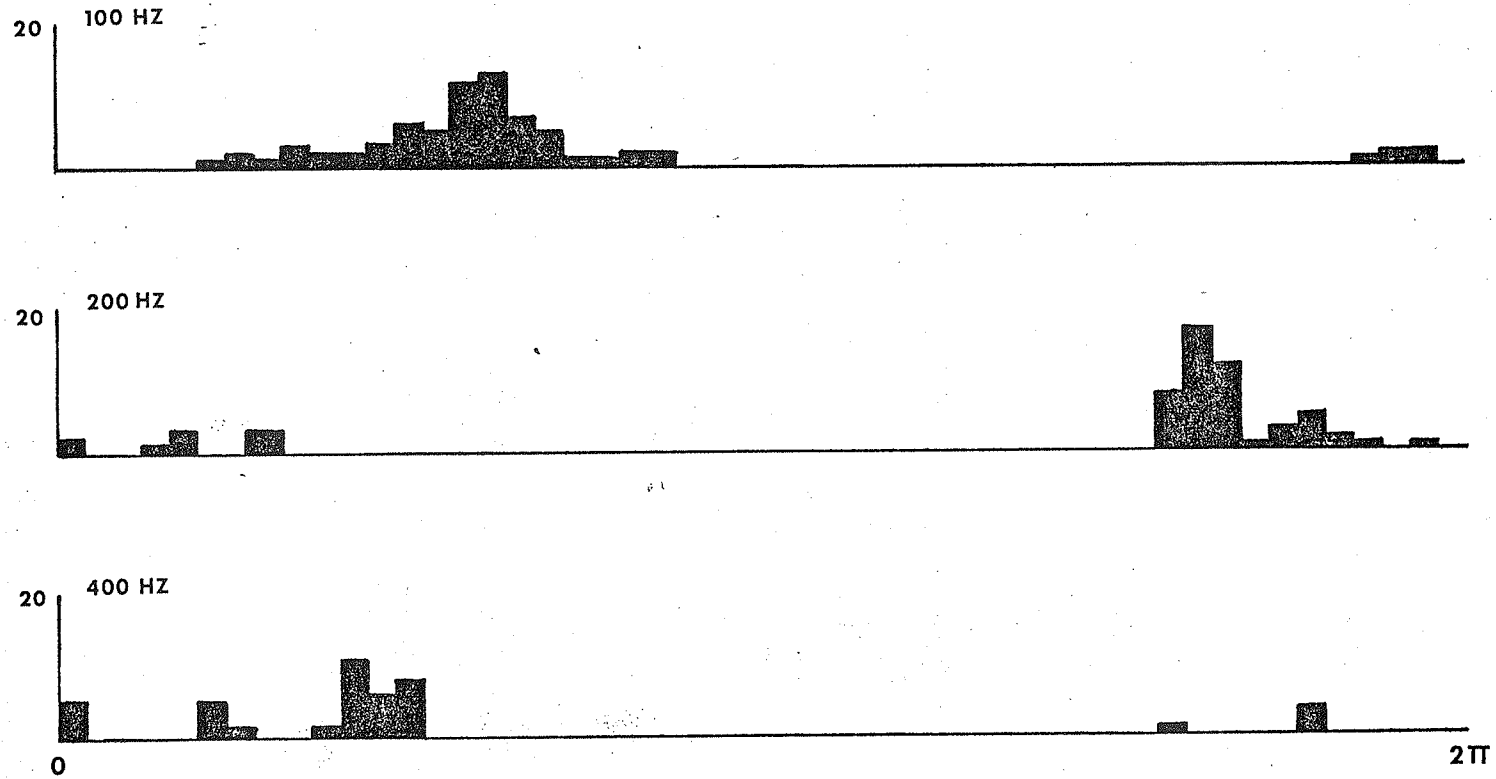
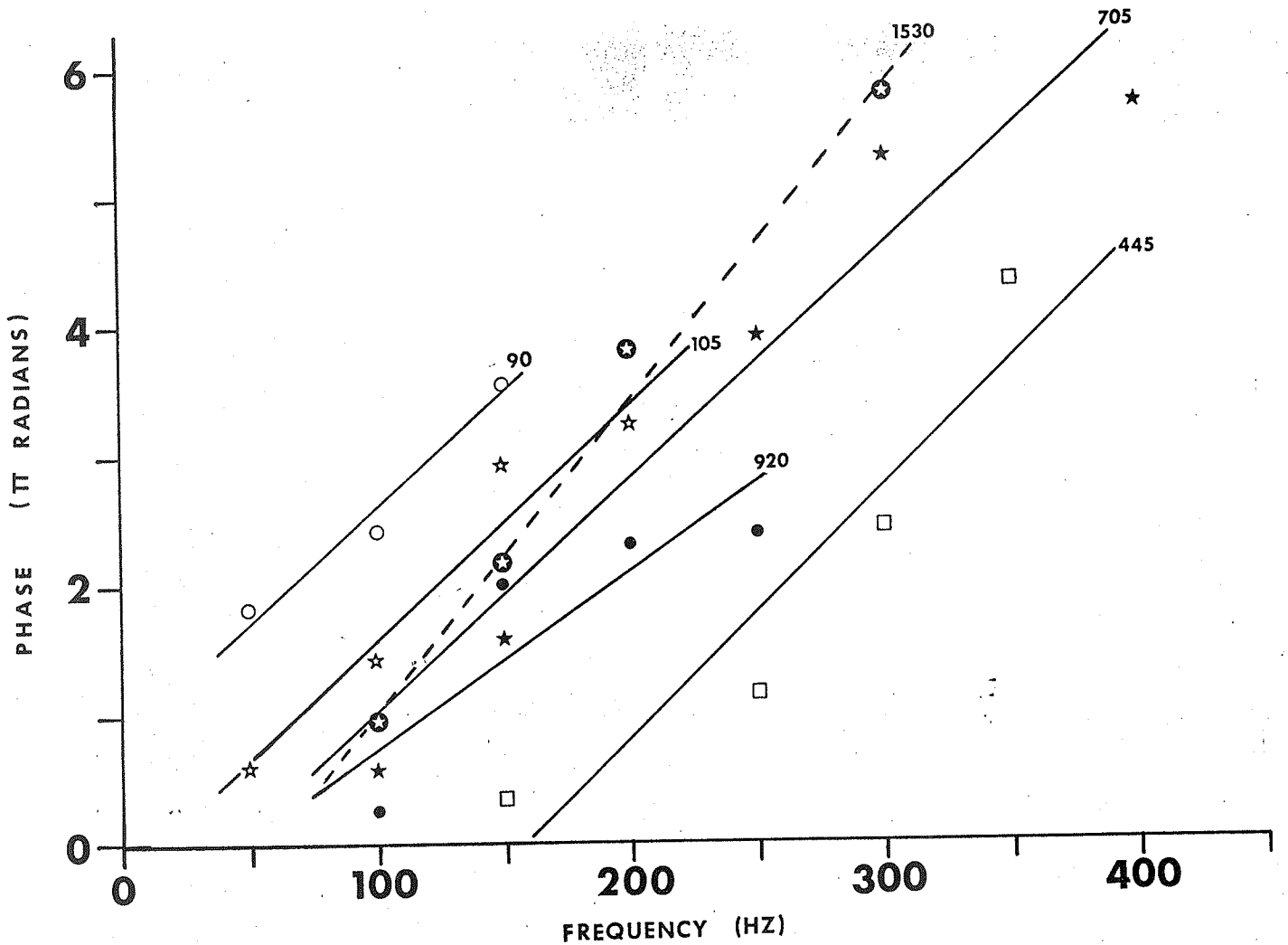

20
100 HZ
20
200 HZ
20
400 HZ
0
2π

Figure 2. The cumulative phase shifts as a function of stimulus frequency for 5 amphibian papilla fibers (solid lines) and 1 basilar papilla fiber (dashed line) from the same animal. The BEF of each fiber is given at the top of each phase-frequency plot. Data points have been fit with regression lines.


PHASE (TT RADIANS)
6
4
2
0
0
100
200
300
400
FREQUENCY (HZ)
1530
705
445
90
105
920

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SECTION VI. CONCLUDING REMARKS

CONCLUDING REMARKS

Summary of the Results

During the post-metamorphic development of the bullfrog, there is a gradual downward shift in the frequency selectivity of the peripheral auditory system. This downward shift is the consequence of developmental changes in the sizes of the various peripheral auditory structures. As these structures increase in size during post-metamorphic development, their physical properities are modified resulting in changes in the frequency selectivity of the peripheral auditory system.

The decrease in the upper limit of hearing with age appears to be primarily the result of the increase in the area (and mass) of the tympanic membrane and the mass of the middle ear bones. As the middle ear structures become more massive, there presumably is an attenuation of high frequency vibrations in the middle ear resulting in a decrease in the upper cut off frequency of the middle ear response. Mechanical measurements of the vibration of the tympanic membrane are necessary in order to determine the frequency response of the middle ear during post-metamorphic development. Only then can the contribution of the middle ear to the shift in frequency selectivity be resolved.

While the increase in the mass of the middle ear bones may be an important factor in decreasing the upper limit of hearing during post-metamorphic development, it is unlikely to be the primary contributor to the decreases in the frequency selectivities of the three populations of auditory fibers in the VIIIth nerve. Although a decrease in the upper cut off frequency of the middle ear response with post-metamorphic age may result in the elimination of the

high frequency population observed in juveniles, it cannot account for the shifts in the frequency selectivities of the low and intermediate frequency populations due to the low pass filter characteristics of the middle ear (Saunders and Johnstone, 1972; Moffat and Capranica, 1978). Thus, the tuning properties of the individual auditory organs are important factors in shaping the distribution of best excitatory frequencies (BEFs) during post-metamorphic development.

The downward shift observed in the frequency selectivity of the high frequency population of auditory fibers from the basilar papilla seems to be the result of two factors. First, the basilar papilla apparently acts as a tuned resonator under conditions where fluid viscosity is the important component. Based on calculations, changes in the acoustic inertance and acoustic compliance of this resonator seemingly occur during post-metamorphic development as a result of the enlargements of the resonator opening (contact membrane) and volume (lumen). As a consequence of the changes in acoustic characteristics of the basilar papilla, there is a decrease in the calculated resonant frequency of the organ. In addition to the changes in the resonance properites, there is a dramatic increase in the mass of the tectorial membrane during this period of development, which presumably results in a decrease in the frequency selectivity of the hair cells associated with this structure by increasing the load on the stereocilia. While the shift in the upper limit of hearing can be attributed to the middle ear, it is the resonance characteritics of the basilar papilla that determine the frequency selectivity of the high frequency populations of fibers, and not any resonance characteristics of the middle ear.

The intermediate frequency population of auditory fibers is derived from the caudal tail of the amphibian papilla, and the downward shift in their frequency selectivity during development appears to be due in part to an increase in the mass of the tectorial membrane associated with this region of the papilla.

As in the basilar papilla, the increase in tecorial membrane mass presumably results in an increase of the load on the hair cell stereocilia. In addition to the enlargement of the tectorial membrane, there is also a lengthening of the stereocilia of the predominant hair cell type in the caudal tail of the amphibian papilla. This increase in stereocilia height theoretically results in a decrease in the stiffness of the stereociliary bundle. Both of the above factors, namely the increase in tectorial membrane mass and the increase in stereocilia height, result in a decrease in the frequency selectivity of the hair cells in the caudal tail of the amphibian papilla.

The overlap between the frequency selectivity of the juvenile low frequency with that of the adult intermediate frequency population appears to be due in part to the absolute mass of the tectorial membrane which has similar values in the S-segment of the amphibian papilla rostral to the tectorial curtain in juveniles and caudal to the tecotial curtain in adults. Low frequency selective fibers in both groups of frogs show two-tone inhibition, but the frequency selectivity for inhibition shifts downward with post-metamorphic age. This shift appears to be related to the increase in the mass of the tectorial membrane in the caudal tail of the amphibian papilla, since the frequency selectivity for inhibition corresponds to the excitatory frequencies in this region of the sensory epithelium. Thus, the mechanism underlying two-tone inhibition may involve a nonlinear interaction between the tectorial membrane in the caudal tail and rostral triangular regions of the papilla.

In addition to the changes in frequency selectivity, differences in the sensitivity of some auditory fibers of the various populations are found between the two groups of frogs. The threshold of excitation of the majority of auditory fibers are similar between juveniles and adults with the most sensitive fibers in both groups having thresholds around 20 dB SPL and suggests that the impedance transformer action of the middle ear is mature in juvenile bullfrogs. Thus, the middle ear sensitivity may not be able to account for the populations of high threshold fibers that are present in juveniles, but

absent in adults. Mechanical measurements of the middle ear are necessary to establish its sensitivity during post-metamorphic development, nevertheless.

It is likely that changes within the inner ear are in part responsible for the sensitivity changes of some fibers. In the basilar papilla, the increase in the sensitivity of the higher threshold fibers during post-metamorphic development does not appear to be due to changes in the number of hair cells innervated by a single auditory fiber, since one axon innervates only one hair cell in the adult (Lewis etal., 1982a). However, if a nerve fiber innervates immature hair cells in the juvenile, then the threshold of excitation may be high. On the other hand, the increase in sensitivities of some amphibian papilla fibers may possibly be the result of changes in the innervation pattern of the hair cells. For instance, in the adults, a single fiber innervates 1-15 hair cells (Lewis etal., 1982a; 1982b). If there is an increase in the number of hair cells innervated by a single auditory nerve fiber during post-metamorphic growth, the sensitivity of the fiber may be increased. Furthermore, some hair cells in the amphibian papilla appear to differentiate into other hair cell types. If this apparent transformation is accompanied by changes in the innervation pattern, increases in the sensitivity of the fiber may result. Intracellular staining of single auditory fibers in the juvenile bullfrog are required in order to relate the sensitivity of the fiber to its innervation pattern of the hair cells.

In addition to the possible changes in the innervation patterns of the hair cells, the tectorial membrane may also play a role in this increase in sensitivity. Since a basilar membrane does not exist in the anuran auditory papillae, the bending of the hair cell stereocilia in response to acoustic stimulation in the anuran auditory organs is presumably due to the viscous coupling between the stereociliary bundle and endolymph. The tectorial membrane may serve to increase the effective surface area over which the frictional

coupling between endolymph and stereocilia can act upon, thus increasing the sensitivity of displacement of the stereociliary bundle. If this is the case, the enlargement of the tectorial membrane during post-metamorphic development may further increase the effective area for viscous coupling resulting in an increase in the sensitivity of the hair cells associated with the tectorial membrane.

Behavioral Significance

The behavioral significance of the frequency selectivities of the three populations of auditory fibers has been described for adult anurans. The dominant spectral energies of anuran calls are closely matched to the frequency selectivities of the basilar and amphibian papillae such that the auditory periphery is specialized for the detection of biologically signifigant sounds. Thus, the central auditory system is only processing acoustic information which contains the same spectral features as those found in anuran calls.

The spectral energies found in the mating call of the adult male bullfrog are bimodally distributed (Capranica, 1965; 1966; 1968). There is a low frequency peak of energy centered around 200-300 Hz, and a broad peak of spectral energy is typically centered around 1400-1500 Hz. In addition to these two spectral peaks, a relative absence of acoustic energy is found from 500-700 Hz. However, other calls produced by both male and female bullfrogs such as territorial calls, release calls and warning calls possess substantial spectral energy around 500-800 Hz (Capranica, 1965; 1968).

When the bullfrog mating call is presented to a group of adult male bullfrogs, it evokes a high level of mating calling from the males (Capranica, 1965; 1966). However, the playback of mating calls from other anuran species fails to evoke mating calling in the male bullfrogs, thus demonstrating that the bullfrog can recognize its own species mating call from those of other species (Capranica, 1965). Female bullfrogs do not exhibit this evoked vocalization in response to the mating call, but presumably would show phonotaxis toward the loudspeaker (or male bullfrog) as described for other families of anurans (Martof and Thompson, 1958; Gerhardt, 1974; 1981; Ryan , 1980). Thus, the mating call is attractive not only to adult males, but presumably to adult females as well.

Using synthetic mating calls, Capranica (1965; 1966) has demonstrated the essential spectral features of the mating call which stimulate the evoked vocal response in male bullfrogs. When either the high or low frequency peak of energy is filtered from the call, evoked mating calling by males does not occur. When an additional frequency component of 500 Hz is added to the synthetic call, the evoked vocal response can be partially or totally suppressed depending on the relative amplitude of the 500 Hz component to the low frequency peak. Furthermore, if both low and high frequency peaks are present, but the low frequency energy is systematically shifted toward 500 Hz, the evoked vocal response systematically falls off. Interestingly, smaller younger males (3-4 inches) produce a bimodal mating call with a low frequency peak of energy around 500-700 Hz rather than at 200-300 Hz as in larger adult bullfrogs (6-7 inches), and this call of the younger frogs is unattractive to the adults (Capranica, 1965; 1966).

The results of Capranica (1965; 1966) demonstrate that the simultaneous presence of low frequency energy centered around 200-300 Hz and high frequency energy centered around 1400-1500 Hz is required in the spectral distribution of the mating call in order to render this call attractive behaviorally. Physiologically, the low frequency peak in the mating call corresponds to the frequency selectivity of the low frequency population of auditory fibers, which innervate the rostral triangular patch and the initial S-segment of the amphibian papilla sensory epithelium (Lewis etal., 1982a; 1982b). The high frequency energy in the mating call matches the frequency selectivity of the high frequency population of auditory fibers, which are derived from the basilar papilla (Lewis etal., 1982a; Feng etal., 1975). Thus, in order to evoke the behavioral response in adult male bullfrogs, the rostral regions of the amphibian papilla and the basilar papilla must be simultaneously excited. In addition to the presence of low and high frequency energy, an absence of acoustic energy around 500-700 Hz is a prerequisite for the evoked vocal response

to occur. This frequency peak corresponds to the frequency selectivity of the intermediate frequency auditory fibers derived from the caudal tail of the amphibian papilla (Lewis, etal., 1982a; 1982b) as well as to the frequency selectivity for two-tone inhibition of low frequency auditory fibers.

The excitation of the low and high frequency fibers by the two dominant spectral energy peaks in the mating call provides a peripheral mechanism for the stimulation of the behavioral response. In addition, these two populations project to different regions of the dorsal medullary nucleus (Lewis etal., 1980; Fuzessery and Feng, 1981) and their inputs appear to converge in higher auditory centers. For example, many auditory neurons in the thalamus show nonlinear excitatory responses to combinations of low and high frequency tones, which may serve as a central mechanism in the stimulation of this behavior (Mudry etal., 1977; Fuzessery and Feng, in press). The suppression of the neural activity of the low frequency auditory fibers by intermediate frequencies via two-tone inhibition serves as a peripheral mechanism in suppressing the evoked calling response (Capranica, 1965). In the central auditory system, the intermediate frequencies also inhibit the neural responses of many thalamic neurons (Fuzessery and Feng, in press).

In view of the close match between the frequency selectivities of the adult amphibian and basilar papillae to the dominant spectral energies present in the bullfrog calls, it raises the question as to what might be the behavioral signifigance of the frequency selectivities of the juvenile auditory organs. The juveniles used in the present study are much less than one year old based on their body length (Howard, 1978) and will not begin to produce a mating call until they are one year past metamorphosis (Dickerson, 1969). While newly transformed bullfrogs have been heard to give an alarm or warning call (Wright, 1914), the spectral structure of this juvenile call is unknown. In adults, however, the warning call has a broad unimodal distribution of spectral energy

below 1500 Hz and a monotonic decrease in energy at higher frequencies (Capranica, 1965; 1968). Thus, it is unknown whether the frequency selectivity of the juvenile peripheral auditory system is matched to the dominant spectral energies of supposed juvenile calls. Nevertheless, it is interesting to note that the dominant spectral energies of the bullfrog mating call are closely matched to the frequency selectivities of the low and intermediate frequency populations of auditory fibers in the juveniles. Moreover, juvenile bullfrogs apparently are present during the adult mating season.

In the bullfrog, the transformation from tadpole to frog takes place from June to August (Wright, 1914; Dickerson, 1969), while the mating season lasts from May through July in northern states (Willis etal., 1956; Howard, 1978), but extends from April to November in Florida (Willis etal., 1956). Thus, newly metamorphoses bullfrogs are present during at least part of the mating season. However, juvenile bullfrogs are not reproductively mature (Willis etal., 1956; Howard, 1978) and hence, will not be participating in mating. Typical behavior for the newly transformed frogs is to hide themselves during the day and to venture out at night (Dickerson, 1969). It is at night, however, when the males are generally calling for a sustained period (Howard, 1978). In view of this, might the detection of the adult mating call have some behavioral significance for the juveniles?

It is possible that when juveniles come out of hiding that the detection of the adult mating call may serve in the recognition of the calling males as potential predators. Adult bullfrogs are voracious eators and do prey on smaller frogs (Dickerson, 1969). If the same peripheral and central mechanisms which process the acoustic information in the adult are also present in the juvenile, the the mating call is presumably unattractive to juvenile bullfrogs. Since the two dominant spectral peaks of the mating call are matched to the frequency selectivities of the juvenile low and intermediate frequency populations of auditory fibers, the call presumably excites the rostral and caudal

amphibian papilla in juveniles, rather than stimulating the rostral amphibian papilla and basilar papilla as in adults. Furthermore, since the 1400-1500 Hz component of the mating call corresponds to the frequency selectivity for inhibition of low frequency fibers in the juveniles, then this spectral component of the call presumably suppresses the neural activity of the low frequency population. Thus, two-tone inhibition may serve as a peripheral mechanism for the recognition of calling males as potential predators. Moreover, the excitation of the intermediate frequency population by the 1400-1500 Hz component presumably inhibits the neural activity of the juvenile thalamic neurons rather than exciting them as in adults. This presumptive central inhibition in juveniles may serve as a central mechanism in the recognition of the males as predators. Systematic behavioral studies are required to establish whether or not this behavioral significance in juvenile bullfrogs actually exists.

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