J. comp. Physiol. 118, 249–260 (1977)

Journal of Comparative Physiology · A

Response Patterns and Peripheral Origin of Auditory Nerve Fibers in the Monitor Lizard, *Varanus bengalensis*

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Received January 25, 1977

Summary. 1. The activity patterns of primary auditory nerve fibers in *V. bengalensis* were studied with microelectrode recordings from the proximal and distal portions of the nerve.

2. Spontaneous firing rates varied from 0.65 to 52.1 spikes/s, and decay rates were exponential (Fig. 1).

3. A low characteristic frequency (CF) group of units had smooth, sharp tuning curves, whereas a higher CF group had less sharp, more complex tuning curves (Figs. 2 and 3). These tuning curves had no particular asymmetry (Figs. 4 and 5).

4. Recordings from fibers near the basilar membrane allowed for accurate mapping of the peripheral origin of different CF's (Fig. 6). The lower CF group was localized in the dorsal portion of the papilla, with the lowest CF's at the ventral end. The constricted-off ventral portion of the papilla had abnormally high CF's, and gave rise to the high CF group. Only the dorsal portion of the papilla was tonotopically organized. It is suggested that the constriction in the papilla allowed independent evolution of the ventral part of the papilla.

5. Click polarity reversal data (Fig. 8) and pure tone response pattern data (Fig. 7) indicate the ventral third of the dorsal papilla has unidirectional hair cell orientation. It is suggested that bidirectional hair cell orientation is a peripheral specialization for sound onset emphasis.

Introduction

A good deal of current research work in the physiology of the auditory periphery is directed towards attempting to understand the influence of the accessory structures of the ear on the response patterns of primary auditory fibers. The ears of various reptiles provide a diversity of arrangements of these accessory structures (e.g., basilar membrane, tectorial membrane), in contrast to the relative stability of structure in birds and mammals (Baird, 1970; Manley, 1974;

Miller, 1966; Wever, 1967). The lizards, in particular, provide a series of 'natural experiments' where a species can be chosen for study on the basis of the unique arrangement of structures in the ear. The combined study of a number of species should help answer questions concerning the functional significance of the presence or absence of a tectorial membrane, unidirectional or bidirectional hair cell orientation, etc. (Manley, 1970; 1974).

Comparisons with mammalian structures and auditory nerve responses should be possible, given the general stability of structure of the hair cells themselves. It should be expected, however, on the basis of our knowledge of the evolution of the ear (Manley, 1973) that a number of patterns of response seen in reptiles will be unique.

Lizards of the family Varanidae have a moderately well developed basilar papilla more or less divided by a constriction into two areas (Manley, 1976; Miller, 1974). In *Varanus bengalensis* (the bengal monitor) the auditory receptor is a papilla a little more than 2 mm long (Miller, personal communication) with the constriction one-third of the length from the ventral (apical) end. A thick tectorial membrane is present throughout the papilla.

In a previous report, it was demonstrated (Manley, 1976) that the activity patterns of units in the cochlear nuclei of this species provide a basis for division of the units into two functional groups. The numerically larger group of cells has low frequency responses and smooth frequency-intensity tuning curves. The smaller group has higher frequency responses and broader, somewhat more complex tuning curves. The present report deals with the response properties of fibers in the distal and proximal portions of the auditory nerve and with the mapping of the origin of these responses onto the papilla itself.

Methods

Varanus bengalensis were obtained directly from Thailand and weighed 46 to 740 g when used in experiments. Seven animals were used to record from the proximal auditory nerve. They were anaesthetized with ethyl carbamate (urethane, 0.22 g/kg, i.p.) and held dorsal side down. After surgical exposure of the roof of the mouth and the middle ear cavity and ensuring free ventilation of the trachea, the skin and bone overlying the ventral surface of the medulla on one side was removed without using power drilling equipment. Using tiny balls of tissue paper, the brain was gently moved medially by a small amount to expose the eigth nerve. Great care was taken not to stretch the nerve. Experiments were conducted with the animal in an anechoic and sound-attenuating chamber. A DC heating pad maintained a rectal temperature of 32°C. Sound stimuli were delivered in an open acoustic system, pure tones and pink noise from a speaker 1 m from the ipsilateral tympanum and clicks (140 μs monophasic pulses to the amplifier, optimized as to acoustic waveform) by a small speaker 30 cm from the ear.

Using 2/s 50 ms bursts of pink noise as search stimuli, 3 M KCl-filled glass micropipettes were advanced slowly through the nerve by means of a remote hydraulic microdrive. Once stimulus-locked responses were detected, the single units were classified according to their responses to tone bursts (2/s, 50 ms, 5 ms rise-fall times) of different frequencies and intensities. A 4-channel tape recorder was used to preserve spontaneous activity and tone and click responses for subsequent computer analysis. Tone intensities were calibrated using a Brüel and Kjaer 0.5 in condenser microphone placed adjacent to the ipsilateral tympanum. A recording wave analyzer (Bandwidth 10 Hz) was used to plot a sound intensity profile for all frequencies used.

Standard neurophysiological criteria were used for delineating fiber activity. Encounters with cells in the anterior and dorsal portions of the nerve were interpreted as originating from part

of the vestibular ganglion. These cells gave very large spikes, had very regular discharge patterns, never responded to even very intense sounds and showed pronounced injury discharge. Good data for the present purposes were obtained from 52 units in the proximal portion of the nerve.

In four additional animals a modified ventral approach was used to expose the basilar papilla. In these animals, bone and membrane overlying the ventro-medial surface of the *recessus scalae tympani* were removed to provide direct visualization of the papilla. Using this approach, almost all of the smaller ventral (apical) portion of the papilla and more than half of the dorsal (basal) portion of the papilla was visible and accessible for recording without further surgery.

Following exposure of the papilla, glass electrodes were used to record activity from primary dendrites coursing down the neural limbus towards the cochlear ganglion. Previous histological study had shown that although dorsal papilla fibers form a thick band running from the papilla more or less at right angles, ventral papilla fibers form thinner bands running obliquely dorsalwards from the papilla. In order to obtain recordings of activity originating in hair cell-neural junctions near the electrode (and hence to map more accurately), an attempt was made to place the electrode tip within 50 μm of the visible edge of the basilar membrane. In two cases, in order to reduce fluid pulsations and hence movement of the whole papilla, animals were paralyzed with 'alloferin' (4 mg/kg, i.p.) and artificially respirated by a one-way flow of humidified air which left the lungs through small lateral punctures. In other respects the recording and calibrating systems were as in the proximal nerve recordings as described above. Data are reported here from a further 50 units in the dorsal and ventral neural limbus.

Results

a) Spontaneous Activity

The spontaneous activity was computed for 22 units in the auditory nerve. The rates of firing in the absence of controlled stimuli varied from 0.65 to 52.1 spikes/s. Time interval histograms of firing interval distributions generally had exponential decay patterns (Fig. 1).

b) Frequency Response Characteristics

Frequency-intensity threshold tuning curves from both recording sites conform to the pattern previously observed in the cells of the cochlear nuclei. That is, there was a low frequency group with characteristic frequencies (CF's) from 250 Hz to 1.1 kHz and having sensitive, smooth V-shaped tuning curves and another group with CF's above 1.3 kHz, less sensitive and with more complex tuning curves (Fig. 2). As in the cochlear nucleus, the high CF group made up about 20% of the nerve units. The Q_{10dB} quotients of sharpness of tuning of the low CF group ranged from 0.9 to 6.4 and of the high CF group from 0.9 to 4.0 (Fig. 3). The lowest threshold in the nerve was a CF 700 Hz unit at 19 dB (re. 20 $\mu N/m^2$). In the cochlear nucleus, the lowest threshold was an 800 Hz unit at 8 dB.

The distribution of Q_{10dB} values is very similar in both the cochlear nuclei and the nerve (Fig. 3). An apparent tendency for lower Q values to be seen in the higher CF units in the nerve is probably due partly to surgical damage incurred during exposure of the papilla, as the ventral region is less protected. There is a general trend for higher Q's to be found in the higher CF units of the low frequency group.

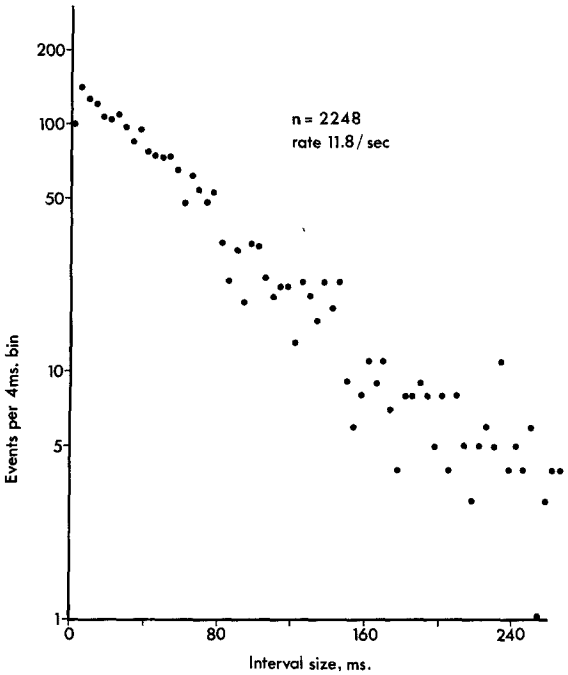

Fig. 1. Typical time interval histogram of primary fiber spontaneous activity, showing the exponential decay pattern. The number of intervals and the spontaneous rate are indicated

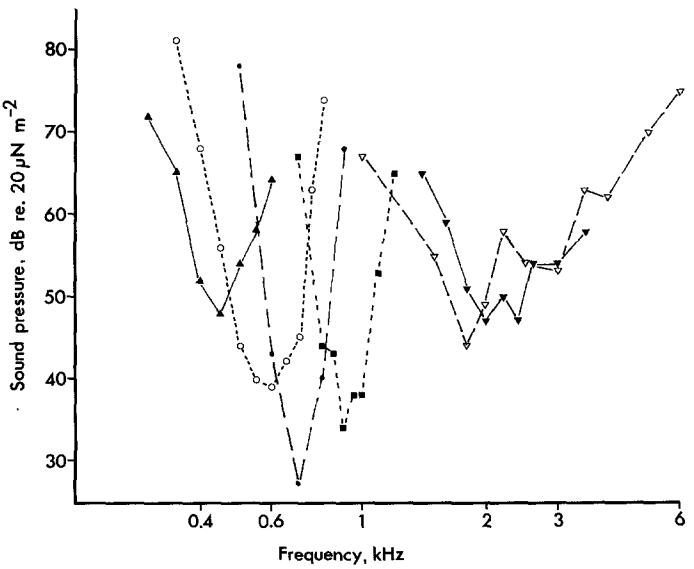

Fig. 2. Sample tuning curves from primary auditory neurons in *Varanus bengalensis*. Four units in the low frequency group and two units in the high frequency group are shown, not all from the same animal

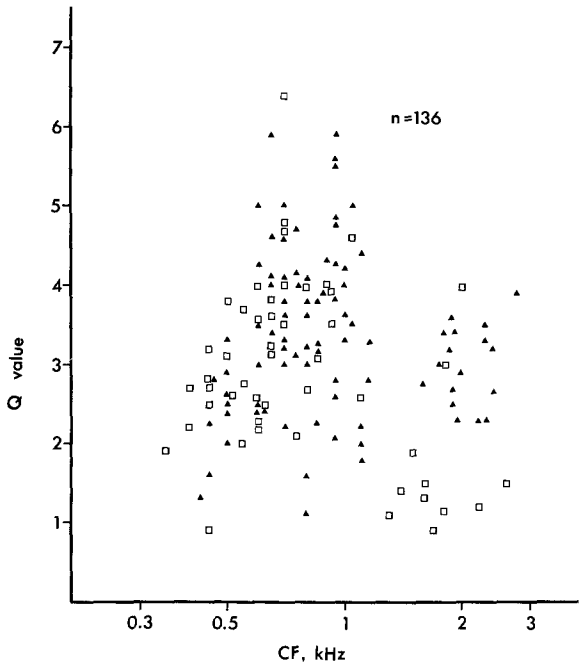

Fig. 3. Q_{10dB} values (CF divided by 10 dB bandwidth) *versus* CF for auditory nerve (open squares) and cochlear nucleus (filled triangles). The low Q values for primary fibers in the high frequency group is probably at least partly due to some damage incurred during exposure of the papilla

As previously noted for units in the cochlear nucleus (Manley, 1976), tuning curves from the nerve of *Varanus* have no particular asymmetry. Plotting the slopes of the tuning curves as measured from 3 to 23 dB above threshold against unit CF indicates slope trends (Fig. 4). These parallel the Q data. Plotted against one another, the slope on the high frequency side of the tuning curve and the slope on the low frequency side produce a scatter of points around a 1:1 ratio line (Fig. 5). The mean slopes in both cases are between 70 and 80 dB/octave.

c) Peripheral Origin of Fibers of Different CF's

The papilla recording experiments directly confirmed that the high CF responses originate from hair cells of the ventral papilla, but with no obvious internal arrangements of the frequency responses along the papilla. Hair cells of the dorsal papilla give rise to lower CF responses with a systematic tonotopic arrangement. The lowest CF's originate in the ventral end of the dorsal papilla and CF's rise as the recording location is moved dorsally (Fig. 6). Unfortunately, the most dorsal 40% of the dorsal papilla is inaccessible by this approach, but presumable gives rise to the higher CF's of the lower frequency group (i.e., CF's from 900 Hz to 1.1 kHz). Some evidence for this was obtained in

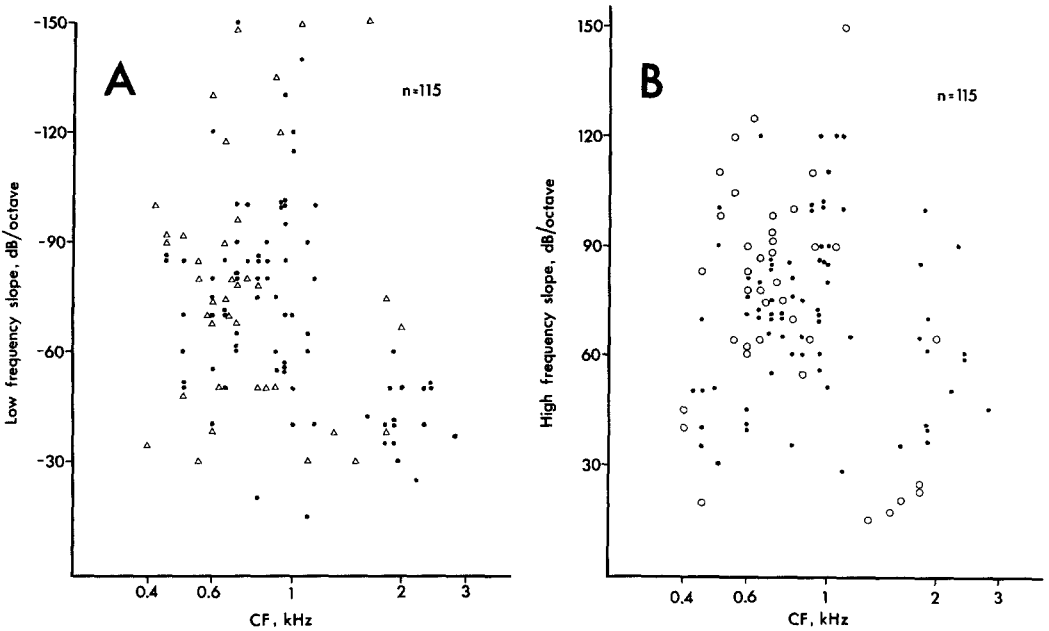

Fig. 4A and B. Slopes of the low frequency side (**A**) and high frequency side (**B**) of the tuning curves in dB/octave calculated from 3 to 23 dB above threshold, as a function of unit CF. In both **A** and **B**, the open symbols refer to data from auditory nerve units, dots refer to cochlear nucleus units

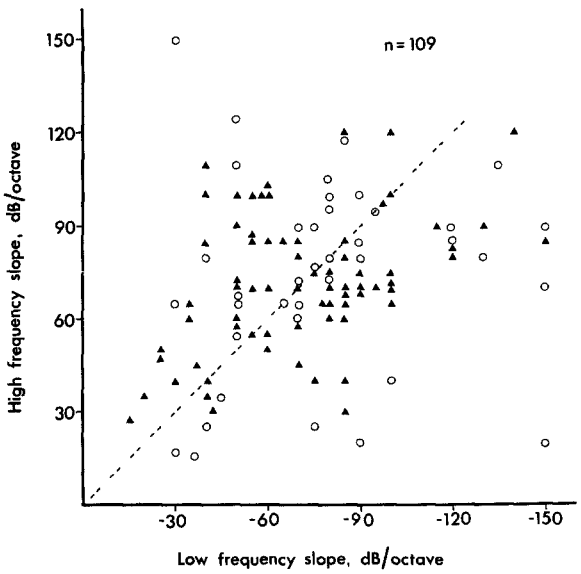

Fig. 5. High and low frequency slopes of tuning curves plotted against each other. The dashed line indicates a ratio of 1:1, or a symmetrical tuning curve. Open symbols refer to cochlear nerve data, closed triangles to cochlear nucleus units

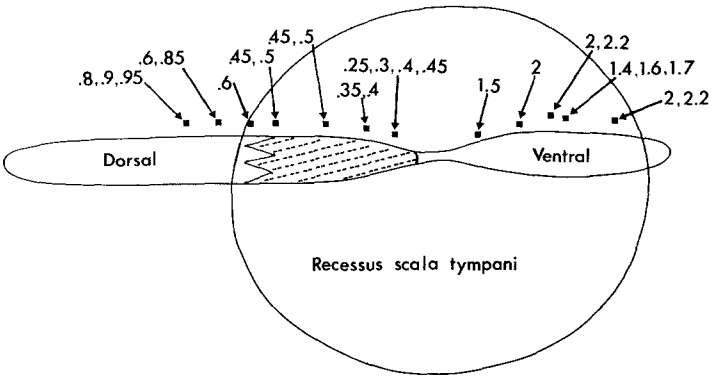

Fig. 6. Schematic drawing of the outline of the basilar membrane of *V. bengalensis,* showing the area visible through the round opening of the *recessus scalae tympani.* Dots on the neural limbus indicate ink spots deposited by the recording electrode. The adjacent numbers are the CF's, in kHz, of units recorded in each location. These data are a composite from four animals. A tilt of the electrode extended the recording beyond the area visible in the dorsal papilla – the two most dorsal ink spots are therefore guesses as to electrode location. Data in this report support the conclusion that the hatched area of the dorsal papilla contains unidirectional hair cells only

one experiment while recording from the cochlear nuclei. After destroying the ventral papilla and the ventral part of the dorsal papilla with a broken electrode, only CF's between 700 Hz and 1 kHz were obtained. The tonotopic arrangement described above is preserved in the nerve in a general way.

d) Discharge Patterns in Response to CF Tones

Pure tone latencies were shortest in the highest CF units and increased systematically to be longest in the lowest CF units, as in mammalian eigth nerve responses (Kiang et al., 1965). Latencies were 2 ms (high CF) to 5 ms (lowest CF) shorter than for units in the cochlear nuclei of corresponding CF. Threshold ranges in individual animals in the low frequency group were up to 32 dB, with a mean of 20 dB; for the high frequency group, up to 25 dB with a mean of 15 dB.

In many cases, peri-stimulus-time histograms (PSTH's) were computed for pure tone CF responses to 30 repeated tone bursts. All the PSTH's for units below CF 600 Hz had a 'filled' shape (Manley, 1976) not unlike that of primary fiber responses of mammals (Pfeiffer, 1966). Units with CF between 650 Hz and 900 Hz have either this filled type of PSTH or a 'peaky' variety (or intermediate) with a lower overall discharge rate and a conspicuous peak at the onset. Above 900 Hz, except for rare exceptions, all PSTH's are of this 'peaky' variety, as previously noted for the cochlear nucleus (Fig. 7). There is a concomitant tendency for the slope of the rate-intensity function to decrease with CF. Units in the high CF group have rate-intensity functions with a low slope and which reach maximum discharge at rates lower than those of the low frequency group.

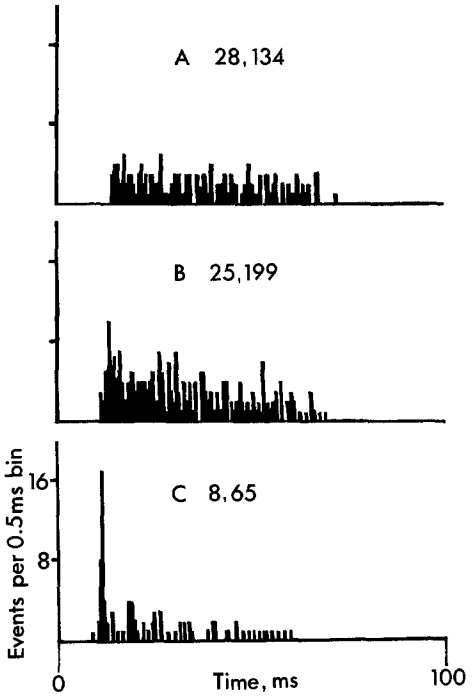

Fig. 7A–C. Sample PSTH's to illustrate the three basic patterns of pure-tone CF responses. **A** 'Filled' type from low frequency CF units. Numbers indicate dB above threshold (28) and total number of spikes to 30 repeated tone bursts. **B** Intermediate type. **C** 'Peaky' type. These patterns do not fundamentally change with intensity

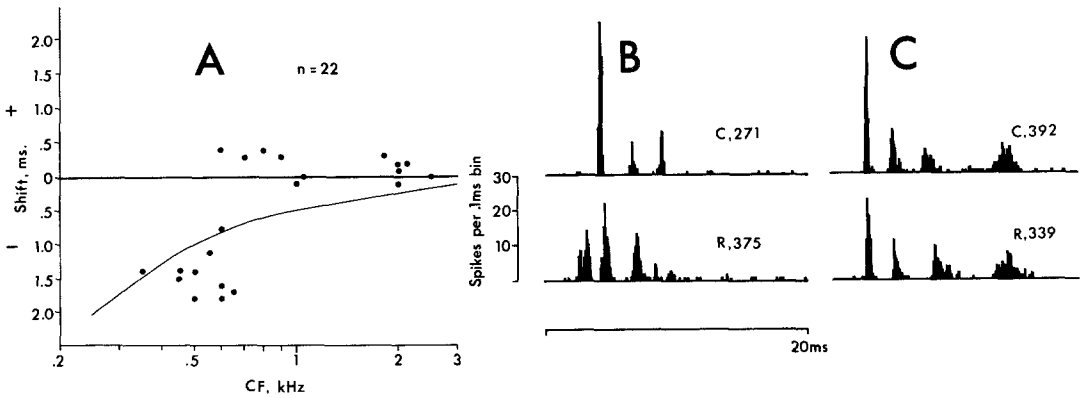

Fig. 8. **A** Graph of latency shifts for click polarity reversal for auditory-nerve units of different CF. Positive shifts indicate that the first condensation peak has a shorter latency than the first rarefaction peak, negative shifts *vice versa*. The thin curved line indicates the half-period shift 'expected' from known mammalian data. **B** PSTH's of click responses, top, condensation (*C*), with number of spikes to 100 repeated clicks (= 271), bottom, same to rarefaction (*R*) clicks, of a low CF unit showing a latency shift. **C** PSTH's of click responses of a high CF unit to (top) condensation (*C*) clicks and (bottom) rarefaction (*R*) clicks, showing no significant latency shift

e) Discharge Patterns in Response to Clicks

High CF units responded easily to a 110 dB SPL click (peak to peak), but low CF units often required up to 125 dB clicks to elicit a good response. This was presumably related to the energy spectrum of the click, which was not measured. For low CF units, the period separating the first and second response spikes was closely related to the inverse of the CF frequency. For higher CF units, however, no clear relationship was found. On reversing click polarity, very few units showed the expected latency shift of one half-cycle of the CF frequency (Fig. 8A). Units with CF 550 Hz or below had a shift generally somewhat larger than expected, but less than one full cycle (Fig. 8B). Those of CF 600 and 650 Hz had either no shift or a shift larger than expected, while units of CF over 700 Hz showed no significant shift (Fig. 8C).

Discussion

The simple visualization and mapping technique employed here resembles that used previously in the basal turn of the guinea pig cochlea (Robertson and Manley, 1974) but no bone or cartilage covers the papilla fibers in *Varanus*. A basically similar approach has very recently been described by Weiss et al. (1976) for the alligator lizard papilla.

If it is assumed that afferent fibers in this species innervate more than one hair cell, a shift in the click response latency following click polarity reversal would only be expected for fibers innervating a region of the papilla which has unidirectionally oriented hair cells. All lizard papillae have both unidirectional and bidirectional hair cell orientation patterns, the actual distribution varying between and within families (Miller, 1974). Miller (1974) showed that specimens of *Varanus* sp. have an area of unidirectional orientation in and near the constriction between dorsal and ventral papillae. The click reversal data presented here are most easily explained by assuming that the area of the papilla devoted to CF's below 550 Hz is unidirectionally oriented. The papilla localization data also presented here (Fig. 5) shows that these CF's are found in the most ventral third of the dorsal papilla (hatched in Fig. 5). The prediction that this area has unidirectionally oriented hair cells has very recently been confirmed by M. Miller (personal communication) using the scanning electron microscope.

Further possible correlates of this kind of distribution of hair cell orientation patterns are, firstly, that the PSTH shape shifts from the 'filled' pattern for CF's below 600 Hz through transition CF's to being almost exclusively 'peaky' for CF's above 900 Hz. Secondly, the rate-intensity function also shows a parallel fall in slope as the CF of the unit rises. No special correlation was found between the tuning curve shape and the hair cell orientation patterns, neither is there any obvious tendency for CF to correlate with mean rates of spontaneous discharge. Spontaneous rates in the nerve have the same range as those previously reported for the cochlear nuclei.

There are no obvious anatomical differences which can easily account for

the variation in mean Q_{10dB} values between the ventral and dorsal papillae responses. Both have areas of bidirectional hair cell orientation and a continuous thick tectorial membrane covering all cells (Miller, 1974). The main factor may be the instrinsic frequency selectivity of the papilla in each location. As to the question of the functional significance of kinocilial or hair cell bidirectionality, which is not a primitive feature of reptilian auditory receptors, these data from *Varanus* perhaps provide a partial answer. The only prominent feature of the response patterns which can be attributed to input to the nerve fiber from oppositely oriented hair cells is that the response PSTH is 'peaky'. That is, the discharge pattern tends to emphasize the onset of the sound. Such 'peaky' PSTH's are never seen in *Caiman* (Manley, 1974), which has only unidirectionally oriented hair cells (von Düring et al., 1974). On the other hand such PSTH's reach a very high state of development in the nerve of *Gekko* (Manley, 1974, and unpublished data) where most of the papilla is bidirectionally oriented in a very regular fashion (Miller, 1974). The tectorial membrane also differs in *Gekko*. In all cases where the onset of the sound is thus emphasized, the rate-intensity function has a low slope and can even be flat a few dB above threshold. The cells are thus relatively insensitive to the actual sound intensity.

Recently, Weiss et al. (1976) described tuning curves from primary afferents of the auditory papilla of the alligator lizard, *Gerrhonotus multicarinatus*. This species has a basilar papilla which is about 400 μm long and is anatomically separable into two regions, but with no constriction. The apical (ventral) portion has unidirectionally oriented hair cells having short cilia attached to a tectorial membrane. The basal (dorsal) region has bidirectionally oriented hair cells with long cilia and no tectorial membrane. The apical region gives rise to low frequency responses with asymmetrical tuning curves and the basal region to higher frequency responses with poorly tuned, symmetrical tuning curves. Because there are no obvious differences between the regions in terms of width of the basilar membrane, etc., Weiss et al. suggest that the micro-mechanics of hair cell stimulation (i.e., direct through a tectorial membrane or indirect through the fluids where there is no tectorial membrane) determine the frequency tuning pattern of the primary fibers.

Despite the anatomical differences, the above responses closely resemble in a number of ways those described for *Varanus* in this paper. Although the higher frequency responses in *Varanus* are derived from the opposite end of the papilla, they are less well tuned than the low frequency responses, as in *Gerrhonotus*. Unfortunately, because the slopes of the tuning curves in *Gerrhonotus* were not measured from a standard number of dB above threshold, they cannot easily be compared to the present data. It would appear, however, that the high frequency groups in the two species are similarly tuned. The fact that this tuning in *Varanus* is achieved with a tectorial membrane makes Weiss et al.'s suggestion for the origin of tuning differences less likely to be true. Curiously, while the low frequency groups in the two species resemble each other closely in their Q_{10dB} relationships, the *Gerrhonotus* tuning curves are highly asymmetrical while the *Varanus* curves have no particular asymmetry. This is true in *Varanus* in spite of an obvious tonotopic organization in the low frequency region of the papilla which suggests that travelling-wave analysis of frequency occurs.

There are no features of the tuning properties of the two regions of the papilla of *Varanus* which can be explained by gross anatomical differences except that the tonotopic organization on the dorsal papilla is correlated with the taper on the dorsal papilla. The transition from unidirectional to bidirectional hair cells orientation patterns in the dorsal papilla is not accompanied by any obvious changes in the tuning curves. The PSTH patterns change as described, but as Weiss et al. show no PSTH's, these changes cannot be compared to data from *Gerrhonotus*.

It is important to note that no differences of any kind (except latency) were easily demonstrated in the various response categories between primary units in the nerve and the secondary units of the cochlear nuclei already described (Manley, 1976). This contrasts strongly with the situation in mammals, where there are obvious groupings in the cochlear nuclei, both anatomically and physiologically. This suggests that the cochlear nuclei in lizards do little more than act as a relay station to higher brain centers. It has previously been noted that quite complex response patterns can be recorded from the lizard auditory nerve (Manley, 1974), suggesting that filtering and processing at the peripheral organ is very important in these forms. It is reasonable to suggest that the extraordinary diversity of anatomical variation in reptilian papillae serves to maximize peripheral filtering. Different species, genera and families might thus select important features of the acoustical environment even at this very peripheral level. The extensive development of bidirectional hair cell orientation patterns (Miller, 1974) which appears to emphasize sound onset is just one aspect of this interesting pattern.

Anatomical and physiological evidence indicates that the ventral ends of lizard papillae are primitively sensitive to the low frequency end of the species' spectrum, and the dorsal end to high frequencies. This pattern is preserved in the dorsal portion of the papilla of *Varanus*. It appears, however, as if the constriction in Varanid papillae (and presumably also the complete separation seen in Lacertid papillae and in isolated forms such as the Iguanid *Basiliscus*) allowed the smaller ventral area to evolve independently, so that it now responds to the highest frequencies.

At present, available ethological data on *V. bengalensis* provide no specific clues as to the usefulness of this elaborately developed peripheral auditory apparatus to this species.

This work was supported by grants from the Canadian National Research Council, the Banting Medical Research Foundation and the Quebec Department of Education. I thank Michele Sammaritano-Klein, Lorraine Pawson and Ruth Anne Eatock for expert technical assistance. R. Capranica provided useful criticism of an earlier draft of this paper.

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