THE RELATION BETWEEN WHOLE-NERVE AND UNIT RESPONSES
OF THE AUDITORY NERVE (ALLIGATOR LIZARD)

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1975

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ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his supervisory committee chairman, Dr. Donald C. Teas, for his guidance, support, and patience. Thanks are due to the other members of the supervisory committee, Drs. Daniel E. Sellers, William A. Yost, G. Paul Moore and William E. Brownell. A special thanks is given to Dr. Donald W. Nielsen for his interest in this research.

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August, 1975

Chairman: Donald C. Teas
Major Department: Speech

The papilla of the alligator lizard contains two populations of hair cells: a ventral (apical) population with unidirectional orientation, associated with a tectorial membrane, and a dorsal (basal) population with bidirectional orientation and free standing cilia. Previous work demonstrated that the auditory nerve fibers could be divided into two populations, low frequency ($CF \leq 0.8$ kHz) and high frequency ($CF \geq 0.9$ kHz), on the basis of tuning curve shape. The low frequency fibers were associated with the ventral papilla and the high frequency fibers with the dorsal papilla.

In the alligator lizard, the whole-nerve action potential (AP) response to clicks is characterized by a complex change in the waveform which occurs when click polarity is reversed. Post-stimulus-time (PST) histograms of the response of single high

frequency fibers to click stimuli have a single peak of approximately the same shape and latency for both click polarities. Click histograms for low frequency fibers show multiple peaks which shift in time when click polarity is reversed. High pass (2.0 kHz) clicks significantly reduce the activity of low frequency fibers resulting in an AP whose waveshape is independent of stimulus polarity.

On the basis of the response to low-pass (0.6 kHz) clicks, individual high frequency fibers were classified as Type 1 or Type 2. The response of both types of fibers depended on stimulus polarity. The response pattern of a Type 1 fiber to a stimulus of one polarity is the same as the response pattern of a Type 2 fiber to that stimulus with opposite polarity. Using similar criteria, the responses from low frequency fibers could not be classified into two or more types.

The AP response to tones and tone bursts shows phase-locked activity at frequencies below 1.0 kHz. This wave activity is the same frequency as the sinusoidal stimulus. PST histograms for low and high frequency fibers have peaks which occur once for every period of the sinusoidal stimulus and which shift in time as stimulus polarity is reversed. For frequencies above 1.0 kHz, the low frequency fibers have little response; whereas the high frequency fibers respond significantly, but with little phase-locked response activity.

Amplitude-latency plots as a function of filter frequency were derived from the AP responses to low-pass and narrow-band

clicks. These data show a transition from a predominately polarity-independent response to a polarity-dependent response as filter frequency is decreased from 2.0 kHz to 1.0 kHz.

It is concluded that low frequency fibers innervate hair cells in the ventral papilla, and that because of the unidirectional orientation of these hair cells, the low frequency fiber population contributes a polarity-dependent component to the AP. A high frequency fiber innervates hair cells of only one orientation in the hair cell population of the dorsal papilla. Because of the bidirectional orientation of these hair cells, the high frequency fiber population contributes a polarity-independent component to the AP. The analysis of the AP response to high-pass, low-pass, and band-pass clicks reflects the tonotopic organization of the alligator lizard's papilla.

INTRODUCTION

General Considerations

Hearing loss affects more Americans than any other chronic health problem. Over eight and one-half million Americans suffer from some degree of loss (Rees, 1973). A hearing loss (conductive loss) which results from an abnormal condition of the external or middle ear is usually treatable with medication or surgery. Unfortunately, very few techniques are available for correcting or compensating for a hearing loss (sensorineural loss) which is due to pathologies of the inner ear or auditory pathways. It is estimated that about 120,000 Americans have sensorineural hearing impairments of the severity classifiable as total handicaps (Carhart, 1974). A much larger number suffer from less severe hearing losses.

The majority of sensorineural losses result from abnormalities of the inner ear and/or primary auditory nerve fibers, resulting in the improper coding of acoustic information by the cochlea, or the inability of the nerve to correctly transmit the information. The major help available to individuals with sensorineural hearing losses has been the hearing aid, which often is of limited value. Recently, a new technique has been developed, the cochlear implant, but its success has been minimal (Merzenich et al., 1974). The failure of the hearing

aid and the cochlear implant to adequately compensate for the sensorineural loss results, in part, from an insufficient knowledge of cochlear processes, in particular, the transduction of the motion of the basilar membrane into the activity of primary auditory nerve fibers.

Certainly, much is known about the auditory system as the result of anatomical and physiological research on animals and humans. Primarily, mammals have been used for auditory research because of the similarities among mammals, including humans, in the anatomy of their auditory systems and thus, the applicability of animal research to human problems. Occasionally, a non-mammalian animal demonstrates an anatomy which is advantageous for the study of a particular question. A classic example is the recording of intracellular potentials from single hair cells in the lateral-line organ of amphibia and fish. Recently, researchers have found that the lizard, in particular, the alligator lizard, is a valuable preparation for the study of the peripheral auditory system because of the unique anatomical structure of its inner ear.

Lizard Anatomy

Peripheral Auditory System

The following anatomical description is applicable to many species of lizards: the major exception being the burrowing lizards which have no external ear openings in the skin. A short external auditory meatus terminates at the tympanic membrane (for some species, the tympanic membrane is at the level of the skin). The lizard's middle ear consists

of two bones, the stapes (columella) and the extrastapes (extracolumella), which join in a rod-like manner to provide a direct connection between the tympanic membrane and the inner ear (Fig. 1).

The oval window, containing the stapes footplate, opens into scala vestibuli. Scala vestibuli is connected to scala tympani by the helicotrema, and both scalae are part of the perilymphatic system. Unlike the mammalian cochlea, the inner ear of the lizard is not coiled, nor are the scalae long narrow chambers similar to an "uncoiled" mammalian cochlea. They are, instead, chambers of irregular shape, separated by the cochlear duct (Fig. 2).

The space within the cochlear duct, scala media, is filled with endolymphatic fluid. The medial boundary is formed by supporting structures for two sensory organs: the lagena macula and the basilar papilla. The lateral boundary of the cochlear duct is the vestibular membrane. The function of the lagena is not clear; the lizard's basilar papilla is the analog of the mammalian organ of Corti. The papilla sits on the basilar membrane which separates scala media from scala tympani.

The papilla is a strip of neuroepithelium which consists of hair cells, supporting cells, and the unmyelinated portion of the basilar (auditory) branch of the VIIIth nerve fibers which pass between the supporting cells and terminate at the base of the hair cells.

Fine Structure of the Inner Ear

At a gross level, there is similarity in the anatomy of the peripheral auditory systems of many species of lizards: however, recent studies by Wever (1965, 1967b, 1967c, 1968, 1970a, 1970b, 1971a, 1971d,

Fig. 1. Diagram of a section of the head of a "typical" lizard. This figure is representative for many species of lizards, including the alligator lizard. (Adapted from Weiss et al., 1974a)

INNER EAR OF ALLIGATOR LIZARD

Fig. 2. The inner ear of the alligator lizard. The basic structure of the alligator lizard's inner ear is similar to that of other species. The dotted line indicates the surgical removal of the round window to expose the papilla and nerve. A microelectrode is shown recording from the auditory nerve. (Adapted from Weiss *et al.*, 1974a)

1974), Miller (1966, 1973a, 1973b, 1974), Mulroy (1968), Baird (1967, 1969) and Bagger-Sjoberg and Wersall, (1973), reveal the significant differences in the fine structure of the inner ears of different lizard species. There is a remarkable variation in papilla size, number of hair cells, hair cell orientation, and hair cell associated systems. Papilla size can vary from less than 200 μm in species of Chamaeleonid to more than 2 mm in Varanid. Less than one hundred hair cells are present in some species of Iguanid, while many Gekkonid have over two thousand. A population of hair cells may be orientated in the same direction (unidirectional), adjacent regions of hair cells may be orientated in opposite directions (bidirectional), or hair cells of both orientations may be intermixed in the same region (multidirectional) (Fig. 3).

There are four basic hair cell systems found in the inner ears of lizards. Wever (1971b, 1971c) has proposed that these structures are involved in the mechanics of hair cell stimulation. The first, a tectorial membrane system is most similar to the mammalian structure and is characterized by a thin membrane attached to the neural limbus and connected at the other end to the cilia of the hair cells. The second variety, the sallet system, is similar to otolith systems, consisting of a large mass lying free in the cochlear fluid except for contact with the cilia of one or more hair cells. A variation of the sallet system is the culmen system, a large mass which contacts the cilia of all the hair cells of a population. The culmen system is usually found in association with the sallet system. The free-standing cilia system, the fourth variation, is actually the lack of any addi-

Fig. 3. The anatomy of the papilla in seven families of lizards. Hair cell orientation (indicated by direction of the arrows) is shown for seven families; hair cell associated structures are shown for four families. The alligator lizard belongs to the Anguid family. (Adapted from Miller, 1974)

tional structure associated with the hair cell. The cilia of the hair cell stand free in the cochlear fluid except for, perhaps, a small amount of tectorial like substance found between the cilia.

The three patterns of hair cell orientation could be combined with the four types of hair cell systems to produce many possible patterns of inner ear anatomy. Available data indicate that the inner ear anatomy of species within the same family follows the same basic pattern of hair cell orientation and hair cell systems; however, there is great diversity among different families. The basic pattern of inner ear structure is shown for seven families in Fig. 3.

Alligator Lizard

In the alligator lizard (*Gerrhonotus multicarinatus*), the papilla is slightly larger at both ends than in the middle, varying in width from 50 μm to 75 μm . The basilar membrane is larger than the papilla and is wider in the middle than at the ends, with a width of 60 μm to 100 μm .

Each hair cell in the papilla of the alligator lizard has a single kinocilium and from fifty-five to seventy stereocilia arranged in a random pattern. The length of the stereocilia of a single cell decreases in a direction away from the kinocilium. The morphological polarity (orientation) of a hair cell is defined by the eccentric position of its kinocilium. The alligator lizard belongs to the family Anguid; its pattern of hair cell orientation and hair cell systems is typical for its family (Fig. 3). In the ventral (apical) region of its papilla, all the hair cells (about 50) are morphologically polarized

in the same direction (unidirectional orientation). A tectorial membrane is present and the cilia have a length of 5 μm to 9 μm . In the dorsal (basal) region, the hair cells are divided into two groups of opposite polarity (bidirectional orientation), with about fifty cells in each group. The hair cell group closest to the nerve (neural) is orientated away from the nerve, whereas, the other group (abneural) is orientated towards the nerve (Fig. 41). No tectorial membrane has been found in this region, but a small amount of tectorial substance is found between the cilia (free-standing cilia system). In this region the cilia are longer and vary from 12 μm to 31 μm (Mulroy, 1968; Weiss et al., 1974a). In summary, in the ventral region of the papilla, the alligator lizard has a unidirectional orientation/tectorial membrane system; in the dorsal region, a bidirectional orientation/free standing cilia system.

There are 600 to 1000 afferent auditory nerve fibers innervating the papilla of the alligator lizard. Little is known about the innervation pattern of the afferent fibers in the papilla. Unmyelinated afferent fibers pass through the habenula perforata and become myelinated. They form the basilar branch of the VIIIth nerve which passes anteriorly and medially to enter the brain stem. Efferent fibers have been observed forming synapses on the hair cells in the ventral region but not the dorsal region of the papilla (Weiss et al., 1975).

Physiology

Overview

The most extensive physiological data have been provided by

Wever (1965, 1967a, 1968, 1970a, 1970b, 1971a, 1971b, 1974) and his associates (Wever et al., 1964, 1965, 1973; Wever and Hepp-Reymond, 1967). They have studied many species of lizards using the standard technique of recording extracellular potentials using a metal electrode placed on the round window. Wever considers these potentials cochlear in origin, and has attempted to answer fundamental question, such as tonotopic organization of the papilla, using these data. Others (Campbell, 1969; Crowley, 1964; Hepp-Reymond and Palin, 1968; Johnstone and Johnstone, 1969b) have used similar techniques in different species of lizards.

Additional physiological data include the measurement of middle ear frequency response in two species (Saunders and Johnstone, 1972), and single unit recordings from the auditory nerve in one species (Johnstone and Johnstone, 1969a) and from higher order auditory fibers in three species (Manley, 1972, 1975; Suga and Campbell, 1967).

Alligator Lizard

Of all the species of lizards, the most extensive physiological data are available for the alligator lizard. Wever (1971a), Crowley (1964), and Campbell (1969) recorded electrical responses to sound with a round window electrode. Intracellular potentials were recorded from individual hair cells in the papilla (Weiss et al., 1974a; Mulroy et al., 1974). The waveshapes of the intracellular responses suggested tonotopic organization of the papilla and appeared related to the orientations of the hair cells in the two regions of the papilla.

Weiss, Mulroy, Turner and Pike (1974b, 1975) recorded single unit activity from primary fibers in the auditory nerve. On the basis of the

tuning curves obtained from individual fibers, they divided the fibers into two populations: a low frequency population ($0.2 \text{ kHz} \leq \text{CF} \leq 0.8 \text{ kHz}$) and a high frequency population ($0.9 \text{ kHz} \leq \text{CF} \leq 4.0 \text{ kHz}$) (Fig. 4). This division was evident on the basis of both tuning curve shape and the distribution of the characteristic frequencies (CF) of the fibers. As indicated by dye-marking experiments, the fibers of the low frequency population enter the ventral end of the papilla, while the fibers of the high frequency population enter the dorsal end of the papilla. There appears to be a tonotopic organization of the papilla from the ventral (low frequency) to the dorsal (high frequency) end of the papilla. Although the exact innervation pattern is unknown, it is reasonable to conclude that the low frequency fibers innervate the hair cells in the ventral papilla while the high frequency fibers innervate the hair cells of the dorsal papilla. Electrical responses recorded from individual hair cells in the papilla support this assumption (Weiss *et al.*, 1974a). They also found that the rate of response of a low frequency fiber to a tone at CF could be suppressed by the simultaneous presentation of a second tone whose frequency is above the CF of the fiber (Weiss *et al.*, 1975). This phenomenon, called two-tone rate suppression (TTRS), was not found for high frequency fibers.

Research Implications

In the mammalian cochlea, there are two populations of hair cells, inner and outer hair cells, which differ in anatomical structure and which are innervated by different afferent auditory nerve fibers

Fig. 4. Typical tuning curves for primary auditory nerve fibers in the alligator lizard. Note that the two tuning curves with CF greater than 1.0 kHz have a broader shape than the three tuning curves with CF less than 1.0 kHz. (From Weiss *et al.*, 1975)

(Spoendlin, 1973). The relationship of these significant anatomical differences to the discharge patterns of individual nerve fibers is not clear. There is significant research interest in this question and the closely related question concerning the relationship between the motion (displacement and velocity) of the basilar membrane and the initiation of auditory nerve activity (Dallos et al., 1972; Dallos and Wang, 1974; Sokolich and Zwislocki, 1974; Zwislocki and Sokolich, 1973; Konishi and Nielsen, 1972, 1973).

The great diversity in the anatomy of the inner ears of lizards offers exciting possibilities for the study of cochlear transduction processes, including questions similar to those discussed above. A comparative study, involving species from many families, could result in an understanding of the relationship between particular anatomical structures in the inner ear and the discharge patterns of individual auditory nerve fibers. Such a study would require extensive time and effort and would rely primarily on single fiber recordings. Other techniques are needed which would permit the quick survey of many species for the answers to a few fundamental questions. One possibility is the recording and analysis of the whole-nerve action potential (AP). There has been only limited interest in recording the AP in the lizard (Weiss et al., 1974a; Campbell, 1969; Hepp-Reymond and Palin, 1968). A wire electrode on or near the round window was used to record the AP response in several species of lizards. For two species, the alligator lizard and the tokay gecko, the AP response to condensation and rarefaction clicks is shown. Unlike the mammal where the waveshape of the

AP is independent of click polarity, there is a complex change in the waveshape of the response in these two species of lizards when click polarity is changed. It has been proposed that this complex change in response results from the complex structure of the papilla (Hepp-Reymond and Palin, 1968). These data strongly imply the potential value of recording the whole-nerve action potential in the lizard.

Whole-Nerve Action Potential

The whole-nerve action potential (AP), recorded near the inner ear or auditory nerve, is an electrical signal which reflects the summed activity of individual auditory nerve fibers. In the mammal, the most popular recording technique has been the differential electrode pair. Other techniques include recording from the round window or the auditory nerve with a wire electrode.

The AP is largest when many fibers fire in a short period of time. Transient stimuli, such as clicks or tone bursts with fast rise times, are best for producing the synchronous firing of the individual nerve fibers needed to produce the AP. Teas, Eldredge and Davis (1962) proposed that the waveshape of the AP is the convolution of the basic response unit of an individual fiber with the temporal firing patterns of the fibers. The shape of the basic response unit is affected by the recording electrode configuration. For an electrode pair in the cochlea of a mammal, the basic response unit is diphasic, first negative, then positive.

In the mammal, the AP response to a click primarily reflects the

activity of the nerve fibers in the basal end of the cochlea (Kiang et al., 1965). Fibers in the basal turn have similar latencies and fire synchronously to a transient stimulus. Differences in latency among fibers become greater closer to the apex causing the activity of these fibers to cancel. It cannot be concluded that the activity of the more apical fibers does not affect the waveshape of the AP. Teas, Eldredge and Davis (1962) used bands of noise to reduce the synchronous firing of a small group of the more apical fibers and found that this altered the AP. Legoux and Pierson (1974) also presented evidence that there was some contribution to the AP from basal and apical fibers.

Tasaki (1954) proposed that the source of the AP is the nerve trunk in the basal turn of the cochlea. Teas, Eldredge and Davis (1962) concluded that the internal auditory meatus forms an insulating tube and that the origin of the AP is the nerve as it emerges from the meatus. Dallos (1973) agreed with Teas et al., (1962) and developed a model to explain the initial negative-positive complex. The complex results because the nerve is first contained within an insulator and then emerges into a conducting fluid which is in contact with the recording electrode.

With the development of better techniques for the recording from individual nerve fibers, the research interest in the AP decreased. Recently, there has been renewed interest in the AP because of electrocochleography (ECoG). ECoG is the clinical recording of the AP in man to determine the condition of the peripheral auditory

system. Much of the work in humans has concentrated on determining an audiogram using the AP. The AP response to a click reflects, primarily, the condition of the basal turn fibers; other stimuli, such as filtered clicks and tone bursts, have been used with some success to measure the condition of fibers from all turns of the cochlea.

Experiments

The potential value of the AP in ECoG and to study the peripheral auditory system of the lizard has increased the need to better understand and interpret the AP. The objectives of this research are to analyze the alligator lizard's AP response to clicks and to investigate the use of the AP as a research tool.

The click AP in the alligator lizard demonstrates a complex change when click polarity is reversed. It has been suggested that this phenomenon is related to the complex anatomy of the papilla. The analysis of the click AP will be in terms of the contributions of the low and high frequency fiber populations and the relationship of these contributions to the anatomy of the inner ear. The investigation of the AP as a research tool will consist of the analysis of the AP response to carefully selected stimuli, to determine which stimuli provide the best information concerning the activity of certain groups of fibers and the physiological significance of inner ear anatomical structures.

The parameters of the AP response of interest will be waveshape, amplitude and latency. Click, filtered click, tone and tone burst stimuli will be manipulated in terms of polarity, intensity and spectral

composition. Other attempts to analyze the AP have relied primarily on stimulus manipulation (Teas et al., 1962; Eggermont and Odenthal, 1974). Since the AP reflects the activity of individual nerve fibers, it seems advantageous to record single unit activity simultaneously with the recording of the AP. A knowledge of single nerve fiber activity should greatly facilitate the interpretation of the AP.

The basic strategy of the experiments is as follows:

1. Stimulus manipulation (polarity, intensity, spectrum).
2. Record single unit activity.
3. Record the AP.
4. Analyze the AP in terms of waveshape, amplitude and latency.

METHODS

Animal Selection

The alligator lizards were obtained from Hermosa Reptile, Inc., Hermosa Beach, California. There are approximately ten species of alligator lizards (Genus *Gerrhonotus*) found in the United States (Smith, 1946). Considering the geographical location in which the lizards were captured, it can be concluded that the majority of lizards used in the research were *Gerrhonotus multicarinatus webbia* (San Diego alligator lizard) with the possibility of a few *Gerrhonotus multicarinatus multicarinatus* (red-backed alligator lizard). There is no information available concerning anatomical variations among subspecies of *Gerrhonotus multicarinatus*.

Surgical Procedures

The experiments were performed on twelve alligator lizards which weighed from 11 to 31 grams. The animals appeared in good health; the external auditory meatus and the tympanic membrane were examined under a Zeiss operating microscope and the ears were cleared of any parasites.

The animals were anesthetized for surgical preparation with sodium pentobarbital (Nembutal) administered intraperitoneally in doses of 25 mg/Kg of body weight. Additional injections of one-half the initial

dose were administered as needed to maintain the proper anesthetized state.

The middle ear of the anesthetized lizard was exposed by removing the skin and muscle of the ventral wall of the pharynx. A cannula was inserted into the trachea, and the posterior end of the retroarticular process of the lower jaw, together with the associated muscles, was removed. Scala tympani was opened by removing the ventral bony edge of the round window and the round window membrane (Fig. 2). The basilar papilla and the basilar (auditory) branch of the VIIIth nerve could be seen with an operating microscope (Weiss et al., 1974a).

Acoustic Stimulation

The stimuli were normally clicks, filtered clicks, tones and tone bursts. The standard click was a 100 μ s pulse, which was filtered (Krohn-Hite 3550) to produce a low-pass, high-pass, or narrow-band click (24 dB per octave slope (s)). The standard tone burst was 50 ms in duration with no shaping (fast rise time). The tone burst was triggered on the positive-going zero-crossing of the sine wave. Stimulus presentation rate was 10/sec. The amplitude and polarity of the stimulus could be controlled.

The acoustic stimulus was generated by a 1.0-inch condenser microphone (Bruel and Kjaer 4132) fitted into a speculum which was sealed to the entrance of the external auditory meatus. Within each speculum there was a small diameter probe tube that permitted sound pressure measurements close to the tympanic membrane. Acoustic calibrations were obtained

with the speculum sealed to the external meatus and the probe tube connected to a calibrated 0.5-inch condenser microphone (Bruel and Kjaer 4134) (Fig. 5).

Click and filtered click intensities are in decibels (dB) relative to 45 volts-peak into the earphone (1.0-inch condenser microphone), and tone and tone burst intensities are in dB relative to 27 volts rms into the earphone.

Electrical Recording

The experiments were conducted in a sound-proofed, electrically-shielded chamber. The whole-nerve action potential (AP) was recorded by a nichrome wire electrode placed on the bone near the round window membrane. This electrode also recorded the cochlear microphonic (CM), the activity of higher auditory elements and electrical and physiological artifact. The electrode was connected to the input of a high gain (20,000 or 50,000), AC-coupled amplifier (Grass P511). The output of the amplifier was displayed on an oscilloscope, recorded on tape and led to a LAB-8e computer for on-line averaging.

The spike activity of an individual nerve fiber was recorded using a glass microelectrode. The reference electrode for the microelectrode and the wire electrode was a steel wire inserted into a muscle in the head. The electrodes were filled, using the fiber-fill technique (Tasaki, 1968), with 3 M KCl. Tip diameter was less than 1 μm , and resistance at 25 Hz was 20 to 100 M Ω . Once the inner ear was opened, visual observation was used to insert the electrode, which was connected

ACOUSTIC CALIBRATION

Fig. 5. Typical acoustic calibration curve for the sound pressure near the tympanic membrane. The curve was generated by a 9 volt rms tone into the one-inch condenser microphone earphone.

to a hydraulic microdrive (Kopf 1207B), into the fluid above the nerve. The electrode was lowered into the nerve using the microdrive at a location peripheral to the internal auditory meatus and most of the cell bodies of the ganglion. This procedure insured recording from primary auditory nerve fibers.

The microelectrode was connected to the input of a high impedance, low gain amplifier with capacitance neutralization (Keithley 605). The output of this amplifier was connected to an AC-coupled Grass amplifier with a gain of 500, 1000, or 2000. The output of the Grass was displayed on an oscilloscope, passed through a band-pass filter (Krohn-Hite 3100), fed to an audio monitor, recorded on tape, and led to a LAB-8e computer for on-line calculations of poststimulus time (PST) histograms.

Data Collection and Analysis

Most of the data analysis was performed off-line from tape recordings of the data. The FM channels of the tape recorder (Ampex FR-1300) have a band-width of DC to 1.6 kHz when recording at 3.75 in/sec. Comparisons of averages of the AP computed on-line and off-line from tape recordings revealed little distortion of the AP waveform due to the limited high frequency response of the tape recorder. This also had little effect on the processing of single unit data. The following signals were recorded on separate channels of the tape recorder:

1. Amplified output of the microelectrode
2. Amplified output of the wire electrode

3. Voice commentary
4. Master timing pulse
5. Electrical stimulus to earphone

A LAB-8e computer was used off-line to produce averages of the AP and the electrical stimulus and PST histograms of single unit activity.

Figures

Most of the data are presented in the figures as averages of the AP or PST histograms of single fiber activity. Except where noted, the rarefaction and the condensation stimulus conditions are shown together in the figures with rarefaction above condensation. The amplitude of the response is indicated for the rarefaction condition but also applies to the condensation condition.

When the AP average is shown by itself or with the stimulus waveform, the amplitude of the AP response is indicated by the calibration bar. When the AP is presented with a PST histogram, the amplitude of the AP is not indicated; however, the AP average shown was recorded simultaneously with the single unit activity represented by the histogram. An AP average always represents 100 averages; positive voltage up.

A PST histogram always represents 300 stimulus presentations. The number of bins of the histogram is always 200 except for the histograms with a 10 ms time base; here the number of bins is 100. The bin width is evident from the number of bins and the time base. The number of spikes contained within a bin is indicated by the scale shown with the

histogram; note, this number represents the total for 300 stimulus presentations.

When a stimulus contained very little energy above 1.0 kHz, an average of that stimulus is shown in the figures with the AP average and the PST histogram. Stimuli with significant energy above 1.0 kHz are not shown because their waveshape is distorted by the limited bandwidth of the FM tape recorder. The stimuli presented were always recorded simultaneously with the corresponding AP response or single unit activity.

Zero time in the averages and histograms and for the measurement of latencies corresponds to the master tuning pulse recorded on tape, not the arrival of the acoustic stimulus at the tympanic membrane. To facilitate comparison of the AP response and single unit activity to the stimulus waveform, the stimulus average in a figure is shown delayed 2.0 ms. The stimulus actually began at zero on the AP average or PST histogram time scale, even though, it appears to have begun at 2.0 ms.

Experimental Protocol

The following general procedure was used during the experiments:

1. Initial surgery. The first part of the surgery was completed so as to expose the middle ear.
2. Acoustic calibration.
3. Recording the AP. The wire electrode was placed on the bone near the round window membrane and the AP was recorded for the stimuli of interest.

4. Final surgery. The surgery was completed so as to expose the papilla and the auditory nerve.
5. Measurement of the visual detection level. The wire electrode was again placed on the bone in approximately the same location as before. A visual detection level (VDL), the intensity at which a response to clicks is just visible in the unaveraged AP, was determined. The VDL was monitored throughout the experiment and served as an estimate of the condition of the ear. Data were not used if the corresponding VDL was poorer than -40 dB.
6. Recording from single units. Using clicks as a search stimulus, the microelectrode was advanced into the nerve until either spike activity was recorded or the electrode passed completely through the nerve. Spikes recorded by the microelectrode were typically monophasic, positive in voltage, and had a width of about 1 ms. Single unit data were obtained from 160 units, with the units divided equally below low and high frequency fibers. Because of the thinness of the nerve near the papilla, less than four units were normally encountered during one pass through the nerve. It was difficult to hold a unit very long; the time varied from a few seconds to an hour, with the average about five minutes.
7. Determination of fiber population. Once a unit was encountered, a 2.0 kHz tone burst was used to assign the

fiber to either the low or high frequency fiber population. A high frequency fiber would show obvious response to the stimulus; a low frequency fiber would not.

8. Estimation of characteristic frequency. The frequency of the tone burst was quickly varied from 100 Hz to 4.0 kHz to provide an estimate of the characteristic frequency (CF) of the fiber. A more accurate measure of CF was not made because of the time required.
9. Data collection. Single unit activity and the AP were simultaneously recorded for the stimuli of interest.

RESULTS

Click Stimuli

AP Response

Typical whole-nerve action potential (AP) responses to clicks are shown in Fig. 6A. The most obvious feature is that the waveforms of the responses depend upon the polarity of the clicks. The response to both polarities demonstrates a large initial negative-positive diphasic complex (N_1-P_1), which is larger for condensation than rarefaction. The rarefaction P_1 peak is typically broadened, as indicated by the arrow in Fig. 6A. The initial N_1-P_1 is followed by two smaller diphasic complexes, N_2-P_2 and N_3-P_3 . The major activity in the AP response is over before 10 ms. Also note that there is little cochlear microphonic (CM) or electrical artifact evident in the average. The gross response recorded with the wire electrode consists almost entirely of neural activity.

The waveform of the AP response depends upon click intensity (Fig.7). At -80 dB, little synchronous activity is evident in the AP. At -70 dB, the response is not well defined. For -60 dB through -20 dB, the qualitative features described above are evident; however, at -10 dB, complex changes have occurred in the response. Some of the changes present at -10 dB are evident at -20 dB.

Fig. 6. AP response to clicks. A: Pictured are typical AP responses to rarefaction and condensation clicks showing the large $N_1 - P_1$ and smaller $N_2 - P_2$ and $N_3 - P_3$ complexes, and the broadening of the rarefaction P_1 peak (arrow). The two responses are shown to the same amplitude scale. B: The AP responses to rarefaction and condensation clicks are shown superimposed with the rarefaction $N_1 - P_1$ amplitude made equal to the condensation $N_1 - P_1$ amplitude by adjusting the scale factor of the AP averages. Even though the two responses appear very similar in Fig. 10, differences are evident in this figure. All AP responses represent 100 averages. The stimulus was a 100 μ s duration click presented 10/sec.

AP RESPONSE TO CLICKS

Intensity: -30 dB

Lizard 9

A

Lizard 14

B

Fig. 7. AP response as a function of click intensity. Note the change in response waveform as intensity is increased from -30 dB to -10 dB. Click intensity is in decibels relative to 27 volts peak into the earphone. The amplitude calibration bar shown for each intensity applies to both the rarefaction and condensation response.

AP RESPONSE TO CLICKS
Lizard 15

Another useful measure of the click AP is the amplitude of the N_1 - P_1 complex and the latency of the P_1 peak. Amplitude-latency plots as a function of click intensity are shown for four lizards in Fig. 8. The N_1 - P_1 amplitude increases with intensity up to about -30 dB; at higher intensities, the amplitude decreases. In the mid-intensity range, the N_1 - P_1 amplitude is larger for condensation than rarefaction; however, at the lower and higher ends of the intensity range, the amplitudes tend to be the same for both polarities. The latency of the N_1 peak decreases with increasing intensities, reaching a minimum of about 2.0 ms.

There is some variation in the click AP with time during the experiment (Fig. 9). The AP responses shown represent a time period greater than twelve hours. The first AP pair pictured was recorded before the final surgery, where the round window is removed to expose the nerve. The remaining four AP pairs were recorded simultaneously with single unit activity, after the completion of all the surgery. The click AP remains fairly stable with time, always demonstrating the qualitative features typical of its waveform.

There is some variation in the click AP recorded from different lizards (Fig. 10). The amplitude of the N_1 - P_1 complex varies, for constant click intensity of -30 dB, within a range of approximately 10 μ v to 40 μ v. Even with all the variation in waveform evident in this figure, the qualitative features described for the responses in Fig. 6A are present in these responses. The condensation N_1 - P_1 amplitude is always greater than the corresponding rarefaction N_1 - P_1 amplitude. The amount of broadening of the rarefaction P_1 peak varies with animal. There is significant broadening for lizard 9 and lizard 12,

Fig. 8. Amplitude-latency plot as a function of click intensity. The AP amplitude data for each lizard are plotted in percent of the amplitude of that lizard's AP response to -30 dB condensation clicks.

AP RESPONSE TO CLICKS

EFFECT OF TIME ON AP

Stimulus: -30dB Click

Lizard 10

Fig. 9. Variation with time of the AP responses to clicks. The first responses (before final surgery) and the last responses (U 10-23) were recorded more than twelve hours apart in time. Click intensity was -30 dB.

Fig. 10. Variation of the AP response to clicks across animals. Click intensity was -30 dB.

AP VARIATION ACROSS ANIMALS

Stimuli : -30 dB Clicks

with little present for lizard 6 and lizard 14. The AP responses for lizard 14 are superimposed with the N_1 - P_1 amplitudes made equal (Fig. 6B). It is clear that the P_1 peak is broader for rarefaction than condensation, even though this is not obvious in Fig. 10. Other, more subtle differences with polarity are evident in Fig. 6B.

The effects on the AP waveform of click duration and presentation rate were examined (Fig. 11, Fig. 12). As click duration was changed, click intensity was adjusted to maintain equal energy in the electrical stimulus to the earphone. For these conditions, a variation of click duration from 25 μ s to 200 μ s had little effect on the AP waveform (Fig. 11). For click presentation rates of 1/sec to 20/sec, the AP waveshape is similar, although the amplitude begins to decrease at rates greater than 10/sec. At rates of 80/sec and 200/sec, the AP response is significantly decreased in amplitude and modified in waveform (Fig. 12). Clicks of 100 μ s duration presented 10/sec were acceptable stimuli to use in these experiments since the AP response to this stimulus condition is the same as the AP response to clicks of shorter duration and slower presentation rates.

Single Unit Activity

The AP in the lizard, as in any animal, can be better understood by examining the activity of individual auditory nerve fibers since the AP is the weighted sum of the response of single fibers. The post-stimulus time (PST) histogram for a high frequency ($CF \geq 0.9$ kHz) fiber is characterized, at a gross level, by a single peak which has the same shape and latency for both click polarities (See U8-3 in Fig. 13 for a

EFFECT OF CLICK DURATION ON AP

Lizard 14

Fig. 11. Effect of click duration on the AP. The intensity of the click was adjusted to maintain equal energy in the electrical signal to the earphone. Click duration varied from 25 μ s to 200 μ s.

EFFECT OF CLICK RATE ON AP

Intensity: -30dB

Lizard 8

Fig. 12. Effect of click presentation rate on the AP. The click intensity was -30 dB. Click presentation rate varied from 1/sec to 200/sec.

Fig. 13. Single unit response of high frequency fibers to clicks. The amplitude of the AP response is not indicated; however, the AP responses for rarefaction and condensation clicks are shown to the same amplitude scale. The number of spikes in a bin for 300 stimulus presentations are indicated by the scale shown for each pair of histograms. The scale applies to both the rarefaction and condensation histogram. The bin width is determined from the number of bins, which is 100 for a 10 ms time scale and 200 for any other time scale, and the time scale. For these histograms, the bin width is 100 μ s. See the text for explanation of the Type 1 and Type 2 categorization of high frequency fibers. AP: whole-nerve response; PST: post-stimulus-time histogram.

HIGH FREQUENCY UNITS **RESPONSE TO CLICKS** Intensity (I): -30dB (unless noted)

Type 1

Type 2

typical PST histogram). The maximum amplitude of the peak occurs in time between the N_1 and the P_1 peaks of the AP response (Fig. 13).

The response of a fiber depends upon click intensity (Fig. 14). As click intensity is increased up to -30 dB, the peak in the histogram for the high frequency fiber increases in amplitude, decreases in latency, and becomes sharper. At -20 dB, the amplitude of the peak decreases slightly. Multiple peaks are not present for any of the intensities shown in the figure, although some spontaneous activity is evident. The amplitude and latency of the N_1 - P_1 complex in the AP appears well correlated with the activity of high frequency fibers.

PST histograms of low frequency ($CF \leq 0.8$ kHz) fibers responses to click stimuli are characterized by multiple peaks (Fig. 15, Fig. 16, Fig. 17). The time between the peaks is equal to $1/CF$ of the fiber (Turner and Weiss, unpublished research). For some units, these peaks can occur greater than 35 ms after the presentation of the click stimuli (Fig. 15, Fig. 16). The latencies associated with the various peaks are shown in Fig. 18. For a rarefaction click, intensity equal to -30 dB, there is an early peak (R_0) which occurs for some units, but not all, at about 2.0 ms. There is an additional peak (R_1) which is present for all units. The latency of this peak varies, probably with the CF of the fiber, but the minimum latency is about 3.0 ms. Additional peaks may be present; the latencies of these peaks depend upon the CF of the fiber.

For a condensation click, there is an initial peak (C_1) at 2.5 ms, whose latency varies little across fibers. This peak may be followed by additional peaks whose latencies depend on the CF of the fiber.

The response of low frequency fibers also depends upon stimulus

HIGH FREQUENCY UNIT RESPONSE TO CLICKS

Unit 15-2

Fig. 14. Single unit response of a high frequency fiber as a function of click intensity.

Fig. 15. Single unit response of low frequency fibers to clicks. Click intensity was -30 dB.

LOW FREQUENCY UNITS RESPONSE TO CLICKS

Fig. 16. Single unit response of low frequency fibers to clicks.
Click intensity was -20 dB and -30 dB.

Fig. 17. Single unit response of low frequency fibers as a function of click intensity.

Fig. 18. Latencies of the peaks in PST histograms
for low frequency fibers. The stimuli was a -30 dB clicks.

LOW FREQUENCY UNITS RESPONSE TO CLICKS

intensity. In general, the discharge rate of the fiber increases with increasing click intensity; however, the relative amplitude of the peaks can change in a complex way. There is a tendency, at higher click intensities, for the early peaks, R_0 , R_1 , and C_1 to decrease in amplitude relative to the later peaks (Fig. 16, Fig. 17). This is best illustrated by U10-3 (Fig. 16) and U8-7 (Fig. 17).

Contributions of the Two Fiber Populations to the AP

The PST histograms of the response of a high frequency fiber to clicks are similar for both click polarities. The response pattern of a low frequency fiber changes significantly when click polarity is reversed. Since the AP is related to the activity of individual auditory nerve fibers, the following conclusion can be made. For click stimuli, the high frequency fiber population contributes a polarity-independent component to the AP; whereas, the low frequency fiber population contributes a polarity-dependent component to the AP. A more detailed analysis of the click AP waveform in terms of single fiber activity is presented in the Discussion.

High-Pass (2.0 kHz) Click Stimuli

AP Response

It is possible to test the conclusions stated above concerning the contributions of the low and high frequency fiber populations to the AP. If the low frequency fibers do contribute a polarity-dependent component to the AP, then elimination of this component from the AP

should produce an AP waveform that is the same for both click polarities. It may be possible to eliminate the polarity-dependent component by significantly reducing the activity of the low frequency fibers.

The high frequency slope of the tuning curve for a low frequency fiber is sharp; there is little response area of the tuning curve above 1.0 kHz (Fig. 4). Treating the nerve fiber as a linear filter, a click, high-pass filtered at 2.0 kHz, should excite the high frequency fibers, yet have little effect on the low frequency fibers. The AP responses to high-pass clicks are compared to the responses to clicks (Fig. 19). The most striking feature of the data is that the waveform of the AP response to the high-pass click is the same for both polarities; whereas, the response to a click shows the normal changes with polarity. Also, the broad rarefaction P_1 peak has been eliminated by the high-pass click. The filter used to generate the high-pass clicks did not have infinite slopes (24 dB/octave), thus the stimulus contained energy in frequencies below 2.0 kHz. At -10 dB, the high-pass click contained sufficient energy below 2.0 kHz to significantly excite low frequency fibers, resulting in the change in AP waveform with stimulus polarity.

Amplitude-latency data as a function of stimulus intensity is presented in Fig. 20 for click and high-pass click stimuli. Not only is the waveform the same for both high-pass click polarities, but, as indicated in the figure, the N_1 - P_1 amplitude and the latency of the N_1 peak is the same, except at high intensities.

Single Unit Activity

The AP responses to the high-pass clicks supported the conclusions concerning the contributions of the low and high frequency fibers to

Fig. 19. AP response to clicks and high-pass clicks. Cut-off frequency of the high-pass clicks was 2.0 kHz. The AP responses to high-pass clicks were recorded from the same animal immediately following the recording of the AP responses to clicks. At each click intensity, the calibration bar applies to all four AP responses.

AP RESPONSE TO CLICKS AND HIGH-PASS CLICKS (2 KHz.)

Lizard 10

CLICK HIGH-PASS
CLICK
Cut-Off Frequency: 2.0 KHz.
Intensity (dB)
-70

AP RESPONSE TO CLICKS AND HIGH-PASS CLICKS [2.0 KHz]

Fig. 20. Amplitude-latency plot for clicks and high-pass clicks as a function of stimulus intensity. The latency data is plotted only for the condensation stimulus condition since the latencies for the rarefaction stimulus condition are essentially the same. Cut-off frequency of high-pass click was 2.0 kHz.

the AP. That test was based on the assumptions that the low frequency fibers would not be significantly excited by the high-pass click and that the response of the high frequency fibers would be similar for click and high-pass click. The best way to test those assumptions is to record the responses of single high and low frequency fibers to high-pass clicks. Even at the relatively high intensity of -20 dB, the spike activity of a low frequency fiber is significantly reduced relative to its response to a click (Fig. 21). The single unit response of a high frequency fiber to a high-pass click is very similar to its response to a click; although there are small changes in discharge rate and latency (Fig. 22).

Contributions to the AP for Low Frequency Stimuli

The data presented thus far have shown that the high frequency fibers contribute a polarity-independent component to the AP and that the low frequency fibers contribute a polarity-dependent component for a click stimulus. For low frequency fibers, that result can be extrapolated to any general, non random stimulus. It is not clear that the contribution to the AP by high frequency fibers would be polarity-independent for a low frequency stimulus (a stimulus which has most of its energy below 1.0 kHz). This is related to the issue, to be discussed later, of the innervation pattern of afferent fibers in the papilla. An inspection of the AP response to a low frequency stimulus reveals little, if any polarity-independent component (Fig. 25). Either the contribution of the high frequency fibers is polarity-dependent, or it

**LOW FREQUENCY UNITS
RESPONSE TO CLICKS & HIGH-PASS CLICKS**

Cut-Off Frequency: 2.0KHz.

Intensity: - 20 dB

Fig. 21. Comparison of the response of low frequency fibers to click and high-pass click stimuli. The amplitude scale applies to both the click and high-pass click histogram. Cut-off frequency of high-pass click was 2.0 kHz. Stimulus intensity was -20 dB.

HIGH FREQUENCY UNITS RESPONSE TO CLICKS & HIGH-PASS CLICKS

Cut-Off Frequency: 2.0 KHz.

Intensity: -30 dB

Fig. 22. Comparison of the response of high frequency fibers to clicks and high-pass clicks. Cut-off frequency of high-pass click was 2.0 kHz. Stimulus intensity was -30 dB.

is polarity-independent, but not evident in this response.

The best way to resolve this question was to record the activity of individual fibers in response to a low frequency stimulus. A low-pass (0.6 kHz) click was used as the standard stimulus. PST histograms for a sample of eight high frequency fibers are shown in Fig. 23. The fibers have been classified as Type 1 or Type 2. The responses of both Type 1 and Type 2 fibers are dependent upon stimulus polarity. The response of a Type 1 fiber to a rarefaction low-pass click is similar to the response of a Type 2 fiber to a condensation low-pass click. Likewise, the response of a Type 1 fiber to a condensation low-pass click is similar to the response of a Type 2 fiber to a rarefaction low-pass click. The histogram of the response of a Type 1 fiber to a rarefaction low-pass (0.6 kHz) click (or a Type 2 fiber to a condensation low-pass click) is characterized by a single peak; whereas, the histogram of the response to the opposite polarity shows two peaks. For most units, the two peaks have approximately the same amplitude; however, the relative amplitudes of the two peaks can vary, as in U9-21 and U10-11 (See arrows in Fig. 23). The effect of polarity on a Type 1 fiber's response is opposite the effect on the response of a Type 2 fiber. A total of 39 fibers were classified as Type 1 or Type 2 on the basis of their response to a low-pass (0.6 kHz) click; 22 fibers were Type 1 and 17 were Type 2.

In Fig. 13, the fibers are separated into Type 1 and Type 2 on the basis of their response to a low-pass (0.6 kHz) click; however, the histograms shown represent the responses of those fibers to clicks, not low-pass clicks. Careful examination reveals small differences in

Fig. 23. Single unit response of high frequency fibers to low-pass clicks. Cut-off frequency of low-pass click was 0.6 kHz. See text for explanation of Type 1 and Type 2 categorization of high frequency fibers. St: stimulus waveform; AP: whole-nerve response; PST: post-stimulus-time histogram.

HIGH FREQUENCY UNITS RESPONSE TO LOW-PASS CLICKS

Cut-Off Frequency: 0.6 KHz.

Type 1

Type 2

the latencies of the peaks for rarefaction and condensation clicks. In general, the response of a Type 1 fiber to a rarefaction click has a shorter latency than that of a Type 2 fiber.

The responses of low frequency fibers to low-pass (0.6 kHz) clicks are shown in Fig. 24. While there are variations in the discharge patterns of low frequency fibers, most likely related to fiber CF, the fibers cannot be divided, using the same criteria used for high frequency fibers, into two or more types.

The response of a Type 1 or a Type 2 high frequency fiber depends upon stimulus polarity; however, because of the unique symmetry in the response of the two types, the net contribution to the AP of all the high frequency fibers is polarity-independent. The response of a low frequency fiber changes with stimulus polarity; the net contribution of all the low frequency fibers to the AP is polarity-dependent.

Low-Pass Click Stimuli

AP Response

With the knowledge gained in the analysis of the click AP, and the understanding that the contribution to the AP of high frequency fibers is polarity-independent while the contribution of low frequency fibers is polarity-dependent, it was possible to investigate the use of the AP as a research tool. AP data for low-pass clicks are shown for various cut-off frequencies (f_c) and intensities in Fig. 25 and Fig. 26. For f_c equal to 1.0 kHz or less, the AP consists almost entirely of a polarity-dependent component. For f_c greater than 1.0 kHz, the AP has a significant polarity-independent component.

Fig. 24. Single unit response of low frequency fibers to low-pass clicks. Cut-off frequency was 0.6 kHz. Even though there are some differences in the response patterns of the fibers, the fibers cannot be separated into two types in the same way as the high frequency fibers.

LOW FREQUENCY UNITS RESPONSE TO LOW-PASS CLICKS

Cut-Off Frequency: 0.6 KHz.

AP RESPONSE TO LOW-PASS CLICKS

Fig. 25. AP response to low-pass clicks as a function of stimulus intensity.

Fig. 26. AP response to low-pass clicks as a function of cut-off frequency. Stimulus intensity was -48 dB. Stimulus not shown for cut-off frequencies greater than 1.0 kHz.

AP REPOSE TO LOW-PASS CLICKS

Intensity: -48 dB

Lizard 11

Cut-Off Frequency (KHz.)

An amplitude-latency plot as a function of cut-off frequency is shown in Fig. 27. Of particular interest is the discontinuity in the amplitude plot for the rarefaction stimulus and the obvious change in the nature of the latency data which occurs between 1.0 kHz and 1.5 kHz. There is a difference in amplitude with polarity for all cut-off frequencies; however for f_c greater than 1.0 kHz, the difference in amplitude is small relative to the amplitude for a rarefaction stimulus. For f_c less than 1.5 kHz, the difference in amplitude is larger than the amplitude for the rarefaction stimulus. This suggests a large polarity-independent component for f_c greater than 1.0 kHz and a small component for lower cut-off frequencies. The latency of the response is independent of stimulus polarity for f_c greater than 1.0 kHz, and dependent upon polarity for lower cut-off frequencies. These data indicate a transition from a predominately polarity-independent response to a polarity-dependent response as f_c is decreased. Decreasing f_c to 1.0 kHz should affect the response of the high frequency fibers more than the low frequency fibers; thus, this amplitude-latency plot supports the conclusion that the contribution of high frequency fibers to the AP is polarity-independent while the contribution of low frequency fibers is polarity-dependent. It is clear from the single unit data in Fig. 23 that high frequency fibers do respond to a low-pass (0.6 kHz) click. The lack of an obvious polarity-independent component in the AP is a problem; but, there is an explanation. First, for a low frequency, low-pass click, the low frequency fibers have a higher discharge rate and may tend to dominate the AP (Fig. 23, Fig. 24). Second,

AP RESPONSE TO LOW-PASS CLICKS

Fig. 27. Amplitude-latency plot for low-pass clicks as a function of cut-off frequency. $N_1 - P_1$ was the first negative-positive complex evident in the AP responses in Fig. 26.

a comparison of the response of high and low frequency fibers demonstrates that the response patterns for a low frequency fiber and a Type 2 high frequency fiber are similar (Fig. 28). The total contribution to the AP of these two fiber groups probably obscures the contribution from the Type 1 high frequency fibers. In Fig. 28, the Type 1 fiber response may be reflected in the small dip (arrow) in the AP response to the rarefaction low-pass click. At higher low-pass (0.6 kHz) click intensities, the AP response to the condensation stimulus shows a broadening of the first negative peak and then a decrease in latency until, at -18 dB, the latency is the same as for the first negative peak in the AP for the rarefaction stimulus (Fig. 25). This suggests that the polarity-independent contribution of the high frequency fibers is more obvious at high intensities.

Single Unit Activity

Single unit data for the response of high frequency fibers to low-pass clicks of various cut-off frequencies are shown in Fig. 29. The main point to observe is that the classification of a high frequency fiber as Type 1 or Type 2 is consistent with the response of that fiber to low-pass clicks with cut-off frequencies other than 0.6 kHz. Low-frequency fibers cannot be separated into two types even if the cut-off frequency of the low-pass click is varied (Fig. 30).

As the cut-off frequency of the low-pass click is increased above 1.0 kHz, the responses of low frequency fibers demonstrate a pattern that is similar to the phenomenon observed for low frequency fibers when click intensity is increased above about -30 dB (Fig. 30,

UNIT RESPONSE TO LOW-PASS CLICK

Cut - Off Frequency : 0.6 KHz.

Intensity : - 38 dB

Lizard 10

Fig. 28. Comparison of the AP response and single unit activity of individual auditory nerve fibers. Stimulus was a -38 dB low-pass click; cut-off frequency was 0.6 KHz.

Fig. 29. Single unit response of a Type 1 and a Type 2 high frequency fiber as a function of low-pass click cut-off frequency. Note the similarity in the response of the two types of fibers to stimuli of opposite polarity.

HIGH FREQUENCY UNITS RESPONSE TO LOW-PASS CLICKS

Fig. 30. Single unit response of two low frequency fibers as a function of low-pass click cut-off frequency. Stimulus not shown for cut-off frequencies greater than 1.0 kHz.

LOW FREQUENCY UNITS RESPONSE TO LOW-PASS CLICKS

Fig. 31). The early peaks are decreased in amplitude relative to the later peaks. From a linear systems viewpoint, adding energy above 1.0 kHz should not affect the response of low-frequency fibers since their tuning curves show little response area above 1.0 kHz.

Narrow-Band Click Stimuli

It may be possible to excite a smaller group of fibers with a narrow-band click than a low-pass click because the energy in a narrow-band click is contained within a smaller frequency range than the low-pass click. For narrow-band center frequencies of 2.0 kHz and 4.0 kHz, the AP response is the same for both polarities and, therefore, it consists entirely of a polarity-independent component (Fig. 32). For lower center frequencies, the AP consists largely of a polarity-dependent component. It is clear in the plot of amplitude-latency as a function of narrow-band click center frequency that there is a significant change in the AP response as the center frequency varies from 1.0 kHz to 2.0 kHz (Fig. 33). Above this frequency region, amplitude and latency are independent of stimulus polarity; below this region, they are dependent upon polarity.

Tone and Tone Burst Stimuli

AP Response

For low frequency tone bursts, the AP shows synchronous, phase-locked neural activity in the form of regular, diphasic complexes (Fig. 34). A comparison with the stimulus waveform reveals that this

Fig. 31. Comparison of the single unit response of low frequency fibers to clicks and low-pass clicks. Cut-off frequency of low-pass clicks was 1.0 kHz.

**LOW FREQUENCY UNITS
RESPONSE TO CLICKS & LOW-PASS CLICKS**
Cut-Off Frequency: 1.0 KHz.

Low-Pass Click

Click

U9-7
Intensity: -30 dB

U9-29 I:-20

U13-18 I:-20

U10-7 I:-20

Fig. 32. AP response to narrow-band clicks as a function of center frequency. Stimulus intensity was -50 dB. Stimulus is not shown for center-frequencies greater than 1.0 kHz.

AP RESPONSE TO NARROW-BAND CLICKS

Lizard 15

Intensity: -50 dB

Center Frequency (KHz.)

AP RESPONSE TO NARROW-BAND CLICKS

Fig. 33. Amplitude-latency plot for narrow-band clicks as a function of center frequency. Data based upon the AP responses shown in Fig. 32.

Fig. 34. AP response to tones and tone bursts as a function of stimulus frequency. Averages were computed by triggering to the positive zero crossings of the sinusoidal stimuli. Tone bursts had fast rise times and a duration of 30 ms.

AP RESPONSE TO TONES & TONE BURSTS

Lizard 13

Intensity: -40 dB [unless noted]

phase-locked activity occurs once-per-period of the sinusoidal stimulus (Fig. 35), and shifts 180 degrees as stimulus polarity is reversed. As tone burst frequency is increased, the diphasic complexes move together in time, until around 0.4 kHz they begin to overlap. As the frequency is increased, the net result, except at the onset of the tone burst, is sinusoidal in appearance. For frequencies of 1.0 kHz or higher, there is little phase-locked activity evident in the AP. The above description applies as well to the AP response to tones (Fig. 34).

The sinusoidal component in the AP at frequencies of 0.4 kHz to 0.8 kHz could be cochlear microphonic (CM) or electrical artifact. If this was true, then for a tone burst stimulus, the CM or artifact should be evident before the synchronous neural activity begins. Evaluation of the data in Fig. 35 does not reveal any CM or artifact-like component in the first 2 ms of the recorded response. An attempt was made to record electrical artifact. The speculum was removed from the external auditory meatus and plugged. The earphone was rotated slightly and the speculum placed against the side of the lizard's head. The "AP" recorded in this condition is compared to the AP recorded under normal conditions just prior to this test (Fig. 36). The electrical artifact is small compared to the recorded neural activity.

Single Unit Activity

The spike activity of an individual high frequency fiber is phase-locked, once-per-period, to the sinusoidal stimulus (Fig. 37). The phase-locking is present for frequencies as high as 1.0 kHz, but by 2.0 kHz, it is not present in the processed data. The responses of four

AP RESPONSE TO TONE BURSTS Intensity: -40 dB (unless noted)

Fig. 35. AP response to tone bursts as a function of stimulus frequency. The right most column illustrates the DC-like positive potential evident in the AP response for some frequencies.

Fig. 36. AP response to tone bursts as a function of stimulus intensity. The electrical artifact was recorded by the wire electrode after plugging the speculum to significantly reduce the acoustic stimulus. Stimulus intensity was -20 dB for frequencies of 0.2 kHz and 0.6 kHz.

AP RESPONSE TO TONE BURSTS

Lizard 14

Frequency (KHz.)

Intensity (dB)

HIGH FREQUENCY UNIT RESPONSE TO TONE BURST

U15-2

Frequency (KHz.)

Fig. 37. Single unit response of a high frequency fiber to tone bursts of different frequencies. The histograms were triggered on the positive zero-crossings of the stimulus. Stimulus not shown for frequencies greater than 1.0 kHz.

high frequency fibers are shown in Fig. 38. Careful examination reveals that the phase-locked activity of U15-4 and U15-5 is 180 degrees out of phase with the activity of U15-7 and U15-8. While there is insufficient data to relate this result to the Type 1, Type 2 classification of high frequency fibers, the result does indicate that the once-per-period, phased-locked activity in the AP is associated with the low frequency fibers.

Individual low frequency fibers exhibit once-per-period, phased-locked activity to low frequency sinusoidal stimuli, and little response to frequencies above 1.0 kHz (Fig. 39). While there is some variation in the phase of the response of the low frequency fibers to the same stimulus, this variation is much less than 180 degrees (Fig. 40). This supports the above statement that the phased-locked AP activity is contributed primarily by low frequency fibers.

HIGH FREQUENCY UNITS RESPONSE TO TONE BURSTS

Intensity: -50 dB

Frequency: 0.6 KHz.

Fig. 38. Single unit response of high frequency fibers to tone bursts. Tone burst frequency was 0.6 kHz.

LOW FREQUENCY UNIT RESPONSE TO TONE BURST

U15-12

Intensity :- 50 dB (unless noted)

Frequency (KHz.)

Fig. 39. Single unit response of a low frequency fiber to tone bursts of different frequencies. Stimulus not shown for frequencies greater than 1.0 KHz.

LOW FREQUENCY UNITS RESPONSE TO TONE BURST

Fig. 40. Single unit response of low frequency fibers to tone bursts.

DISCUSSION

Analysis of the AP Response to Clicks

The first objective of this research was to analyze the whole-nerve action potential (AP) response to click stimuli and relate the results to the anatomy of the inner ear. Examination of post-stimulus-time (PST) histograms revealed the polarity-independent and polarity-dependent contributions to the AP of high and low frequency fibers. A more detailed analysis of the AP is possible using the histograms. PST histograms reflect the discharge patterns of the fibers and it is these discharge patterns that determine the AP waveform. The regular discharge of a fiber at a certain time following the onset of the stimulus results in a peak in the histogram. If the histograms of a large percentage of fibers in a sample have peaks with the same latencies, then this suggests synchronous activity of the fibers and a contribution to the AP.

Consider the AP response to a rarefaction click (intensity = -30 dB or less). The N_1 - P_1 complex results from the synchronous activity of high frequency fibers, which occurs at about 2.5 ms as indicated by the peak in the histogram. For low frequency fibers, the R_1 peak varies in latency, with a minimum of about 3.0 ms. This results in synchronous nerve fiber activity around 3.0 ms which produces

the broadening of the P_1 peak. The R_0 peak occurs about 2.0 ms. This peak is not always present (Fig. 18), and when it does occur, it is usually smaller than the R_1 peak (Fig. 15, Fig. 16, Fig. 17). It is not clear from these data how the R_0 peak affects the AP waveform. There is no obvious difference around 2 ms in the AP response to condensation and rarefaction clicks which can be associated with the R_0 peak. The histograms for low and high frequency fibers reveal no synchronous activity after 4.0 ms following the stimulus. Low frequency fibers do have peaks in this time period, but the latencies of these peaks vary with fiber CF, resulting in no net time varying component in the AP. The N_2 - P_2 and N_3 - P_3 complexes result, primarily, from synchronous activity of higher auditory elements.

Next, consider the AP response to a condensation click (intensity = -30 dB or less). Again the high frequency fibers have synchronous activity about 2.5 ms. The C_1 peak for low frequency fibers also occurs about 2.5 ms (Fig. 18). The two fiber populations combine to produce a N_1 - P_1 complex larger than that produced for a rarefaction click, when only high frequency fibers contribute to the N_1 - P_1 complex. The latencies of the remaining peaks (low frequency fibers) depend upon fiber CF and produce no net AC component in the AP.

At high click intensities, complex changes occur in the AP waveform; in particular, there is a decrease in the N_1 - P_1 amplitude for both click polarities, and an increase in AP activity beyond 4.0 ms. These changes result primarily from the changes in the discharge patterns of low frequency fibers. The amplitudes of later peaks (> 4.0 ms) increase at high intensities relative to the amplitudes of the

R_0 , R_1 and C_1 peaks. For some units, there is a decrease in the absolute amplitudes of the R_0 , R_1 and C_1 peaks.

The analysis of the click AP is summarized in the following :

1. High frequency fibers contribute a polarity-independent component to the AP: low frequency fibers contribute a polarity-dependent component.
2. The large N_1 - P_1 complexes result, primarily, from the synchronous activity of the high frequency fibers.
3. The N_1 - P_1 amplitude is greater for a condensation click, because, for this polarity the low frequency fibers contribute to the N_1 - P_1 complex.
4. For a rarefaction click, the low frequency fibers contribute a component to the AP at about 3.0 ms which results in the broadening of the P_1 peak.
5. The N_2 - P_2 and N_3 - P_3 complexes reflect the synchronous activity of higher order auditory neural elements.
6. The complex changes in the AP which occur at high click intensities result, primarily, from complex changes in the discharge patterns of low frequency fibers.

Relationship of the AP to Inner Ear Anatomy

It has been shown that the high frequency fibers contribute a polarity-independent component to the AP and the low frequency fibers a polarity-dependent component for any non-random stimulus. One objective of this research was to relate this analysis of the (click)

AP to the anatomy of the inner ear. The most striking anatomical feature in the alligator lizard's inner ear is the presence of two hair cell systems. The ventral hair cell population is a unidirectional orientation/tectorial membrane system, while the dorsal hair cell population is a bidirectional orientation/free standing cilia system. The low frequency and high frequency fiber populations, which have been associated with the ventral and dorsal hair cell populations, demonstrate differences in their physiological response on the basis of their tuning curves. This research also indicates that the physiology of the two fiber populations also differs in terms of the response of individual fibers to click, low-pass click, and tone burst stimuli. No doubt, some of these physiological differences are associated with the tectorial membrane or free standing cilia anatomical structures; but, the relationship between this anatomy and the physiology is not clear.

There are some data to indicate the effect of hair cell orientation on physiological responses. It has been proposed that displacement of the hair cell cilia towards the kinocilium produces a depolarization of the hair cell and a displacement in the opposite direction produces a hyperpolarization (Lowenstein and Wersall, 1959; Flock and Wersall, 1962; Flock, 1971). Studies of bidirectional hair cell systems in the fish and amphibia demonstrate their physiological significance. Microphonic-like potentials recorded from these organs have a large second harmonic, reflecting a frequency doubling phenomenon (Furukawa and Ishii, 1967a, 1967b; Flock, 1965). Single nerve fibers

in these organs responded, in a phase-locked manner, twice per period, or formed two populations whose phase-locked responses were 180 degrees out of phase (Furukawa and Ishii, 1967a, 1967b; Harris and Milne, 1966).

The effect of bidirectional hair cell orientation is the excitation of some hair cells and nerve fibers for the condensation phase of the stimulus; some for the rarefaction phase; and in some organs, some for both phases. The high frequency fibers in the auditory nerve of the alligator lizard contribute a polarity-independent component to the AP because of the bidirectional orientation of hair cells in the dorsal papilla; the low frequency fibers contribute a polarity-dependent component because of the unidirectional orientation of hair cells in the ventral papilla.

Innervation Pattern for Afferent Fibers

Result of dye-marking experiments associated the high and low frequency fibers in the auditory nerve of the alligator lizard with the two hair cell populations in the papilla (Weiss et al., 1974b). Unfortunately, this type of experiment provides no information concerning the innervation pattern of afferent fibers in the papilla. Three possible innervation patterns are shown in Fig. 41. High frequency fibers synapsing to hair cells in both the dorsal and ventral populations would explain the broad shape of the tuning curves (Fig. 41c). In this case, high frequency fibers should contribute a polarity-independent component

POSSIBLE INNERVATION PATTERNS

Fig. 41. Possible innervation patterns of afferent auditory nerve fibers in the papilla of the alligator lizard. The high frequency fibers are associated with the dorsal papilla and the low frequency fibers are associated with the ventral papilla. Pattern B is the most consistent with the physiological data of this study. Neural refers to the dorsal hair cell population closest to the nerve; abneural, the dorsal population away from the nerve.

to the AP for high frequency stimuli, and a polarity-dependent component for low frequency stimuli. Since the contribution of high frequency fibers is polarity-independent for all stimuli, this innervation pattern is not appropriate for the alligator lizard.

Both Pattern A and Pattern B in Fib. 41 are consistent with the AP responses recorded in this research. The two patterns can be distinguished only on the basis of the activity of single fibers in the auditory nerve. If high frequency fibers synapsed on both groups of hair cells in the dorsal papilla (Pattern A), then the discharge patterns of single fibers should be independent of stimulus polarity and constitute one response type. However, the high frequency fibers in the alligator lizard were classified as Type 1 and Type 2 and the response of a high frequency fiber depends upon stimulus polarity. Pattern B best represents, on the basis of physiological data, the innervation pattern of afferent fibers in the papilla of the alligator lizard.

In the bidirectional hair cell systems of the goldfish (Furukawa and Ishii, 1967a) and the toad (Harris and Milne, 1966), nerve fibers were found which appeared, on the basis of physiological data, to synapse only to hair cells of one orientation; although, some fibers in the goldfish synapsed to hair cells of both orientations. There is no physiological evidence for the alligator lizard of nerve fibers synapsing to dorsal hair cells of both orientations.

Basic Neural Response Unit

If the AP is considered to be the convolution of a basic neural response unit with the discharge patterns of the fibers, then the AP

will resemble this basic response unit to the degree that the discharge patterns (represented by the PST histograms) resemble an impulse.

(Convoluting a function with an impulse yields the function) For a high-pass (2.0 kHz) click, the activity of a low frequency fiber is significantly reduced (Fig. 21). The discharge patterns of high frequency fibers to high-pass (2.0 kHz) clicks approximate an impulse (Fig. 22); therefore, the corresponding AP should resemble the basic response unit (Fig. 19). If the N_2 - P_2 and N_3 - P_3 complexes are removed from the AP waveform, because they represent the activity of higher order fibers, the diphasic N_1 - P_1 is left. Therefore, the basic neural response unit as recorded by a wire electrode near the round window is a diphasic, negative-positive potential.

Inspection of the AP in Fig. 19 reveals that the P_1 peak is larger than the N_1 peak. This can explain the DC-like positive potential present in the AP response to tone burst of some frequencies (Fig. 35). Asynchronous activity of the nerve fibers produces no AC component in the AP, but does result in a DC-like component.

New Technique for Analyzing the AP

During one of the experiments, the tip of the microelectrode was damaged resulting in a low impedance ($< 2.0 \text{ M}\Omega$) electrode. When the electrode was placed on or near the nerve, it appeared to record the AP from a limited region of the nerve. In position 1, the electrode recorded a signal which changed with stimulus polarity (Fig. 42). The electrode was moved in a medial direction to position 2. The signal recorded here was relatively independent of stimulus polarity.

Fig. 42. AP response to clicks recorded with a low impedance microelectrode. The AP response recorded with the wire electrode is shown for comparison. The absolute amplitude of the responses is not indicated; however, for each click intensity, the responses to the rarefaction and condensation stimulus condition are to the same scale. For all intensities, the same relation of microelectrode response amplitude to wire electrode response amplitude is maintained.

AP RECORDED WITH MICROELECTRODE

Lizard 13

Stimulus: Click

Intensity (dB)

Position 1

Position 2

-40

It is proposed that in position 1, the electrode primarily recorded the activity of low frequency fibers, while in position 2, the activity of high frequency fibers. This is feasible since the nerve near the papilla forms a sheet with the low and high frequency fibers spatially separated (Weiss et al., 1975). The data in Fig. 42 support the conclusions above concerning the contributions of the low and high frequency fibers to the AP response to a click.

When the peripheral portion of the auditory nerve is accessible to an electrode, as is probable in many species of lizards, then a low impedance electrode can be used to record the contribution to the AP from limited populations of nerve fibers. This is a promising new technique for analyzing the AP and determining the tonotopic organization of the papilla.

AP as a Research Tool

Filtered Click Stimuli

The second objective of this research was to investigate the use of the AP as a research tool. The basic strategy was to examine the AP response to filtered-click, tone and tone burst stimuli to determine what features of the response could be related to the anatomy of the inner ear.

High-pass clicks were used to selectively excite the high frequency population of nerve fibers, simplifying the analysis of the AP. Low-pass clicks could not be used to selectively excite the low frequency fiber population in the alligator lizard because of the broad shape of the high frequency fiber tuning curve; however, this

may be an effective technique for use in other species of lizards which have several hair cell populations. Low-pass and narrow-band clicks were used to generate amplitude-latency plots (Fig. 27, Fig. 33). Both plots indicate a transition from a polarity-dependent AP response to a polarity-independent response as filter frequency (cut-off or center) is increased. This is more obvious in the plot for narrow-band clicks. This transition is most evident from 1.0 kHz to 1.5 kHz.

On the basis of only these two amplitude-latency plots, the high frequency fibers in the alligator lizard could be associated with the dorsal papilla and the low frequency fibers with the ventral papilla. The plots also indicate that the frequency range 1.0 kHz to 1.5 kHz is associated with the transition from one fiber population to the other. Much of the information obtained using complex experimental procedures could have been more easily derived using the AP had it been better understood. The use of filtered clicks to generate the AP could easily provide information concerning the tonotopic organization of the papilla in other species of lizards.

Tone and Tone Burst Stimuli

Wever used a wire electrode on the round window to record responses to tones in many species of lizard. He determined the magnitude of the fundamental frequency of this response, assuming that it was cochlear in origin. These data for the alligator lizard (Wever, 1971a) show the ear to be most sensitive at 0.4 kHz to 0.5 kHz; a result not consistent with single unit data. The AP response to a tone or tone burst shows periodic diphasic complexes at low frequencies. Around

0.4 kHz to 0.5 kHz these overlap to produce a very sinusoidal response. At higher frequencies, they cancel to produce little net response. The data presented by Wever for the alligator lizard probably reflects the fundamental frequency of the synchronous, phase-locked neural activity. This is supported by the fact that in the AP data presented in this paper, very little CM like response is evident. Data presented for other species may also primarily reflect neural activity, not cochlear potentials.

Weiss, Mulroy, Turner and Pike (1975) reported two-tone rate suppression (TTRS) in the alligator lizard. A similar phenomenon was observed in the AP response to a low frequency tone (Fig. 43). The phase-locked activity evident in the AP response to a 0.2 kHz or a 0.6 kHz tone resulted from the activity of low frequency fibers. A continuous 2.0 kHz was mixed with the tone burst stimulus. As the intensity of the high frequency tone was increased, the AP activity is significantly decreased; even though, the high frequency tone was well above the response area of the low frequency fibers. The exact nature of this phenomenon is not known; but, it does suggest the possibility of using the AP to study TTRS type phenomena.

Fig. 43. AP response to a tone burst plus a tone. T₁ refers to the tone burst, T₂ to the tone.

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BIOGRAPHICAL SKETCH

Robert Graham Turner was born on August 20, 1946, at Miami, Florida. In April, 1967, and August, 1968, he received his B.S. and M.S. degrees in Engineering Science from the Florida State University. From June, 1968 to August 1970, he was employed in Chattanooga, Tennessee, as an engineer by the E. I. duPont de Nemours Company. In September, 1970, he returned to school as a graduate student in the Electrical Engineering Department at the Massachusetts Institute of Technology. During the two years at M.I.T., he was involved in auditory research at the Eaton-Peabody Laboratory of Auditory Physiology. In September, 1972, he began his doctoral work as a N.I.H. trainee in the Communication Sciences Laboratory, Speech Department, University of Florida. He is married to the former Polly Ann Foshee, and has three children: Lauren, Kristen, and Trey.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A handwritten signature in cursive script, reading "Donald C. Teas", written over a horizontal line.

Donald C. Teas, Chairman
Professor of Speech and Psychology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A handwritten signature in cursive script, reading "D. Paul Moore", written over a horizontal line.

D. Paul Moore
Professor of Speech

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

A handwritten signature in cursive script, reading "William A. Yost", written over a horizontal line.

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Speech

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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