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Inherited Genetic Deafness in the Hedlund White Mink: An Electrophysiologic and Morphologic Study

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INHERITED DEAFNESS IN THE HEDLUND WHITE MINK :
AN ELECTROPHYSIOLOGIC AND MORPHOLOGIC STUDY

By

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ABSTRACT

INHERITED DEAFNESS IN THE HEDLUND WHITE MINK :

AN ELECTROPHYSIOLOGIC AND MORPHOLOGIC STUDY

By

Susan Marie Stejskal

In this research project, the clinical development of the auditory system of standard dark mink (Mustela vison) (SDM) and the Hedlund white mink (HWM) was characterized. The presence of an auditory brainstem evoked responses (ABR) was used as an indicator of the integrity of the auditory system. The development of auditory lesions in HWM was characterized by histologic examination of cochleas from mink at various ages. Although hearing was well established in the SDM by 26 days old, a defined "window" of hearing onset was found to occur in the HWM during the 34th and the 42nd days of age. This was followed by a progressive loss of hearing evidenced by a lack of ABR after 55 days of age. Prior to complete loss of waves, the presence and persistence of the first ABR wave may suggest that while cochlear function persists, anomalies within the auditory brainstem pathways may be occurring simultaneously.

Histologically, the auditory lesions in HWM were very similar to those in other animals with cochleosaccular

degeneration. As this condition is reputedly caused from a failure of melanocytes to establish in the stria vascularis of the cochlea, a principal change was thinning of the cellular layers of the stria vascularis with progression to complete atrophy. These changes were accompanied by collapse of Reissner's membrane and obliteration of the scala media, which began in the basal turn. Degeneration of the organ of Corti progressed to a point where the normal architecture was effaced. Changes in the spiral ganglion included neuronal loss and replacement by connective tissue. These auditory changes were similar to those reported earlier for the HWM. They are also similar to those reported for the blue-eyed white cat and the Dalmatian dog, but mink have later onset of hearing. For this reason, mink may prove useful in the study of developmental auditory anomalies. Consistent and natural occurrence of deafness in the HWM is a feature which make the mink a model for the study of genetic deafness and the use of cochlear implants.

Dedicated with all my love to my husband, Andrew Rosenbaum and to our son, Joseph Kiva Rosenbaum.

ACKNOWLEDGEMENTS

I would like to thank several people who helped me accomplish completion of this research project. First, I want to thank Dr. Richard Aulerich for his unending patience and guidance in the progression and completion of this project. I also wish to thank Dr. Richard Altschuler, Dr. Steve Bursian, Dr. James Render, Dr. Duane Ullrey for their suggestions and assistance as members of my committee.

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LIST OF ABBREVIATIONS

ABR	auditory brainstem evoked response
AC	alternating current
AP	action potential
BAER	brainstem auditory evoked response
BC	basal cell (stria vascularis)
CD	cochlear duct
CM	cochlear microphonics
dB	decibels
DC	direct current
EDTA	ethylenediamine tetracetate
EEG	electroencephalogram / electroencephalographic
EP	evoked potential
HC	hair cell
HWM	Hedlund white mink
Hz	Hertz
IC	intermediate cell (stria vascularis)
IHC	inner hair cell
KHz	kiloHertz
MC	marginal cell (stria vascularis)
MSO	medial superior olive
msec	millisecond
OofC	organ of Corti

LIST OF ABBREVIATIONS (continued)

NA	not available
OHC	outer hair cell
RM	Reissner's membrane
ScM	scala media
ScT	scala tympani
ScV	scala vestibuli
SDM	standard dark mink
SG	spiral ganglion
SP	summating potential
SPL	sound pressure level
SV	stria vascularis
TEM	transmission electron microscope
TM	tectorial membrane

INTRODUCTION

The primary objective of this research project was to investigate the possible use of the mink as an animal model for hearing research. Although there are only a few citations in the literature about the auditory system of the mink, there were some published about the Hedlund white mink (HWM) in the 1960's and 1970's. According to the publications, the deafness condition in the HWM appears to be very similar to that found in some Dalmatian dogs, blue-eyed white cats and human beings with Waardenburg's syndrome. Although the condition is probably a pigment-associated hereditary trait (autosomal dominant), it appears very sporadically in the cat, dog and human. Most if not all, Hedlund mink appear behaviorally deaf as they fail to respond to noise stimuli.

The HWM are somewhat abundant as evidenced by United State Department of Agriculture's statistics for 1990. In that year, approximately 60,000 white mink were pelted and 13,300 females were kept to be bred in 1991. Because of the availability of the animals and consistency in genetic expression of the hearing loss in the animals, the Hedlund white mink could be very useful in auditory research, especially in the area of cochlear prosthesis implantation.

Cochlear implants (prosthesis) are currently being surgically implanted in both children and adult human beings to correct for some conductive or sensorineural hearing losses. Early implantation is sometimes recommended in young deaf children to help maintain the integrity of the auditory neural pathway. However, important questions remain unanswered concerning the effects of long-term electrical stimulation of nerves within the auditory pathway by the prosthesis. With the use of an animal model like the Hedlund white mink, these questions may be answered and lead to greater use of implants in hearing-impaired children.

The goal of this research project was to study and characterize the auditory system of the "normal" mink (Mustela vison) and to study and define the hearing loss and deafness in the Hedlund white mink. By using anatomic and physiologic methods to study the animal, the value of the mink as an animal model for auditory research may be determined. This project focused on two major areas : (1) the chronological development of the auditory system of the normal mink, and (2) the physiologic and histologic differences in the auditory system of "normal" and Hedlund white mink. Some discrepancies exist in the literature about the onset of hearing in the Hedlund white mink. By using more sophisticated electrophysiologic methods to test the animals, an attempt was made to address these discrepancies. An effort was also made to compare morphologic changes observed during critical timepoints with electrophysiologic findings in the Hedlund white mink and other species demonstrating Waardenburg-like syndrome or similar pigment-associated auditory anomalies.

Although the disadvantages of the mink as an animal model may be numerous, the Hedlund white mink may prove to be a useful model for studies of genetic deafness or for projects that require consistently and reliably deaf animal models.

LITERATURE REVIEW

Anatomy of the Ear: Introduction

A basic review of the anatomy, physiology and embryology of the auditory system is necessary in order to understand the potential application of the Hedlund white mink as an animal model. The auditory system is very similar among mammals and is most simply divided into three parts - the outer, middle and inner ear. The following synopsis is based on texts edited by Altschuler *et al*. (1986), Jahn and Santos-Sacchi (1988), Hole (1990) and Lewis *et al*. (1985).

Anatomy and Embryology of the Outer Ear

The outer ear is made up of three major parts - the pinna or auricle (ear flap), the external auditory meatus (ear canal) and the lateral surface of the tympanic membrane (eardrum). The pinna is an elastic cartilaginous structure covered by skin on both sides. In the animal, the lateral side is covered with hair and sebaceous sweat glands; the medial side has much less hair and a greater concentration of sebaceous glands. The function of the pinna is to help "catch" sound waves and direct them down the external auditory meatus (Figure 1).

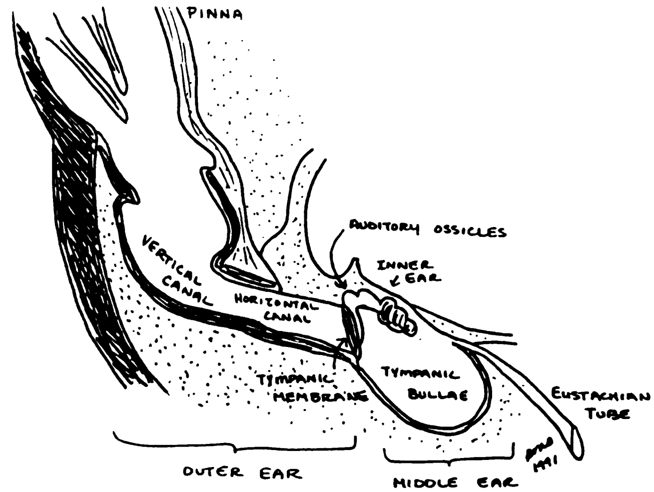


Figure 1. Diagram of parts of the auditory system.

The external auditory meatus amplifies the sound waves and directs them to the tympanic membrane. The canal is capable of amplification of up to 15 to 20 decibels in sound intensity when the frequency is about 2000 Hertz. The external portion of the canal is supported by a partial cartilaginous and osseous frame which allows some flexibility; the innermost portion is supported by bone. The canal is lined with thin skin which contains small hair follicles and sweat, sebaceous and ceruminous glands. The ceruminous and sebaceous glands produce cerumen - a dark waxy material that provides protection for the external ear by acting as a physical barrier and a bactericide. Slow, progressive migration of the cerumen laterally toward the pinna provides a mechanism to remove small foreign material from the tympanic membrane.

The tympanic membrane is the most medial structure of the outer ear. The lateral side is covered with thin skin which lacks hair and glands; medially it is covered with epithelial mucosa of the middle ear. The purpose of the tympanic membrane is to receive the sound waves, vibrate and transfer the waves to the middle ear.

Embryologically the external auditory meatus is formed from both ectodermal and mesenchymal sources. It eventually lengthens and widens to form the mature auditory meatus (Figure 2). The tympanic membrane is formed from three different tissue sources : a) endoderm - external layer; b) mesoderm - middle layer and c) ectoderm - inner mucosal layer.

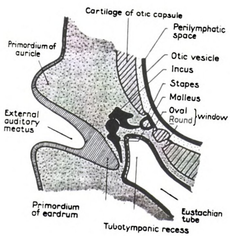


Figure 2. Embryologic development of the outer and middle ear. Used with permission from Springer Verlag Publishing and authors Tuchmann-Dupliessis _et_ _al_.; from _Illustrated Human Embryology (Volume 3)_, (1974).

Anatomy and Embryology of the Middle Ear

The middle ear is a hollow, air-filled chamber within the petrous temporal bone which is lined with simple squamous or low cuboidal epithelium. The tympanic cavity contains three auditory ossicles. The malleus (hammer) is the first of the auditory ossicles within the middle ear. It is firmly attached to the medial surface of the tympanic membrane and moves as the tympanic membrane vibrates from sound waves. As the vibration continues, a chain reaction follows as the incus (anvil) and stapes (stirrup) also move in response to the pattern of the sound wave. This results in movement of the footplate of the stapes in the oval window of the vestibule. It is at this point that the conduction of sound waves shifts from an air to a fluid medium.

The middle ear also contains the auditory or Eustachian tube which extends between the tympanic cavity and the pharynx. The function of the tube (which is lined with pseudostratified ciliated columnar epithelium) is to provide for equalization of pressure between the tympanic cavity and the external environment.

The middle ear is formed from the invagination and upward growth of the tubotympanic sulcus from the first branchial cleft. This sulcus arises from both ectoderm and mesodermal mesenchyme. The tympanic cavity is formed from the enlarging of the sulcus.

The auditory ossicles arise from different sources. The malleus and incus originate from the first branchial arch while parts of the stapes emanate from the second branchial arch. The stapedial footplate forms in the oval window within the stapedial annulus. After the auditory ossicles form in a cartilagineous framework, ossification takes place. The vascular supply, muscles and ligaments of the middle ear originate from mesoderm.

Anatomy and Embryology of the Inner Ear

The inner ear begins with the junction between the footplate of the stapes in an opening in the osseous labyrinth of the inner ear called the oval window. The stapes is held in place by a membrane in the oval window. Vibration of the footplate and the membrane transfer the mechanical energy of the sound waves to the inner ear.

The functional part of the inner ear is actually enclosed in bone called the osseous labyrinth. The cellular portion is contained within the bony tubes in the temporal bone and is called the membranous labyrinth. The osseous labyrinth has three parts - the vestibule, the semicircular canals and the spiral cochlea. Embryologically, the inner ear begins from the formation of the otic placode on the sides of the head. This tissue originates from ectoderm from the rhombencephalon with some assistance from nearby neural ectoderm (from the neural crest). The otic placode invaginates and forms the otic cup (Figure 3). As the invaginated cup closes off from the surface of the head, a fluid - filled otocyst is formed. Eventually it lengthens into the superior, intermediate and inferior portions (Figure 4). These eventually form the vestibular (superior) system and semicircular canals, the cochlea (inferior portion) and the vestibule with the utricle and saccule (intermediate portion).

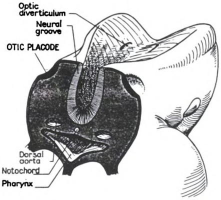


Figure 3. Embryologic development of the inner ear. Used with permission from Springer Verlag Publishing and authors Tuchmann-Dupliessis <u>et</u> <u>al</u>.; from <u>Illustrated Human Embryology (Volume 3)</u>, (1974).

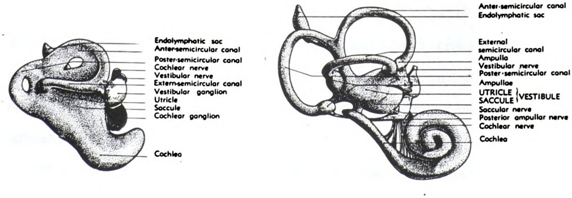


Figure 4. Embryologic development of the cochlea and vestibular system. Used with permission by Springer Verlag Publishing and authors Tuchmann-Dupliessis <u>et</u> <u>al</u>.; from <u>Illustrated Human Anatomy (volume 3)</u>, (1974).

Anatomy and Embryology of the Vestibular System

The vestibular system is made up of three anatomic structures that function in positional orientation, balance and coordinated locomotion. Within the structures, there are sensory neuroepithelium and support cells that transduce the mechanical energy (from gravitational, positional or locomotor changes) to electrical energy which is then delivered to the higher cortical centers for interpretation and utilization.

The sensory cells are called "hair cells" due to the presence of stereocilia and kinocilia on the top surface of the cells. The stereocilia are embedded in a layer which exaggerates the movement and accentuates the changes that occur. When the stereocilia bend due to the physical movement, the environment within the cell changes initiating the transduction of energy.

The three parts of the vestibular system are the semicircular canals, the utricle and the saccule. Each of the individual parts functions separately. The utricle (macula) detects horizontal and gravitational changes, the saccule (macula) vertical and gravitational changes and the semicircular canals (ampullae) rotational and angular changes.

The sensory cells of the utricle and saccule are located in a structure called the macula; the sensory cells and support cells of both the saccule and the utricle are covered by the otolithic membrane. This membrane is gelatinous in nature and has a meshwork of calcium carbonate crystals. The function of the crystalline layer is probably to help accentuate the bending of the stereocilia and transduction of the mechanical energy.

In each ampullae of the semicircular canals there is a transverse ridge (called the cristae) which is covered with hair cells and support cells. The stereocilia and kinocilia are embedded in a gelatinous mass called the cupula which also helps to accentuate sensitivity of the hair cells by moving the entire "hillock" within the fluid environment. All of the sensory parts of the vestibular system are contained within a fluid called endolymph.

Initially the bony labyrinth is formed from a cartilaginous framework which eventually ossifies; the semicircular canals are the last structures to calcify. As the framework forms, the ampullae "grow" at the end of each semicircular canal. The membranous labyrinth forms inside the osseous structure from the ectodermal tissue. The sensory and support epithelium then develop. Although there are two types of sensory hair cells in the vestibular system, type 1 is phylogenetically older than type 2. Type 1 cells are found primarily on the crest of the cristae while type 2 cells are found on the sides. Both the flask-shaped type 1 and the cylindrical-shaped type 2 cells are found in the macula sacculi and the macula utriculi.

Anatomy and Embryology of the Cochlea

The cochlea arises from both ectoderm and mesoderm by the lengthening of the inferior portion of the inner ear. The bony labyrinth forms both the otic capsule (outer covering of the cochlea) and the modiolus (the inner center bony support of the cochlea). The membranous structures form from the ectoderm inside the osseous labyrinth and make three distinct compartments: the scala vestibuli, scala media (cochlear duct) and scala tympani.

The cochlea lengthens from the apex where mitotic activity first begins. Basal mitotic activity follows the apical with cellular differentiation occurring first at the basal end. The oldest undifferentiated cells are located in the apex of the cochlea.

The scala vestibuli and scala tympani connect with each other at the apex of the cochlea or the helicotrema. Both chambers are filled with perilymphatic fluid. The innermost compartment, the scala media or the cochlear duct, does not communicate with either of the other chambers and contains endolymphatic fluid (Figure 5). The basal end of the cochlear duct terminates in the ductus reuniens which is a canal which joins with the saccule of the vestibular system. The apex of the cochlear duct ends in the last turn of the spiraled cochlea.

The cochlear duct is formed by distinct boundaries between the scala vestibuli and the scala tympani. Reissner's membrane lies between the scala vestibuli and the scala media. It is two cells thick and divides the perilymphatic space from the endolymphatic space. Reissner's membrane runs from the lateral wall medially where it attaches just above the spiral limbus. The basilar membrane separates the scala media from the scala tympani and is divided into two distinct parts. The first is the spiral osseous lamina which is a bony shelf on the medial side extending from the modiolus. The second part is a collagenous continuation which finally attaches to the spiral ligament on the lateral wall of the cochlear duct. The organ of Corti is located on the basilar membrane.

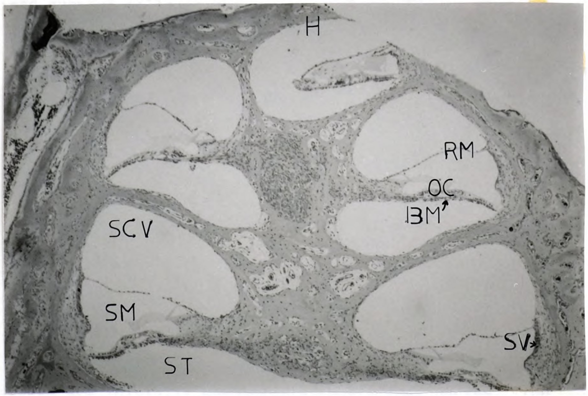


Figure 5. Mink mid-modiolar cochlear section. (ScV - scala vestibuli, SM - scala media, ST - scala tympani, RM - Reissner's membrane, OC - organ of Corti, BM - basilar membrane, SV - stria vascularis, H - helicotrema. 40 x, 1% toluidine blue.)

The stria vascularis lines the lateral wall of the cochlear duct overlying the spiral ligament. There is no basement membrane separating the stria vascularis and the spiral ligament. The stria vascularis contains many blood vessels and three primary types of cells:

marginal cells - innermost cells lining the cochlear duct; probable site of endolymphatic fluid production

intermediate cells - located between the marginal and basal cells; probably are melanocytes

basal cells - located most laterally.

The cochlear duct is the site of the sensory hair cells and support cells in the organ of Corti (Figure 6). Embryologically the differentiation of the support and sensory cells occurs at about the same time. The hair cells differentiate into two types - first the inner hair cells followed by the outer hair cells. Both the inner and outer hair cells initially contain many stereocilia of which some are lost during development. Both cell types develop a single kinocilium, but it is also lost during maturation and is not present in most species after birth.

The inner hair cells (IHC) develop in a single row along the entire cochlear duct. Each cell has approximately 60 stereocilia aligned in a straight row. The inner hair cells are initially connected with afferent nerves. The outer hair cells (OHC) contain three rows of stereocilia. Mature OHC have approximately 75 to 100 stereocilia. The rows of stereocilia are arranged in a "W" configuration in most mammals. Most of the stereocilia of the OHC are embedded in a gelatinous mass called the tectorial membrane. The tectorial membrane extends from the spiral limbus and overlies the OHC.

There are several types of support cells in the cochlear duct with many anatomical and functional differences including :

Deitter's cells - initially are in close connection with the OHC, but as development takes place, the connections dissipate and the Deitter's cells provide primarily structural support for the OHC

Outer and inner phalangeal cells - located between the inner hair cells providing them with structural support

Outer and inner pillar cells - as the organ of Corti matures, the pillar cells "pull" apart and divide the rows of the inner and outer hair cells

Boettcher cells - lie just under the Deitter's cells on the basilar membrane

Henson cells - lie extremely lateral on the basilar membrane and are the least differentiated of any of the support cells.

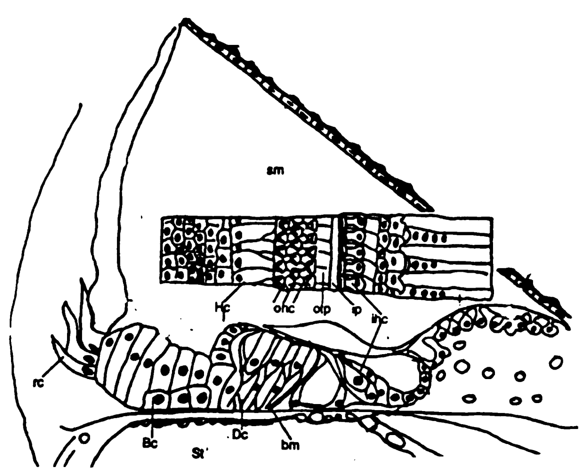


Figure 6. Cells of the organ of Corti. Used with permission of J.A. Render (1991). (Hc : Henson cells; Bc : Boettcher cells; OHC : outer hair cells; OTP : outer pillar cells; IP : inner pillar cells; IHC : inner hair cells; Dc : Deitter's cells)

The function of the cochlea is the transduction of the sound waves' mechanical energy into electrical energy. This is done primarily by the inner hair cells with possible mechanical amplification and attenuation from the outer hair cells. The sensory cells are stimulated by the movement of the stereocilia, as well as the vibration of the basilar membrane moving along the cochlear duct.

The cochlear fluids are necessary for transfer of sound waves to the sensory components in the cochlea. As previously mentioned, there are two types of fluids - endolymph and perilymph. The endolymph is similar to intracellular fluid but is contained within the cochlear duct. It is most likely produced by the marginal cells in the stria vascularis. The fluid bathes and provides nutritional support for the cells in the organ of Corti. It has a high positive endocochlear potential of approximately +80 millivolts. It provides potassium ions to be transported to the hair cells to allow for depolarization and eventual release of neurotransmitters to the afferent nerves. The perilymphatic fluid is similar to cerebrospinal fluid. It is contained within the scalas tympani and vestibuli and may be a source of potassium ions for the strial cells.

Anatomy of the Auditory Neural Pathway

The cochlear neural supply consists of both afferent and efferent nerves. The afferent supply enters the cochlea after originating in the neural crest. It then "grows" up the modiolus and outward from the spiral ganglion where it stretches radially through the channels of the immature habenula perforata in the spiral osseous lamina. The afferent nerves then grow further outward in the basilar membrane and upward toward the inner hair cells and eventually encounter the outer hair cells. After the afferent supply is well established, the efferent nerves grow outward from the crossed olivary bundles (ganglion) into the cochlea.

The afferent neural supply undergoes changes which result in 95% of the afferent supply innervating the inner hair cells and only 5% supplying the outer hair cells. The inner hair cells are furnished with many afferent nerves. One afferent neuron will form multiple branches supplying many outer hair cells. The efferent neurons synapse directly on the membrane of the outer hair cells and on the afferent dendritic processes leading from the inner hair cells.

The spiral ganglion is the site of bipolar neurons that receive afferent innervation from the cochlear duct and then transmit the impulse to the cochlear nerve and the central nervous system. Both type 1 and 2 neurons are found in the spiral ganglion. They are differentiated by the presence of myelin and their numbers within the ganglion. Type 1 neurons have multilaminar layers of myelin and make up about 95% of the population in the spiral ganglion. They are the neural supply for the inner hair cells. Type 2 neurons have little to no myelin and make up only 5% of the ganglion's population. After receiving the "electrical message" from the stimulated cochlear hair cells, an action potential is generated and transferred from the periphery up the ascending auditory neural pathway.

The auditory neural pathway continues from the hair cells, via the afferent bipolar neurons, through Rosenthal's canal to the spiral ganglion. Eventually, all the neural fibers join to form the cochleovestibular nerve (eighth cranial nerve) which continues to the brainstem and onto cortical centers where psychoacoustics (or the actual perception of hearing) takes place. Moller (1985) described the parts of the auditory pathway as follows (Figure 7) :

1. eighth cranial nerve
2. cochlear nuclei - dorsal and ventral (medulla)
3. superior olivary complex (pons)
4. lateral lemniscus (pons)
5. inferior colliculus (midbrain)
6. medial geniculate (thalamus)
7. auditory radiations (thalomocortical regions)
8. auditory cortex.

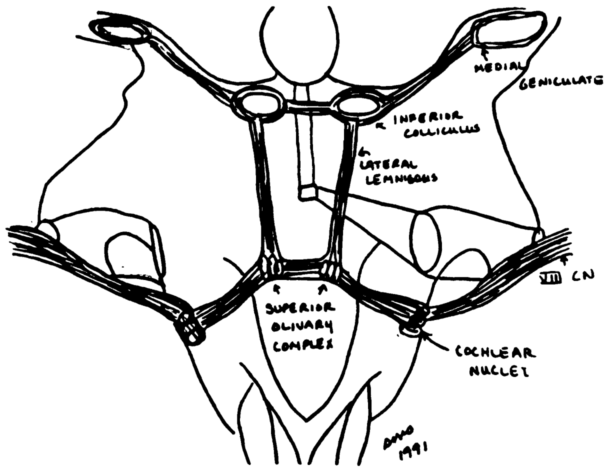


Figure 7. Parts of the auditory neural pathway.

Evaluation of the Auditory System: Introduction

The purpose of evaluating the auditory system is to detect hearing loss, deafness or anomalies within the vestibular system. Both the cochlea and the vestibular system can be assessed using behavioral and/or electrophysiologic methods to detect partial or complete loss of function. Hearing loss is often described as conductive, sensorineural or mixed based on the location or type of anomaly.

Conductive deafness is described as a failure of transmission of sound waves from the environment through the outer and middle ear to the sensory component of the cochlea. Sensorineural deafness can occur even though the sound transmission may be successful from the environment through the outer and middle ears. The sensory cells may be unable to transduce the mechanical energy to electrical energy. This type of deafness may also occur if the electrical energy is not able to be transmitted to the higher auditory neural centers. A combination of both sensorineural and conductive anomalies may occur and is referred to as mixed hearing loss.

Behavioral Assessment of the Auditory System

Behavioral assessment of the auditory system in animals is fairly easily completed by observation of the animal following acoustic stimulation. The animal may respond in many ways: by turning toward the source, by acknowledging presence of the source via a startle response or by the Preyer's reflex which is an involuntary twitch of the pinna following the sound stimulus. It is difficult to differentiate unilateral from bilateral hearing loss as animals acclimate to loss in one ear and almost imperceptibly, respond to sound. It is also difficult to determine the degree of hearing loss.

Electrophysiologic Assessment of the Auditory System

There are many methods used to evaluate the functions of the auditory system. Several texts by Moore (1983), Glasscock *et al*. (1987), and Glattke (1983) describe various methods used for physiologic examination of the auditory system. For assessment of the function of sound conduction through the outer and middle ear, impedance audiometry is used. To assess cochlear function there are many tests, varying in specificity and invasiveness. Beginning with fairly invasive methods, electrocochleography can be used to assess the cochlea itself. Using acoustic stimulation, reference electrodes are placed on the mastoid and an active electrode is placed within the middle ear or the external auditory meatus. Stimulus dependent potentials are generated as they occur following sound stimulation.

There are two main types of electrical potentials generated in the cochlea: cochlear microphonics and summating potentials. The cochlear microphonics (CM) potential is an alternating current (AC) potential that follows the waveform of the stimulating sound. The CM is generated primarily by the OHC with little to no time delay after the stimulus begins and ends. Clinical application of the CM may be limited as it records primarily from the hair cells closest to the electrode.

The summating potential (SP) is a continuous direct current (DC) response generated by both the OHC and IHC. It usually is recorded as a small negative baseline shift that lasts as long as the acoustic stimulus. As it is influenced by the status of hair cells, sustained SP's may indicate cochlear lesions.

There are other types of electrophysiologic methods which can be used to assess the cochlea and the ascending neural pathways. Many of them include invasive measures requiring electrode placement on the eighth nerve or directly in the auditory neural pathway. One non-invasive method routinely used involves using far-field recording to assess the integrity of both the cochlea and the neural pathway. It involves acoustic stimulation similar to that used in electrocochleography, but records from electrodes placed either intradermally or on the surface of the body. The technique utilizes recording of neural responses from the brainstem following acoustic stimulation. The neural responses vary in latency or response time in milliseconds (msec) from fast (<10 msec), middle (10 to 50 msec) and late (over 50 msec). The auditory brainstem (evoked) response (ABR) occurs in the "fast" phase.

Usually a minimum of three electrodes are placed on the head of the animal with one (positive or active) at the vertex, one (negative or reference) at the ipsilateral mastoid and one ground electrode somewhere else on the body. The acoustic stimulation can vary in intensity and frequency; either a click (usually a broadband frequency) or a pure tone pip can be delivered by the instrumentation at a set intensity level. Following the sound, a series of peaks (or waves) are generated by the cochlea and sites along the ascending auditory pathway. They are actually an averaged collection of a composite of neuronal activity. The instrumentation has the capacity to deliver specific acoustic stimulation, collect and amplify the evoked responses over the ongoing electroencephalographic and electromyographic activity, and average all the responses into one composite wave formation.

With variation in the frequency stimulus, either part or all of the frequency-specific cochlear duct can be stimulated. The area of focus of the ABR may be varied to test for either threshold (level of hearing loss) or the site of a lesion. It is important to remember though, that the ABR tests only the integrity of the cochlea and the neural pathway. A failure to evoke an ABR may not only mean damage to the cochlea, it may also indicate failure of the sound waves to get into the ear as in conductive deafness due to malformations or even cerumen impaction, or problems within the neural pathways (as in demyelinating diseases or auditory neuromas).

General Concepts of Cochleosaccular Deafness

There are many articles in the literature which describe various aspects of genetic deafness, including type of effect, genes responsible for the effect, species involved and the similarities and application of those species to genetic deafness found in humans. Steel and Bock (1985) described four types of inner ear defects; (1) morphogenetic, (2) neuroepithelial, (3) cochleosaccular and (4) central. Schuknecht _et al_. (1977) identified three different origins of cochleosaccular disease in humans and other mammals resulting from inherited anomalies, acquired changes (viral initiated) or aging changes (presbyacusis). Based on their earlier work which described the embryologic development of the inner ear, Schuknecht _et al_. concluded that the cochlea and saccule were phylogenetically younger, and so, were probably more predisposed to metabolic changes. Schuknecht et al. described the histologic changes that occurred in both the cochlea and the saccular region of the vestibular system. Their work showed that the other parts of the vestibular system remain normal and unchanged.

Because of the common occurrence of inherited pigmentation anomalies often accompanied by deafness or hearing loss, it was often concluded that the possible origin of cochleosaccular degeneration was some kind of abnormality involving melanocytes in the inner ear. The presence of melanocytes in the inner ear's stria vascularis has been described by Hilding and Ginzberg (1977). They investigated the embryogenic movement of melanocytes from the spiral ligament to the position of the intermediate cells in the stria vascularis. As these cells contained tyrosinase which is necessary for melanin formation, it was possible to trace the migration of melanocytes in fetal rats using immunohistochemical techniques (Dopa positive reactions). Hilding and Ginzberg found that in early fetal rats, the marginal cells were bordered by the endolymphatic space and a basal lamina. They were unable to trace the migration of melanocytes from the neural crest to the spiral ligament, but found as the feti aged, melanocytes (defined by the presence of premelanosomes and melanin granules) moved from the spiral ligament to the basal lamina. The melanocytes "inserted" processes between the marginal cells with an eventual increase in the interdigitation of the melanocytes and the marginal cells. The basal lamina became more divided and eventually disappeared. The end result was the mature stria vascularis characterized by the marginal cells, intermediate cells (which they termed as melanocytes) and the basal cells.

The marginal cells were found to be darker in appearance and contained large amounts of organelles. The intermediate cells contained fewer organelles but had pigment granules at all stages of maturation and had processes that were commonly in close contact with capillaries in the stria vascularis. The basal cells had the fewest organelles and formed a flat sheet that separated the marginal and intermediate cells from the spiral ligament.

Hilding and Ginzberg (1977) described the melanocytes in the stria vascularis (intermediate cells) as closely resembling melanocytes found in the skin and hair follicles. When examining the stria vascularis of albino animals, Hilding and Ginzberg found that the intermediate cells had small, non-pigmented crystalline organelles which were similar to those found in albino melanocytes in skin and hair follicles. They felt that the similarities between the normal and albino intermediate cells and the normal and albino melanocytes confirmed their hypothesis that the albino intermediate cells were of the same embryonic origin.

LaFerriere _et al_.(1974) investigated the relationship of melanocytes and the microvasculature of the vestibular labyrinth. Using histochemical methods, they compared the concentration of melanocytes in the inner ear with the degree of pigmentation of human skin. Melanocytes are found diffusely spread throughout the human inner ear, including the cochlea (modiolus, osseous spiral lamina, stria vascularis and Reissner's membrane) and the vestibular system (saccule wall, utricle and ampullae). LaFerriere et al. did not find melanocytes in the saccular wall in pigmented guinea pigs, but did find them in concentrated pigmented areas in the utricular wall of the vestibular system. The authors demonstrated the close anatomic relationship of melanocytes and the auditory capillaries, but were unable to determine what it meant physiologically. Hilding and Ginzberg (1977) concluded most succinctly that the melanocytes (intermediate cells) appeared to influence the development of the stria vascularis and made up a major portion of the strial tissue in the adult animal. A lack of melanocytes would probably result in a loss of strial function and potential loss of cochlear function. Whether the absence of melanocytes may result from a failure of the melanocytes to move from the neural crest to the stria vascularis, or from incomplete or arrested development, has not been determined.

Schrott and Spoendlin (1987) gave a comprehensive review of melanin and its interaction with the auditory system, but concluded that the function of melanin has not yet been clearly defined. They stated that melanin is produced by melanocytes and is responsible for pigmentation of the body, except the retina and ciliary body. The melanocytes (as well as a number of other cell types) are formed in the neural crest and are believed to migrate to many parts of the body. Schrott and Spoendlin reported on the possibility of some components of the stria vascularis also arising from the neural crest and not just the placodal ectoderm as originally believed. In order to confirm the origin of the specific layers of the stria vascularis, they used immunofluorescence and histochemical techniques to verify the presence of either keratin (found in cells of epithelial origin) or vimentin (found in cells of mesenchymal origin). These investigators found that the marginal cells of the stria vascularis were of epithelial origin and the intermediate cells were mesenchymal, giving evidence that the cells are derived from the neural crest.

To also verify that the intermediate cells were actually melanocytes, Schrott and Spoendlin (1987) used autoradiography to demonstrate the presence of tyrosinase which is found exclusively in melanin and its precursors. They found positive reactions in the intermediate cells of normally pigmented (and normally hearing) mice, guinea pigs, cats and humans. No positive reactions were found in the marginal cells or other parts of the cochlea. In white spotting (abnormally pigmented) animals where "the formation of melanocytes in the neural crest or the migration to their target organs is disturbed", no melanocytes were found in the stria vascularis.

Introduction to Cochleosaccular Degeneration

Various species have been described with an inherited pigment-associated anomaly which includes hearing loss or deafness. Auditory problems in humans with Waardenburg's syndrome have been described throughout the scientific literature as well as the popular press (Palmer, 1989). The following is a brief literature review of several species in which pigmented-associated auditory anomalies seem to be similar.

Cochleosaccular Degeneration in the Human Being

Waardenburg was the first person to identify a syndrome which he described as a combination of facial abnormalities and deafmutism found among people incarcerated in European institutions. In 1951, he defined and published six characteristics which became part of the "Waardenburg syndrome". They included the following:

1. dystopia canthorum (lateral displacement of the medial canthi)
2. broad root of the nose (bridge)
3. hypertrichosis of the eyebrows (dense, bushy eyebrows which often grow together)
4. partial albinism or leucismus pilorum (white forelock)
5. heterochromia iridum (varied iridal color)
6. congenital (partial or complete) deafness.

He reported that people exhibiting the syndrome often had several of the characteristics, but rarely demonstrated all of them. After studying the mode of inheritance among several families, Waardenburg concluded that the syndrome probably first occurred as a mutation and then was transmitted as a dominant trait. He found one family in which two deafmute parents (showing some characteristics listed above) did produce normal offspring, but that the majority of the families with the syndrome inherited it as a dominant trait. Because of the combination of several traits which occurred in a number of people, Waardenburg believed that the syndrome was inherited by an autosomal dominant mode, but concluded that the possibility existed that more than one gene played a role in the inheritance.

Waardenburg (1951) reported that dystopia canthorum was a major trait which was commonly displayed among people with the syndrome. He estimated that the penetrance for deafness was only about 20%, but did not account for unilateral deafness or hearing loss.

Aasved (1962) reviewed the six characteristics of the autosomal dominant syndrome Waardenburg described and applied it to a Norwegian family. Waardenburg syndrome was seen in three different generations. Dystopia canthorum was observed in all the affected family members. Aasved reported that although dystopia canthorum was observed, most of the family members had normal distances between their pupils and the lateral canthi. Those who were "stricken" with deafness usually lost their hearing between 12 and 30 years of age.

McDonald and Harrison (1965) published a similar report of their search for families with Waardenburg syndrome. By examining children in schools for the deaf in South Africa, they were able to identify only four children with some features described by Waardenburg. McDonald and Harrison decided that a positive diagnosis could be made on the basis of a person having at least two of the six characteristics described in Waardenburg's original paper. Their report was essentially a review of each of the six features as described by other authors.

In 1971, Arias described three categories of Waardenburg syndrome in the human. Type 1 was described as occurring with dystopia canthorum; type 2 occurred without dystopia canthorum, and type 3 called, pseudo-Waardenburg's, was characterized as inherited unilateral ptosis (drooping upper eyelids) without dystopia. Arias concluded that type 2 was often associated with deafness and piebaldism or partial albinism. He found that the pigmentary anomaly only involved the iris and skin and that hyperpigmented borders were often seen around hypopigmented areas in these people. He concluded that it may be due to an increase in melanosomes in the border region.

Arias (1971) set forth three hypotheses for the abnormal pigmentation: (1) the presence of partially undifferentiated melanocytes that are unable to induce a normal response by the target tissue; (2) a defect in the migration of the melanoblasts from the neural crest to the intended area; or (3) local factors which prevent the correct differentiation of the already abnormal melanocytes. He felt that the first hypothesis was discountable and that the true answer existed in either the second or third, because the melanocyte could "turn on" a particular genetic message that could coincide with abnormal local conditions.

In the same year, Penchaszadeh and Char (1971) published a paper describing Waardenburg syndrome in a Negro family. The child of phenotypically-normal parents was bilaterally deaf but had a normal tympanic membrane and external ears. Other "classic" Waardenburg features were seen including the white forelock of hair, white eyelashes and eyebrows and blue irises.

Hageman and Delleman (1977) surveyed over 1000 Waardenburg syndrome patients described in the literature and attempted to evaluate the penetrance of deafness as part of the Waardenburg syndrome. By using the three classifications of Waardenburg's syndrome, they were only able to locate descriptions of two people who matched the third classification. They used only data from 276 people for type 1 and 159 for type 2 due to limitations of information along family lines. For their study, they also required that a person had to exhibit two of the three major characteristics (dystopia canthorum, pigmentary changes and deafness) in order to be classified as a person with Waardenburg's syndrome. Hageman and Delleman reported that the penetrance of bilateral deafness was 28% for type 1 and

53% for type 2 Waardenburg syndrome, while unilateral deafness was 8% for type 1 and 4% for type 2.

Hageman and Dellman (1977) also addressed the necessity of genetic counseling for families affected with Waardenburg's syndrome. As both types of the syndrome are autosomal dominant, they concluded the chances of deafness for a child from a type 1 parent is 1 in 8 while the chances of deafness for a child of a type 2 parent is 1 in 4.

Balkany and Pashley (1986) reported that 20% of humans with Waardenburg's syndrome had hearing loss. They found a wide range of clinical findings. They reported that (1) the syndrome is autosomal dominant, (2) that the offspring of an affected parent has a 50% chance of inheriting the gene, (3) that the severity of the condition may vary and (4) that the child has a 10% chance of suffering from hearing loss. Balkany and Pashley postulated that the problems may result from ectodermal anomalies which occur during embryogenesis, causing pigmentary alterations resulting in defective myelinization.

Hildesheimer _et_ _al_. (1989) studied a population of Waardenburg syndrome people who underwent both hearing and vestibular examinations. Using traditional testing methods on the middle ear and the auditory brainstem evoked response on the inner ear (cochlea) and electronystagmograms on the vestibular system, they reported that 51% of the people had bilateral, symmetrical sensorineural hearing loss. Using the same criteria as previously described to classify the different types of Waardenburg syndrome (type 1 being the more common classic autosomal dominant form with dystopia canthorum, and type 2 being much less common with no dystopia but a white forelock), the authors found more cochlear involvement in type 2. Type 2 people demonstrated progressive, bilateral, moderately severe sensorineural hearing loss. Hildescheimer and co-workers described two different types of audiograms in the population; one showed normal hearing in low frequencies and a sharp drop in higher frequencies while the second had a gradual and progressive decline towards the higher frequencies.

In 1990, Newton examined families with Waardenburg syndrome using auditory brainstem evoked response (47 with type 1 and 32 with type 2) and found that those with bilateral sensorineural hearing loss outnumbered those with unilateral sensorineural hearing loss. The number of people with bilateral hearing loss was greatest in those with type 2, as 88% of type 2 had bilateral hearing loss compared to 69% for type 1. Newton's principle conclusion seemed to be that the percentage of people with sensorineural hearing loss was higher than had been previously reported but that the hearing loss was extremely variable between families in penetrance and expressivity.

Animal Models of Cochleosaccular Degeneration

A comparison of deafness among animal models with hereditary deafness was made by Suga and Hattler in 1970. They compared dogs (Dalmatian) and cats (white in color) using behavioral audiometry, electrophysiology and histology. The behavioral audiometry consisted of an acoustic stimulus delivered from a loudspeaker (using a variety of pure tones and intensities) and observation of behavioral response.

Electrophysiologic evaluation included invasive recordings from the round window (cochlear microphonics, summating potential, auditory nerve action potential) and from the cochlear duct (endocochlear direct current potential). Each of the species was examined individually with some variety in the results found.

Among the white cats, several demonstrated profound deafness and lack of melanin pigment within the inner ear. Melanin pigment was seen in the normal (control) cats in the stria vascularis and near part of the vestibular epithelium. Of the three deaf Dalmatians examined, no melanin pigment was found in the inner ear. As indicated by these results and conclusions by other authors (Hilding and Ginzberg, 1977); Mosher et al. 1979), the absence of strial melanocytes could contribute to dysfunction of the stria vascularis.

Foss and Flottorp (1974) examined hereditary deafness by comparing the development of hearing (and vision) in a

number of species. They tested cats, rabbits, dogs and mink by placing them in front of a loudspeaker and stimulating them with various frequencies (using both pure tones and white noise) and intensities. They assessed a positive response by pinna or cochleo-orbital reflex or by some type of behavioral change. Foss and Flottorp used standard dark mink kits (that had an average gestational length of 46.3 days) and found that in mink, as well as other species tested, hearing begins earlier than vision. Hearing was first detected in the species examined at the following times: cat - 5 days, dog - 14 days, mink - 29 days postpartum, and the human being at 24 gestational weeks.

Several comments made by Foss and Flottorp (1974) were of interest, especially that the middle ear of the mink was virtually undeveloped even after audition occurred and that the sound intensity used as acoustic stimulation was probably not high enough to pass through the middle ear. They concluded that electrophysiologic testing would have been better than the loudspeakers as it would probably bypass the middle ear to eliminate a possible attenuating factor of the immature middle ear.

Foss and Flottorp also set forth a hypothesis of where hearing begins anatomically. Compared to other earlier hypothesis that stated that the basal portion of the cochlea was the first to begin functioning, they felt that the part of the cochlea that had the best blood supply was the first to respond in a "mature fashion" to acoustic stimulation.

This, they surmised, was probably genetically determined which guided the time and site of development of the cochlear vascular supply.

Cochleosaccular Degeneration in the Cat

For many decades it has been recognized that there was some relationship between blue-eyed white cats and deafness. From casual observances by lay people to the first scientific report by Darwin (1859), the connection between the pigment-associated deafness has been studied extensively. Brown et al. (1971) gave a comprehensive account of the anomaly in the cat and a brief description of some other animal models which exhibit the pigment-associated deafness.

Brown et al.(1971) conducted a study to investigate the genetics, physiology and development of a cat animal model which closely resembled Waardenburg syndrome in the human. The cats used were completely white except for an occasional pigmented spot on the forehead (which commonly faded early in life) or that had "high spotting" which is identified by patchy pigmented spots over the body which often grayed early. The cats also demonstrated blue irises either unilaterally or bilaterally. The animals were either normal, had unilateral or bilateral hearing loss or were profoundly deaf. The authors described the genetics of the syndrome as very complex. Correlation of pigmentary changes and degree of hearing loss (if any) was also difficult to determine.

The histologic abnormalities described in the blue-eyed white cat were very similar among researchers (Brown _et al_., 1971; Mair, 1973; Bergsma and Brown, 1973; Rebillard _et al_., 1976; Pujol _et al_., 1977; Elverland and Mair, 1980), regardless of the time of the investigation and the methods of collection or processing used. The following is a brief synopsis of the morphologic changes and observations made in blue-eyed white cats :

1. no anomalies found in the outer or middle ear
2. changes confined to the membranous labyrinth and the spiral ganglion of the inner ear
3. changes began as early as 8 days of age and progressed following a regular pattern which parallels the postnatal maturation sequence of the normal cochlea (beginning first at the upper half of the basal coil at the site of the youngest cells and then spreading towards the apex where the older cells are located)
4. continued as progressive loss throughout the life of the animal
5. initial signs were a progressive collapse of Reissner's membrane which eventually completely contacts the spiral prominence, organ of Corti and the stria vascularis
6. adhesions formed between Reissner's membrane and the underlying structures
7. as Reissner's membrane collapsed, loss of the cochlear duct occurred
8. changes to the organ of Corti were first seen about the same time that Reissner's membrane collapsed; initial anomalies were first noted in the pillar and hair cells
9. the pillar cells collapsed and leaned toward the modiolus with eventual loss of the lumen of the tunnel of Corti
10. the sensory cells matured first and then degenerated following the same order of development
11. the order of degeneration was: 3rd row, 2nd row, 1st row outer hair cells, then finally the inner hair cells

12. the preliminary changes were typical degenerative changes resulting in the hair cells leaning in toward the modiolus
13. the hair cells degenerated until they were virtually unrecognizable
14. the support cells (Deiter's and Hensen) eventually degenerated following the loss of the hair cells
15. eventually the entire organ of Corti was replaced by a layer of simple, undifferentiated cells
16. the tectorial membrane was also abnormal as it initially contacted Reissner's membrane as it moved down and collapsed
17. the tectorial membrane retracted up towards the inner sulcus eventually pulling towards the modiolus and resulting with a globular appearance
18. the stria vascularis appeared normal (when compared to control kittens) at 8 days of age, but by day 12 it began to visibly thin out and became paler (especially in the lower cochlear turns)
19. the stria vascularis eventually became thinner with a progressive obliteration of the vascular spaces.
20. changes within the spiral ganglion included a reduction in the number of neurons (located within Rosenthal's canal in the modiolus) and a decreased neuronal population as the age of the animals increased
21. degeneration within the cochlear nerve fibers
22. the neural degeneration was usually first noticed in the upper basal turn and progressed following the same order of degeneration noted in the cochlea
23. the neural changes occurred long after the cochlear changes first occurred
24. the only vestibular changes observed involved the saccule where the development of the degeneration increased as the animal aged and were seen only in deaf animals
25. the first lesion noted was a decrease in the size of the lumen; the saccule characterized by the collapse of the saccular wall toward the otoconial layer in the macula
26. following a rapidly progressive collapse, the sensory cells began to lose stereocilia
27. the otoconial layer thickened (with the saccular wall still lying on it) and the sensory cells then began to deteriorate and decrease in number
28. loss of the supporting cells resulting in final obliteration of the saccular lumen and the macula sacculi
29. no pathological changes were noted in the other parts of the vestibular system (utricle and semicircular canals).

Because all the tissues affected in the syndrome arise from the neural crest, Brown et al. (1971) suggested that the possible cause for the cochleosaccular degeneration in the cat was due to some anomaly within or resulting from the neural crest. The authors stated that, "the neural crest forms lateral to the closing neural tube in early embryogenesis. The cranial component supplies a facial mesenchyme that develops into the membrane bones of the face and palate as well as local pigment and general sensory components of the cranial nerves". Brown et al. proposed two hypotheses to explain the neural crest defect - (1) either there is a partial failure of the melanocytes from the neural crest to reach their final destination in the stria vascularis? or (2) there could be a limited failure of neural crest interaction between the epithelial and mesodermal parts of the auditory system.

Bergsma and Brown (1973) used conditioned behavioral responses and electrophysiologic testing to evaluate hearing in a population of white cats. (The method of electrophysiologic testing was not described.) They reported that just less than half of the white cats born into their colony were deaf and that those examined histologically demonstrated features similar to those described previously. It was also reported that the deaf white cats were most commonly blue-eyed. Bergsma and Brown concluded that a defect in neural crest migration or in neural crest cell interactions could account for the correlation of deafness with the pigmentary conditions observed.

Pujol et al. (1976) described the presence of unmyelinated neural fibers in the basal lamina spiralis of a white kitten when compared to a large number of myelinated fibers in a normal kitten of equal age. Continuing with this research, Rebilliard et al. (1977) investigated how the presence of unmyelinated fibers correlated with the loss of hearing. They found that when stimulating the cochlear nuclei, they were able to elicit an electrical response from auditory centers within the cortex. They were not sure what the finding meant except that such a lesion could be found in a hereditary neural crest defect.

Later, Mair (1979) addressed some of the questions posed by Pujol et al. (1976) and Rebillard et al. (1977) by histologically examining the higher auditory neural centers. He found little evidence to indicate that the changes were degenerative, but observed that they were caused by atrophy. Elverland and Mair (1980) and Schwartz and Higa (1982) studied the spiral ganglion in the cat and found myelin degeneration occurred before neuronal loss due to the loss of the organ of Corti in the cochlea. Elverland and Mair found that demyelination occurred initially in the type 1 ganglion cells, followed by a transformation stage. The changes were first observed in 75-day-old white kittens where there was an increase in the concentration of unmyelinated cells that contained large numbers of neurofilaments and loss of rough endoplasmic reticulum. Elverland and Mair labelled the cells as type 3 ganglion cells that were a transitional stage in the development into type 2 ganglion cells. In older cats, the spiral ganglion appeared emptier as the neurons degenerated, resulting in an increase in extracellular interstitial space. The neuronal degeneration and loss was not equally distributed throughout the spiral ganglion. The extreme ends of Rosenthal's canal showed less damage evidenced by the presence of more normal neurons. The loss of neurons was greatest in the upper basal and second turns of the cochlea. Whatever the distribution, Elverland and Mair concluded that the loss was not primary in nature, but instead was due to damage to the peripheral sensory component with a resulting "disuse" atrophy of the auditory neural pathway.

Schwartz and Higa (1982) investigated the higher centers of the auditory neural pathways by examining the medial superior olivary nuclei (MSO) in the deaf white cat. They found that marked observable destruction of the spiral ganglion neurons occurred after lesions were noted in the medial superior olive. They observed a decrease in the number of synaptic terminals on the cells in the MSO of the deaf cats. These changes happened about the same time that the degeneration occurred in the organ of Corti. Changes were not seen in the spiral ganglion until months after the organ of Corti was altered. Schwartz and Higa concluded that these lesions were consistent "with the disuse hypothesis" and that the extent of changes in the spiral ganglion should not be used as a method for prediction of the integrity of the central auditory centers.

Cochleosaccular Degeneration in the Dog

Cochleosaccular degeneration in the dog has been primarily identified in animals with pigmentary patterns which are similar to the pigment-associated deafness found in humans with Waardenburg's syndrome and in blue-eyed white cats. The most common breed of dog with this condition is the Dalmatian.

As in the cat, there have been many descriptions of the histologic changes observed in dogs with cochleosaccular degeneration. Over the last six decades, the same basic features have been reported in the literature by several researchers, including Lurie (1948), Hudson and Ruben (1962), Anderson et al. (1968), Igarashi et al. (1972), Branis and Burda (1985) and Gerwitz (1991). Anderson et al. gave the most comprehensive description of the morphologic changes that progressed over time in the Dalmatian. Some of these changes are summarized below:

1. cochlear duct - collapse of Reissner's membrane; membrane almost adhered to stria vascularis and the organ of Corti involving primarily the basal and middle turns
2. organ of Corti - complete loss of hair cells in basal and middle turns; remnants of hair cells in the apical turn; collapse of Corti's tunnel; tectorial membrane collapsed and adhered to the limbus
3. stria vascularis - thin, sclerotic and a decrease in vascularization

4. spiral ganglion - marked reduction in ganglion cells; ganglion filled with fibrous connective tissue
5. slight decrease in the saccular lumen
6. rest of vestibular system normal.

Anderson et al. did not find many more changes utilizing electron microscopy, but reported the following ultrastructural changes :

1. outer hair cells - vacuolization and degeneration of the cytoplasm, degeneration of the mitochondria, reduced chromatin in the irregularly shaped nuclei
2. inner hair cells - fewer overall and earlier signs of degeneration
3. stria vascularis - poorly developed with few capillaries and marginal (dark) cells.

Degeneration of several types of cochlear support cells was described by Igarashi et al.(1972). They examined several breeds of deaf dogs with cochleosaccular defects (including the Border collie, Australian sheepdog and Shropshire terrier) and found that the inner and outer sulcal cells, Deiter's cells and Hensen's cells were severely degenerated while Claudius and Boettcher's cells were not affected.

Lurie (1948) reported a decrease in melanin pigment and blood supply in the stria vascularis of deaf collies and Dalmatians. The stria was described as a single layer of cells (probably marginal) with little to no capillaries lying beneath. Igarashi et al. (1972) cited similar lesions, but found no evidence of any anomalies in the spiral ligament. Johnsson and Hawkins (1973) studied four litters of Dalmatian dogs and found strial atrophy in the middle and apical turns and "fresh" outer hair cell degeneration in the same anatomic locations along the cochlear duct. They found four stages of cochlear degeneration described as (1) strial atrophy; (2) sagging and collapse of Reissner's membrane accompanied by the onset of OHC degeneration; (3) complete OHC degeneration followed by the onset of IHC degeneration; and (4) nerve degeneration. Strial atrophy was initially diffuse and began at the lower basal turn during the first postnatal week. Atrophy was indicated by thinning of the stria vascularis and narrowing of the strial capillaries that eventually disappeared leaving behind "a network of strands and 'ghosts' of vessels".

Hudson and Ruben (1962) and Igarashi et al. (1972) published descriptions of the changes in the tectorial membrane as appearing absent, unidentifiable or swollen, covered with a layer of epithelial cells and often inserted into the internal sulcus. Branis and Burda (1985) found the same changes, but also noted that occasionally they observed impressions of stereocilia from the OHC on the tectorial membrane in the middle turn. The IHC remained intact in the six-week-old puppies.

A time lag in the degeneration of the spiral ganglion was observed by Gerwitz (1991) who reported that neuronal degeneration in the brain occurred at the same time cochlear degeneration took place. Lesions in the higher auditory neural pathway were seen before loss of the spiral ganglion neurons became evident.

Changes that Lurie (1948) reported in the saccule of the vestibular system included partial or complete collapse of the lumen involving the free wall which lies opposite the oval window. The maculi sacculi often showed degeneration of the neuroepithelial cells. The saccule nerve also demonstrated signs of atrophy. Hudson and Ruben (1962) found no changes in the maculi sacculi or the saccule nerves from two eight-week-old Dalmatian puppies.

Igarashi et al. (1972) found two types of inner ear degeneration among the breeds of dogs they examined. Cochleosaccular degeneration was found in the Border collie and Australian sheepdog. The Shropshire terriers exhibited only cochlear degeneration. Histologically, the Shropshire terriers were only "abnormal" in terms of the organ of Corti which was either partially destroyed or completely replaced by undifferentiated epithelial cells. The cochlear duct was intact as Reissner's membrane was not sagging or collapsed. There appeared to be no changes in the stria vascularis or other parts of the cochlea or the vestibular system.

Although most of the cochleosaccular degeneration research has been conducted in the Dalmatian, there are several other canine breeds that have been reported to have genetic deafness. However, it is unknown whether the origin of loss is due to cochleosaccular pathology. The following is a partial list compiled by Johnston and Cox (1970), Erickson et al.(1978) and Strain (1991) of dogs which exhibit hereditary deafness :

Dalmatian	Australian shepherd
Bull terrier	Dogo Argentino
American foxhound	Boston terrier
Collie	Border collie
Scottish terrier	English setter
Fox terrier	English sheepdog
Australian heeler	Norwegian dunkerhound.

It has been demonstrated that the incidence of bilateral deafness in the Dalmatian is approximately 8% and of unilateral deafness about 22%. Anderson et al. (1968) tested 53 Dalmatians by using conditioned behavioral responses to various clicks and pure tones (and at varying intensities). They reported that five dogs were deaf (9.4%), 10 showed some residual hearing (possibly unilateral - 18.9%) and 38 were normal (71.7%). Gerwitz (1991) indicated that studies were in progress to define the gene(s) and the expressivity and penetrance of the gene(s) for deafness. Over twenty years ago, Anderson et al. (1968) described the condition as being caused by a dominant or a recessive autosomal gene that demonstrated varying expressivity.

Many methods have been used to assess hearing in the dog. As mentioned previously, Anderson et al. (1968) utilized conditioned responses which were learned by applying an electrical pulse at the same time acoustic stimuli were generated. Kay et al. (1984) used the ABR to test a group of dogs. Marshall (1986) used the same method to evaluate a group of Dalmatians and found that over 50% were either unilaterally or bilaterally deaf.

Cochleosaccular Degeneration in the Mouse

Schrott and Spoendlin (1987) published a paper on pigment-associated inner ear deafness in which they addressed specifically the black-eyed white mutant mouse (W/Wv) which was found to lose its hearing early in life. Using a histochemical approach (via dopa or tyrosine positive reactions indicating the presence of melanocytes), they examined both normal and affected mice and found that the black-eyed mutant mice lacked neural crest-derived melanocytes in the stria vascularis. The initial change observed was thinning in the stria vascularis in the mutant mice. Electron microscopy revealed that only the marginal and basal cell layers were present. The intermediate (melanocyte) layer was not present. As the mice aged, the stria vascularis became more atrophic and the outer hair cells began to degenerate in the basal turn. Eventual degeneration of the inner hair cells followed with ultimate loss of the entire organ of Corti. Much later, the spiral ganglionic neurons showed signs of degeneration.

Some of the viable dominant spotting mutant mice (Wv/Wv) studied by Steel et al. (1987) were found to have cochleosaccular degeneration. The mice were evaluated using Preyer's reflex, compound action potentials (collected from the eighth cranial nerve) and cochlear microphonics collected from the round window membrane. The Preyer's reflex appeared to be a poor indicator of "hearing". Almost 50% of the animals that showed a "good" Preyer reflex did not have a measurable endocochlear potential. They attempted to define the amount of strial dysfunction by the level of the endolymphatic potential in the viable dominant spotting mice. These animals have been found to maintain an open cochlear duct for a longer time period compared to other animals that also develop cochleosaccular degeneration. The homozygous mutants were easy to differentiate from heterozygous and normal littermates. By measuring the electrical potential within the endolymphatic fluid, Steel et al. were able to find measurable positive potentials in 25% of the homozygous mice examined. By inserting an electrode into the cochlear duct (by passing through the stria and later verified by microscopic examination), endocochlear potentials were not found in 75% of the mice. Fifteen percent were abnormally low and only 10% were near levels that had been established for normal control mice.

Histologic studies by Steel et al.(1987) revealed morphologic changes in the stria vascularis and the outer hair cells similar to those found in the black-eyed white mutant mouse. The stria appeared narrow compared to normals due to very thin marginal cells. The marginal cells were described as not having as many processes extending into the deeper layers of the stria vascularis and the marginal cells often appeared "to be clearly separated from the other layers". Abnormal regions of the stria in the affected mice were unpigmented, but the authors did not specifically mention the presence or absence of an intermediate cell layer. The presence of cysts in the region of Deiter's cells (support cells) in the organ of Corti was mentioned. Their presence of cysts was presumed to indicate fluid imbalance within the cochlea.

Asher and Friedman (1990) published a descriptive comparison of a hamster and three mouse strains that could be models for Waardenburg's syndrome in man. The homozygous hamster mutation (Wh - anophthalmic white) was described to have many morphologic and physiologic defects which caused the hamsters to be deaf, blind and white. The variations in pigmentation patterns in the hamsters were comparatively similar to the variety of pigmentation in humans with Waardenburg's syndrome. One of the strains of hamsters demonstrated significantly shorter response latencies (as compared to normal hamsters) when conducting auditory brainstem evoked responses.

The three strains of mice exhibited the same phenotypic variation of expression as seen in the human, dog (Dalmatian) and cat (blue-eyed white) which Asher and Friedman (199) felt could be explained by one of the following:

1. single locus with several mutant alleles
2. single locus with several mutant alleles each interacting with modifying genes
3. more than one locus with each having multiple mutant alleles which interact with modifying genes.

They also gave a brief description of the various classifications of Waardenburg's syndrome and described yet another type: (1) with dystopia canthorum; (2) without dystopia canthorum; (3) with upper limb abnormalities; (4) with pigmentary anomalies and megacolon (also called Waardenburg-Shah syndrome).

Among the 2,138 mutant alleles in the house mouse, the authors felt there were 178 which caused pigmentation anomalies. Of the 178, they identified nine which affected the inner ear. Of the nine, four were selected as being comparable to the four types described for Waardenburg's syndrome.

The "Ph" locus of the house mouse (actually two alleles on chromosome 5) causes patches of hypopigmentation and inner ear defects. Some of the patch heterozygotes show a facial phenotype that is similar to humans with Waardenburg's. The "s" mouse has inner ear defects and patches of hypopigmentation. The homozygous mutants also have megacolon, making them similar to humans with Waardenburg-Shah syndrome (type 4). The semidominant mutation Splotch (Sp) causes profound defects in the development of the neural crest. Homozygotes for the trait usually die *in utero*. Heterozygotes show defects which are very similar to Waardenburg syndrome type 3. The last model described was the "Mi (or)" mutation which is located on chromosome 6. The mutation was originally created by radiation and developed as a recessive lethal trait inducing reduced ocular pigmentation, lightly pigmented ears and white markings on the head and neck. Some of the heterozygotes phenotypically compare to Waardenburg syndrome type 1; others resemble type 2, as they exhibit no skeletal changes, but many more are deaf (as in the human type 2 where the penetrance for deafness is greater than in type 1).

As all of the locations of the mouse alleles have been found (chromosome number determined) and because of the similarities to several types of human Waardenburg's syndrome, the authors concluded that it may be possible to identify the location of the allele for Waardenburg syndrome in the human by relating it to the same location found in one of the four mouse models.

Foy et al. (1990) carried out that proposal. By locating the locus for the Sp mouse, the authors examined a similar location in the human. They were able to demonstrate a "close linkage" between type 1 Waardenburg syndrome and a locus on chromosome 2q37. The Sp mouse was originally identified by Deol (1980) as having a morphogenetic defect. However, the Sp mouse was found to have both morphogenetic and cochleosaccular defects that were dependent on the degree of neural crest failure. The authors concluded that it at the time of publication it was necessary to study other characteristics of Waardenburg's syndrome to confirm if the Sp mouse is really a suitable model.

Cochleosaccular Degeneration in the Mink

The first mention in the literature of the mink auditory system was found in a publication by Pocock (1921) describing the auditory bullae and cranial features of mink and other mustelids. Much later, Shackelford and Moore (1954) published information about the Hedlund white mink describing the mink's inability to respond to loud noises and the mother's failure to retrieve kits that cried after inadvertently leaving the nest box. They concluded that "all of these deviations from normal behavior suggest that the Hedlund white mink may be unable to hear". Saunders (1965) provided the first histologic description of the auditory system of mink, including both normal (brown) and Hedlund white mink. Although fixation techniques were relatively crude, Saunders was able to demonstrate histologic changes in the Hedlund white mink's cochleas. The six-month-old animals had pathologic changes of the cochlear duct with degeneration of the organ of Corti (including hair cells) and collapse of Reissner's membrane. Although autolytic changes were apparent in the normal brown mink, they did not have the lesions seen in the Hedlund mink. Saunders did not find any pathologic changes in the spiral ganglion, the cochlear nerve or the stria vascularis of the Hedlund white mink and thus, concluded that inadequate vascular supply was not the cause of the pathologic changes. Most probable, Saunders concluded, was that the changes in the organ of Corti, Reissner's membrane and the tectorial membrane were the cause for deafness in the Hedlund white mink. Saunders deduced that the changes in the organ of Corti's hair cells were recent and so, changes had not yet occurred in the spiral ganglion. He felt that the lesions found in the cochlea of the Hedlund white mink were similar to those in dogs, cats, mice and humans afflicted with hereditary congenital deafness.

Hilding et al. (1967) conducted an electron microscopic study of the Hedlund white mink inner ear. Examining newborn, two-week-old and adult Hedlund white mink and newborn, two- and three-week-old and adult "normal" mink, Hilding et al. described lesions similar to those found by Saunders in the white mink. The newborn mink were unresponsive to auditory stimuli, and microscopically, the cochlea appeared undeveloped. In the normal mink, Hilding et al. described behavioral responses to hand clapping by fourteen days of age while the inner ear still appeared histologically immature, as the tunnel of Corti was still closed and only afferent nerve endings were found. By three weeks of age, they found both afferent and efferent fibers crossing the opening tunnel of Corti. By this age, the mink hair cells' single kinocilium (which was present at birth) had disappeared. The cochleas of the normal adult animals had no morphologic features which were different from other mature mammalian cochleas. The spiral vessel under the organ of Corti which had been pronounced in the immature cochlea, was almost completely gone in older normal dark mink. The cochlear neural "supply" was intact and comparable to other species.

Hilding _et al_. (1967) reported that they were unable to elicit any kind of behavioral response to auditory stimuli in the Hedlund mink. Morphologically at birth, the cochleas of HWM kits showed no differences from the cochleas examined in the normal newborn kits. At two weeks of age, the kits showed an absence of mitochondria within the marginal cells lining the stria vascularis and a sagging Reissner's membrane. Both afferent and efferent neural fibers were in place in the two-week-old Hedlund kit. Other parts of the cochlea appeared normal when compared to similarly aged normal kits. However, in the aged Hedlund mink, profound differences were evident by the collapse of Reissner's membrane and loss of the cochlear duct (scala media). The authors reported that the hair cells appeared abnormal when examined with the electron microscope and that the basal hair cells showed more pathologic changes than the apical hair cells. They described the basal hair cells as being globular in shape with swollen mitochondria and nuclei.

Most remarkable were the changes in the stria vascularis of the adult Hedlund white mink. Very few mitochondria were seen, as well as strial atrophy and adherence to Reissner's membrane. Hilding _et al_.(1967) concluded that the collapse of the cochlear duct was due to decreased production of endolymphatic fluid because of the reduced number of mitochondria in the marginal cells. As Reissner's membrane collapsed, they deduced that the tectorial membrane was squeezed medially from the pressure within the scala vestibuli. Eventually the tectorial membrane curled up within the inner sulcus and left the upper surface of the organ of Corti exposed and in contact with Reissner's membrane.

Like Saunders (1962), Hilding et al. (1967) also compared the lesions found at the light microscopic level of the Hedlund mink to those found in Shaker-1 mice, waltzing guinea pigs, Dalmation dogs and blue-eyed white cats. However, upon examination using electron microscopy, the similarities between the neonatal Shaker-1 mouse and the Hedlund white kit were less evident, as the mouse did not show collapse of the cochlear duct but instead showed a pathologic organ of Corti, indicating changes more representative of neuroepithelial degeneration.

In 1969, Foss published an abstract describing a decrease in the number of capillaries within the stria vascularis of cochleas of Hedlund white mink (as compared to normal hearing mink). This was accompanied by a reduction in the diameter of strial capillaries in all but the apical turn of the cochlea. By examining the Hedlund mink over time (at 26, 32, 46 and 60 days of age), Foss also reported a reduction in size of the organ of Corti and a collapse of Reissner's membrane by 46 days of age.

Sugiura and Hilding (1970) published two papers describing the cochleosaccular degeneration and affected stria vascularis in the Hedlund white mink. They confirmed by loud hand claps and whistles that the normal dark mink responded by three weeks of age. At four weeks, the animals exhibited round window responses to click stimuli. They were not able to elicit any kind of response (behavioral or round window recording) from three Hedlund mink tested at various ages. This, Sugiura and Hilding reported, was unlike any other species with hereditary deafness, demonstrating that early periods of hearing were followed by eventual loss.

The normal mink kits demonstrated nearly mature cochleas by three weeks of age with the tunnel of Corti opened in almost all areas of the cochlea. Histologically, the Hedlund kits demonstrated abnormal findings similar to those reported by Saunders (1965). At three weeks of age, Reissner's membrane sagged, the organ of Corti showed signs of coagulation necrosis in the outer hair cells with vacuolization in the cytoplasm, nuclear swelling and disruption of the mitochondrial membranes. By five weeks of age, changes in the basal hair cells were remarkable, while the afferent and efferent nerves were apparently normal. Preliminary findings in the stria vascularis showed reduced numbers of mitochondria in the marginal cells lining the medial surface of the stria and a loss of endolymphatic spaces between the stria vascularis and Reissner's membrane.

Sugiura and Hilding (1970b) evaluated the stria vascularis more closely in the Hedlund mink. Using ferritin injection methods to study cochlear vasculature, they were able to demonstrate several anomalies within the stria vascularis. Not only were the marginal cells lining the stria populated with fewer mitochondria, but the width of the strial cell layer was markedly thinner. The ferritin was unable to pass from the underlying connective layer into the strial blood vessels and so, was concentrated outside of the stria. Ferritin was found in the strial vasculature of the normal mink. The Hedlund mink had a much simpler vascular pattern than the normals because many of the connecting vessels of the HWM disappeared as the animals aged. The authors also observed intravascular clotting and swollen endothelial cells in the three-week-old Hedlunds' stria. Sugiura and Hilding concluded that the strial vascular atrophy, constricted vessel diameter (due to swollen endothelium) and the exaggeration of the normally sluggish cochlear blood flow contributed to the intravascular clotting and eventual loss of strial vasculature.

Sugiura and Hilding (1970b) outlined similar findings from the earlier literature, describing changes observed in the stria vascularis of the Hedlund white mink. Erway and Mitchell (1975) described changes observed in the auditory system in the pastel-colored variety of mink that demonstrated "screw neck". These mink exhibited a reduction of otoliths within the vestibular apparatus and thus, were characterized by head tilting and abnormal movements.

Flottorp and Foss (1979) described the development of hearing and eventual loss in the Hedlund white mink. They were the first researchers to report that the Hedlund mink could hear for a short period of time. They conducted a three year trial evaluating the hearing of HWM kits, finding that a transitory hearing period occurred between days 34 through 38. The method of testing was placement of the kits in front of a loudspeaker, exposing them to one to two second bursts of white noise (at 108 dB SPL), a noise band at 750 Hz, and pure tones ranging from 250 to 10,000 Hz (with intensity levels varying from 120 to 104 dB SPL). Results were based on behavioral responses to the acoustic stimuli and varied from no response, pinna reflex or a startle response. All but one of the 17 Hedlund kits demonstrated behavioral response when tested at two day intervals from 29 to 43 days of age. On the average, the Hedlund kits first responded at 31 days old compared to the standard minks' response at 29 days of age. The noise bands were most successful in eliciting a behavioral response in the Hedlund kits. From their testing, Flottorp and Foss concluded that hearing of high frequency is probably acquired relatively late in standard mink and presumably not at all in the Hedlund mink. However, by 40 days of age, all the Hedlunds were unresponsive to sounds of either noise (white and noise band) or pure tones. The authors concluded that the presumable transient phase of audition could go undetected without close interval testing at several frequencies and that possibly, the one Hedlund mink that never demonstrated any type of behavioral response may have gained and lost its hearing between testing timepoints.

These authors also compared the hereditary deafness in mink to similar conditions in other species that displayed hypopigmentation (partial albinism) and hereditary sensorineural deafness. Mentioned were blue-eyed white cats, Shaker-1 mice and the waltzing guinea pig. Mair (1973) reported that 51.5% of the cats tested did not demonstrate a round window recording while 12.2% did have a unilateral round window response. Flottorp and Foss surmised that hearing in the mink begins in parts of the cochlea where the best metabolic conditions exist (areas of maximal vascular circulation). Then, as the blood supply cannot keep up with the requirements of the functioning organ of Corti, degeneration begins.

In a recent study, Powell and Zielenski (1989) described the mink's response to acoustic stimuli in ultrasound frequency ranges. Using two mink and a maze apparatus, Powell and Zielinski first tested the mink with audible sound stimuli (approximately 6.7 Khz). Following a training period, the frequency was increased to ultrasound levels (approximately 40 Khz). The mink responded by correctly finding their way through a maze. The ultrasound frequency was a level which was emitted by adult rodents of several species and so, the authors concluded, was audible to the mink to aid in finding their prey. The authors also deduced that while the 40 KHz range is within the audible range of other carnivores (cat, dog, raccoon), the mink's peak sensitivity is closer to peak sensitivity of the other carnivores, being somewhere around the one to 16 KHz range.

Auditory Brainstem Evoked Responses: Introduction

The ABR was first described in the literature in 1963. There are many publications and textbooks describing ABR methods, techniques and analysis. This review covers some of the major highlights presented by Glasscock et al. (1987), Glattke (1983) and Moore (1983).

The ABR is an "evoked" or elicited potential as it occurs following some type of acoustic stimulus. The potential or response is recorded from electrodes placed on the animal's mastoid (reference electrode) and vertex of the head (active electrode). It usually occurs within the first 10 milliseconds after the sound stimulus. The acoustic stimulation can vary depending on the frequency and intensity used, as well as the number of stimuli, the repetition rate, and the character of the sound itself.

The response is usually about five to six peaks or waves which occur as "fast" responses. These are easily reproducible when repeated. The response is influenced by anatomic, physiologic, physical and electronic features.

Several textbooks, journals and literature from workshops have dealt with the principles of the ABR (Brama and Sohmer, 1977; Burkard and Voigt, 1989a and b; Ehle, 1981, Jacobson, 1991; Kay et al., 1984; Marsh, 1986; Yamada et al., 1979). Basically, the ABR unit has the capacity to generate a stimulus (setting specific parameters of the sound itself), collect the very small response (which occurs in the nanovolt range) and amplify it over the background noise generated by the brain (electroencephalic), muscle (electromyographic), the electric 60 Hertz (Hz) and electric noise within the unit itself. The instrument must have the ability to elicit many responses, collect and then average them into one representative waveform that characterizes the individual's brainstem response to the acoustic stimuli as shown in Figure 8.

Ehle (1981) called that principle "signal averaging"; Jacobson (1991) summed it up as:

> "a discrete segment of the EEG following the onset of a sensory event is routed through a set of electrodes and an amplifier to a computer for analysis... the bioelectrical activity at each point along the time axis is digitized and sent to storage... time locked stimulus specific bioelectrical activity will remain and this is the evoked potential".

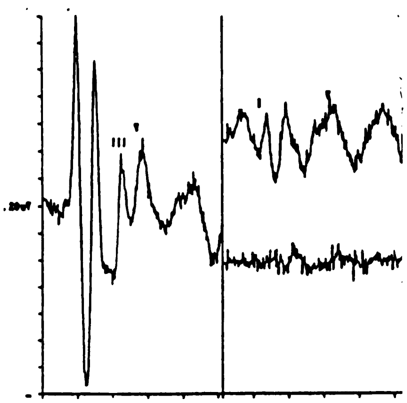


Figure 8. Example of an ABR waveform. (Stimulus: click at 90 dB.)

There are several parameters that enter into conducting an ABR. Each part of the process (stimulus generation, response collection and amplification, averaging and quantification) is made up of several components which influence the success of the ABR. The following is a summary of some of the parameters associated with an ABR.

Instrumentation of the ABR

Before any discussion about sound generation can be complete, various types of sound stimulus should be understood. Sound is defined by Merriman and Webster (1974) as "mechanical energy transmitted by longitudinal pressure waves that is the stimulus to hearing". It is an alternating mechanical current that varies in the number of cycles per unit of time; the frequency of the cycles is either referred to as cycles per second or Hertz (Hz). The frequency specific cochlear duct is arranged anatomically, and physiologically (tonotopically). The basal end "hears" high frequency and the apical end is stimulated by low frequency sounds. The actual frequency range that the cochlear duct is capable of detecting varies from species to species.

Sound can be classified by the frequency or range of frequencies. The following types of acoustic stimuli commonly used as acoustic stimulus for the ABR are described by Glasscock et al. (1987):

1. broad band click - broad frequency range that will stimulate the high-frequency range. Its acoustic response will have peaks reflecting the transducer characteristics

2. filtered clicks - more specific in stimulation as they are generated by ringing a third-octave bandpass filter with a raw click or single sine wave. Filtering allows more selective frequency stimulation (for use in threshold ABR)

3. tone pips - pure tone that produces a narrow peak at the indicated frequency (measured at the transducer)

4. single sine pip - less affected by transducer characteristics, but produces rapid rise time with tone-specific character.

The frequency of the stimulus may alter the ABR in either latency or actual response morphology. If the click is used, generally the basilar membrane will be influenced along the entire cochlear duct. Although all parts of the frequency-specific scala media will be affected, usually one area of the basilar membrane will be especially excited due to the resonance of the rest of the membrane. Depending on the frequencies included in the click and the equipment used to deliver the acoustic stimuli (transducer), the peak frequency (or peak area of the cochlear duct) is approximately 1 to 3 Khz. If pure tones are used as acoustic stimuli, the latencies of the waves of the ABR may vary. As the higher frequencies stimulate the thicker basilar membrane in the basal portion of the cochlea, their latencies of waves 1 and 5 will be less than the wave 1 and wave 5 responses collected from low frequency tones. The differences in latencies are due to the physical nature of the cochlea and nerve. The low frequency action potentials have to travel further (and so take longer) than those generated from high frequency stimuli (Figure 9).

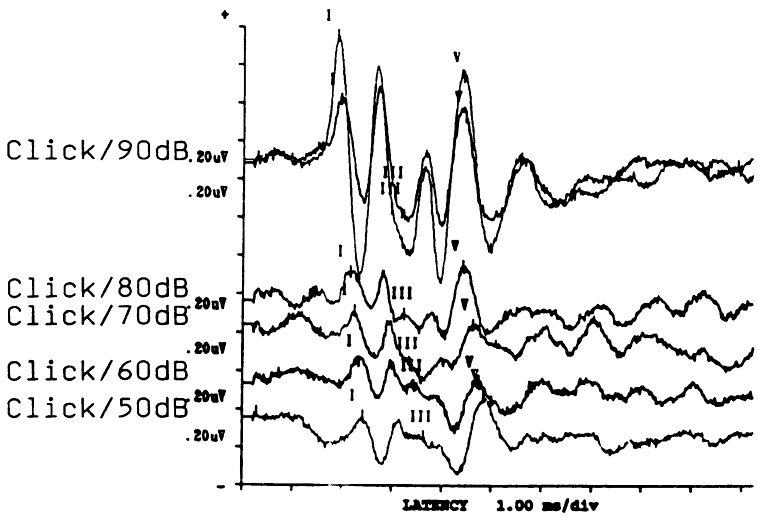


Figure 9. Relationship of intensity and latency. (Note increase in wave 5 latency as intensity decreases.)

The differences in latencies are usually more obvious when using stimuli of lower intensity as high intensity tones can essentially "over energize" the surrounding areas of the cochlear duct.

The response of the auditory nerve and other sites along the auditory neural pathway requires sufficient stimuli to evoke an action potential. Although a specific stimulus at a certain frequency may cause transduction to electrical energy, it may not be of great enough intensity to evoke an action potential of the cochlear nerve. Therefore, it may be necessary to either increase the intensity of the stimulus (if using a click) or use a pure tone pip to activate enough sensory cells in that area to produce sufficient electrical activity to induce a recordable action potential.

As sound is mechanical energy that occurs as a sine wave, it has both a positive and negative component. Martin (1981) used several terms to describe the physical characteristics of sound. They are listed below :

1. condensation - portion of a sound wave where the molecules become compressed together; causes compression of the tympanic membrane as the condensating sound wave hits the membrane (always positive)

2. rarefaction - portion of a sound wave where the molecules become less dense ; causes "pulling" of the tympanic membrane as the sound wave is "pulled" by the rarefacting sound wave (always negative)

3. alternating - combination of both condensation and rarefaction parts of the sound wave (positive and negative alternating output).

Click polarity or phase of stimulus onset is a machine variable and can be set as rarefaction (negative), condensation (positive) or alternating. The alternating acoustic stimulus switches back and forth between the two modes, causing both pushing and pulling of the tympanic membrane. Glattke (1983) reported that one of the consequences of changing an initial click stimulus from condensation to rarefaction is a variation in the wave 1 latency or the action potential of the cochlear nerve. Moore (1983) pointed out that when a stimulus is started in the rarefaction phase, the tympanic membrane is displaced laterally (pulled outward) and is followed by the movement of the ossicular chain inducing the first firing of the cochlear nerve. This coincides with the movement of the basilar membrane towards the scala vestibuli. A stimulus started in the condensation phase would cause the tympanic membrane to be pushed inward causing a "lag time" in the action potential (or wave 1) of the eighth cranial nerve. Glattke noted that the increase in AP latency was most significant when the sound was high frequency sound stimulating the basal area of the cochlea.

The shapes of the wave patterns have been affected by inversion of the acoustic phase. Moore (1983) reported that wave 5 amplitude is increased by stimuli with condensation clicks, whereas waves 1 through 4 are strengthened with rarefaction clicks. Burkard and Voigt (1989a) showed that although condensation and rarefaction click responses were very similar in overall morphology, there was a significant increase in wave 1 amplitude with rarefaction clicks and in wave 5 amplitude with condensation clicks. They reported that latencies for wave 5 were slightly longer for condensation than rarefaction clicks, confirming the information published by Moore.

According to Jacobson (1991), the combination of condensation and rarefaction stimuli can cause alteration in the shape or amplitude of the responses recorded from either of the sound types. An alternating current can reduce the amplitude of a response wave which should be considered when selecting the phase of the stimulus. Sims (1987) reported that the use of an alternating phase stimulus will help reduce the stimulus artifact and make wave 1 more visible.

Another variable which should be considered is the intensity at which the stimulus is presented. This is quantified as decibels which is a unit for measuring the relative loudness of sounds. Martin (1981) presented the following description of terms often associated with dB.

1. NHL - normal hearing level or the number of decibels above the average normal threshold for a given signal.

2. SL - sensation level or the number of decibels above the hearing threshold of a given subject

3. SPL - sound pressure level or an expression of the pressure of a sound.

There are a few generalities pertaining to intensity of the auditory stimulus and the brainstem response that deserve comment. As stated previously, the intensity of the stimuli influences the number of sensory cells which are excited and the amplitude of the action potential of the cochlear nerve. Burkard and Voigt (1989b) found that normally as the intensity of the click stimulus decreased, the latency of the waves increased and the amplitude decreased. As the intensity of the stimulus decreased, the variability of response increased. Thus, as Moore stated, "the robustness of wave 5 causes it to remain long after the other waves have receded" (Figure 10).

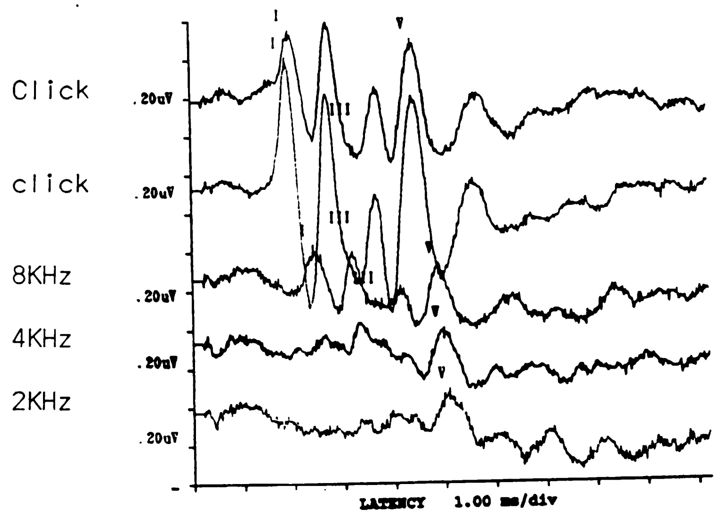


Figure 10 : Relationship of frequency and latency. (Note increase in wave 5 latency as frequency decreases.)

Repetition rate is another parameter which is an instrument variable. According to Marsh (1986), the length of the acoustic stimuli (on and off) is commonly set at 100 microseconds (10 milliseconds). The repetition rate then is usually set as the number of stimuli per second. The "average" ranges from approximately 10 to 50 per second. Moore (1983) indicated that as the repetition rate is increased, there is a resulting increase in the latency of the waves as well as a decrease in the amplitude. Burkard and Voigt's (1989b) work concurred with the principles published by Moore and added that there was also an increase in the wave 1 to wave 5 interval. An additional parameter includes the time element associated with the number of stimuli and responses generated, collected and averaged

(Figure 11). This length of time is referred to as an "epoch" or sweep time. Usually a minimum of 256 epochs or sweeps are recommended as a way to diminish or, as suggested by DeGuire (1986), "to cancel out the random EEG cortical activity of alternating polarities" since the amplitude of the "normal" ABR wave is 1/100 of the size of the EEG waves. Moore (1983) reported that at approximately 8000 responses, there was an inclination for the wave pattern to smooth out the human wave 4 - 5 combination making it no longer easily discernable as a separate wave.

Masking is another possible factor which must be considered when conducting an ABR. It was said by Moore (1983), "to occur when one sound makes another sound difficult or impossible to hear, or when the threshold of the signal (the maskee) has been elevated by a second signal or noise (the masker)". Masking is used to eliminate any possibility of the contralateral ear from "hearing" the acoustic stimulus and responding to it. Masking is done by stimulating the contralateral ear with sound that is "low level" in nature. The sound is usually some kind of background noise that is continuous and does not have an "on or off" as do the click or pure tone pips. Masking does not stimulate any type of ABR. White noise is commonly used to mask as it contains a wide range of frequencies and sounds like hissing.

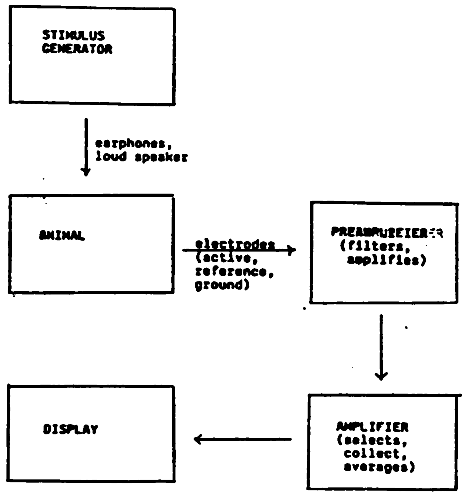


Figure 11. Schematic of ABR instrumentation.

In order to collect the responses, either surface or subdermal electrodes are placed on the animal. As described by Moore (1983), a minimum of three electrodes is used with two connected to the pre-amplifier and the other serving as a system-reference or ground electrode. One of the two electrodes connected to the pre-amplifier acts as the active electrode and is placed on the scalp (usually the vertex) of the animal. Using the 10 - 20 electrode placement system commonly associated with electroencephalography (EEG), this electrode in placed in the Cz position. The second electrode is called the reference electrode and is positioned somewhere near the ear. In animals, it is usually placed on the mastoid in the A1 or A2 position (on the contra and ipsilateral ears) using the 10 - 20 system. Often both A1 and A2 positions are used, resulting in a total of four electrodes used to collect the ABR (Figure

12). The electrodes are metal (either silver, gold or platinum) and have an electrically stable electrode-subject interface which can be minimized easily if the impedance (resistance) is too high between the electrode or the subject. If electrical instability is generated, it usually causes interference which, according to Moore (1983), "manifests as increased movement artifact and occasional large baseline swings even when the patient is still". The continuity between the electrode and the skin must be checked by testing the impedance by sending a very low electrical current between each electrode and the ground electrode. Impedance should be measured for each electrode each time they are placed on a subject and should preferably be less than 5000 ohms. Glattke (1983) stated that an electrode with a high impedance will detect more noise which could possibly interfere with a clear ABR.

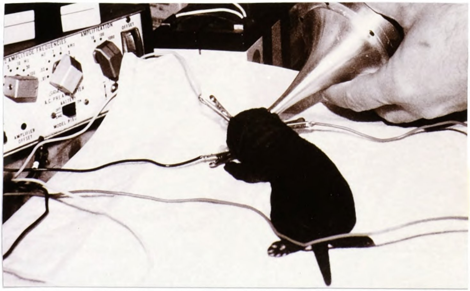


Figure 12. Photograph of four-week-old SDM with electrodes.

The responses detected by the electrodes are fed into a pre-amplifier which functions to differentiate the brainstem response from the electroencephalographic activity and other noise within the system. As the response input is very small when compared to the EEG, it must be increased by reducing the unwanted background noise. The pre-amplifier should have the following characteristics as outlined by Glattke :

1. very low internal electrical noise
2. broad frequency response range
3. selection of a frequency range that is most appropriate for the desired response
4. allows detection of the patient's electrical signals at three electrode locations.

A clear and comprehensive explanation of the function of the pre-amplifier was presented by Glattke as follows:

> "The active electrode placed on the vertex of the skull, is led to the noninverting input of the preamplifier. The reference electrode is led to the inverting preamplifier input. The ground electrode is led to a common input and is placed on the forehead. There are at least three amplifier circuits inside the unit. The noninverting input signal is led to a circuit that amplifies the signal present between the active and ground electrodes. An identical amplifier stage accommodates the signal between the reference and ground electrodes, and it reverses the phase or electrical polarity of that signal. The outputs of the two initial stages are added together on a common electrical pathway before being led to subsequent stages within the amplifier. The effect of the addition of the noninverted and inverted signals on the common pathway is the elimination of voltages that were present with identical characteristics at the active and reference electrode sites. Only the difference between the signals at the active and reference sites is led to the subsequent amplifier stages. This cancellation of electrical signals that are identical at the electrode sites aids in the detection of the desired evoked response

> because much of the unwanted noise in the test environment is present at both the active and reference sites."

In this way, the pre-amplifier attenuates any electrical noise that may be "picked up" and detected by two or more of the electrodes.

Another mechanism for eliminating unwanted noise from interfering with the ABR is by utilizing low and high pass filters within the pre-amplifier. As the input signal enters the pre-amplifier, it is possible to filter out unwanted electrical input. Ehle (1981) reported that filtration of the input signal helps to change the relationship of the signal/noise ratio. As EEG signals are usually in very low frequency ranges, a low band filter would be used to screen out the EEG responses, allowing the ABR response to "pass through". Brama and Sohmer (1977) used low frequency settings (with center frequencies ranging from 250 to 1000 Hz) to positively test for low frequency hearing loss. According to Glattke (1983), the upper frequency limit for the ABR should be approximately 3 Khz or greater, and "the filter setting should span the frequency range from the lowest frequency of interest to a value equal to twice the highest frequency of interest".

There are other characteristics of the pre-amplifier which Ehle (1981) discussed in depth. Removing noise from the signal is very important in the production of reliable and consistent ABRs. Not only does filtration of the incoming signal help, but it also increases the strength of the ABR signal. The low level analog section of the pre-amplifier takes the signal from the animal and increases it to a voltage level that can be recognized by the digital processing unit. After the signal is converted from analog to digital, it is sent to the amplifier where it is increased further. Glasscock _et_ _al_. (1987) stated that the ABR unit usually increases the ABR signal by 10,000 to 1,000,000 times.

Following frequency filtration by the pre-amplifier, the amplifier can sort out artifacts by eliminating any sweeps that have more than a pre-set number of off-scale points and by not including the sweeps with excessive noise in the averaging. This is done after the signal is converted from analog to digital and allows for saving of memory that would otherwise be filled with unusable information. The digital information is stored in the averager's memory and is averaged each time more information comes in. Once the designated number of sweeps is completed, the information is reconverted to analog form so it can be displayed or plotted out.

Analysis of the ABR

Because the ABR is generated by both the peripheral and central auditory neural pathways, all components must be "intact" and functional to produce the waveform. As stated by Jacobson (1991), the presence of the ABR is obviously "dependent upon unimpeded neural transmission and neural synchrony". The evoked response is believed to reflect synchronous activation of "onset-type" neurons within the auditory system and depends on the activation of a functional cochlea and brainstem generators. Although there is some degree of variation in the morphology of the waveform among individuals, the overall pattern is consistent enough to recognize differences. Abnormalities of the ABR can appear as morphologic changes to the waveform, delay in the peaks or decrease in the amplitude of response of each or all of the waves. Variations in latencies can occur by varying either the frequency or intensity of the stimulus. Thomas (1985) described the concept of delays in the ABR when stimulating an ear with low frequency tones. The low frequencies are detected in the apical portion of the cochlea and must travel a longer distance than high frequency stimuli and so have a longer latency. Amplitude changes can be seen by decreasing the intensity of the click response. According to Thomas, wave 5 is the most "robust" of all the waves and decreases less than the other waves when the intensity is decreased.

It is important to remember that precise generator sites for each of the ABR waves have not be defined. Although Moore (1991) indicated that waves 1 and 2 are actually action potentials from the eighth cranial nerve (in the human), the later waves have multiple generators or post-synaptic activity within the auditory brainstem. Waves 1 and 2 are most likely the action potentials of the eighth cranial nerve while waves 3, 4 and 5 are produced from the region of the cochlear nuclei, superior olivary complex and inferior colliculus, respectively.

Jacobson (1991) proposed the following reasons for aberrations within the ABR waveform:

1. selective blocking due to demyelination
2. pressure from tumors
3. ischemia due to tumors or arterial blockage
4. longitudinal stretch from tumors.

Hosford-Dunn (1985) concluded that consistently prolonged waves (with delayed responses or increased latencies) may be suggestive of retrocochlear or conductive anomalies. She also described interpretive criteria for evaluating ABR's. The quantitative methods included measuring peak latency and amplitude and the latencies between the peaks (called the interpeak latencies). Qualitative criteria included the presence of peaks, the stability or reproducibility of the response and the characteristics of the waveforms.

One of the major limitations of the quality or presence of the ABR is the function of the cochlea. Since the response is initiated in the cochlea, it can be difficult to draw any conclusions from the lack of a demonstrable ABR. It may be very difficult to differentiate between a cochlear or retrocochlear lesion if there is no ABR present. Glasscock _et al_. (1987) pointed out that the ABR is not a hearing test, as it only measures cochlear and brainstem neural response to auditory stimuli and it does not reflect any cerebral cortical function or the "psychic" part of hearing. Loss of wave 1 would be the most apparent change in the ABR of animals with conduction hearing disorders. Sims (1987) reported that any change in the outer, middle or inner ear would result in loss of or a delay in the latency of wave 1. According to Glasscock et al. (1987), the presence of only wave 1 and wave 2 can be indicative of auditory pathway pathology. Because waves 1 and 2 are actually action potentials from the eighth cranial nerve, their presence would probably indicate only problems in the brainstem neural conduction system. Glasscock et al. cited a case where a young child demonstrated only wave 1 on one side and wave 1 and 2 on the other which was diagnostic of CNS pathology. Hosford-Dunn (1985) reported similar findings in a young woman with a meningioma involving the acoustic nerve. The presence of only waves 1 and 2 were useful in locating the tumor.

Although the latency between any peaks can be measured, the most common evaluations are made between waves 1 and 3, waves 3 and 5, and waves 1 and 5. According to Sims (1987), the latency between 1 and 3 is the travel time between the cochlear nerve and the pons; the 1 to 5 latency is called the central conduction time or the time necessary for the impulse to travel from the cochlear nerve to a level within the mesenencephalon. Tomasulo and Peele (1988) found that the wave 1 latency was useful in predicting the wave 1 to 5 interval which was found to be the same in both normal persons and those with hearing loss.

The ABR can be used to differentiate the source of lesions within the auditory neural pathway. As waves 1 and 2 probably originate from the eighth cranial nerve and waves 3, 4 and 5 from the brainstem, it is possible to differentiate between cochlear and retrocochlear lesions. Glasscock et al. (1987) felt that a prolonged wave 5 latency represented "the most sensitive detector of eighth nerve dysfunction". This may be represented in the waveform by the presence of dysynchrony and wave 5 abnormalities. Sims (1987) reported that the greatest variation occurred between the third and fifth wave and that in the human, it can form a single 3 - 4 complex or a 4 - 5 complex with wave 5 being the largest. He stated that using the rarefaction click will help separate the 3 - 4 wave complex.

Yamada et al. (1979) examined the wave 5 latency in humans with different types of hearing loss. They found that wave 5 latencies were delayed in people with severe high frequency hearing loss and that the latencies in other people varied, depending upon the severity of the hearing loss. Patients with low frequency hearing loss showed no difference in wave 5 latencies when compared to normal controls. Prosser and Arslan (1987) established a "diagnostic index" to use wave 5 latencies as a predictor of hearing loss caused from retrocochlear lesions. The primary intent of the index was to use it in diagnosing retrocochlear tumors (especially those in the cerebellopontine angle).

Sims (1987) reported that during postnatal maturation in humans, waves 1 and 2 are the first to be seen. In puppies and kittens between one to two weeks of age, he found that wave 5 was seen first. As the ABR approaches normal adult values, the "general pattern is an increase in wave amplitude and a decrease in wave latency". Pujol and Hilding (1973) provided an indepth comparison of the onset of hearing in many species including the morphology and the physiological onset of hearing. By 16 days of age, the mink was reported to have an action potential recorded at the round window, as well as the entire auditory system being responsive to acoustic stimulation. Morphologically, by the time the dog showed similar electrophysiologic responses, it showed an increase in the size of the tunnel of Corti, the spaces of Nuel and integration of the strial epithelium. The authors observed that in the cat and guinea pig, the morphologic maturation preceded the functional capacity by at least two to three days.

According to Romand et al. (1987), most of the signals recorded by the auditory nerve fibers come from the IHC. The OHC may "represent the modulator of the organ of Corti activities with a more mechanical function". Because the cells of the organ of Corti develop at different rates (IHC, OHC, then support cells with Henson's cells developing last), this may be reflected in the success of electrophysiologic assessment at very early stages. By testing single auditory nerve fibers, Romand et al.(1987) described a low frequency stimulus inducing an initial response in the basal cochlea of the one-week-old kitten. However, as the kitten ages, the sensitivity and specificity of the cochlea changes as a high frequency stimulus can then induce a response in the basal cochlea. As the animal ages, the second and third turns of the cochlea mature, demonstrating the "place principle" described as where low frequencies shift from the basal cochlea to the more apical turns.

Using the ABR to evaluate development of the hamster auditory system, Schweitzer (1987) described the decrease in latencies of the major peaks of the ABR as the animals aged. However, the later ABR waves appeared comparatively later in the life of the hamsters studied, indicating that the hamster may be a good model for "studying postnatal development of the central auditory system". As demonstrated by Sims (1987), both waves 1 and 2 appeared first at 18 days of age, followed by the appearance of all five waves by day 22. As the animals aged, latencies decreased and amplitudes increased, demonstrating the accepted pattern of ABR ontogeny.

Moore (1982) described the relatively late onset and maturation of hearing in the European ferret (Mustela putorius). Using behavioral responses (startle response) and an electrode placed in the inferior colliculus, he stimulated neonatal ferret kits with pure tones and white noise (with maximum intensities of 93 to 101 dB SPL) by placing the kits in front of a loudspeaker positioned close to a surgically exposed eardrum. In ferrets less than 32 days of age, the external auditory meatus of the kits was collapsed, the walls were in tight contact with each other and the middle ear was filled with mesenchymal fluid. Although the auditory midbrain exhibited neural discharges, no behavioral responses were noted in kits up to 32 days of age. After 32 days, the meatus opened and most of the ferrets exhibited both behavioral and electrophysiologic responses. By 42 days of age, the animals demonstrated responses similar to those found in adult animals.

EXPERIMENTAL ANIMALS

Standard Dark Mink

The animals used as controls were standard dark or natural dark mink (SDM), (*Mustela vison*). The animals were selected from those kept at the Michigan State University Experimental Fur Farm and were deemed to be "normal" upon visual inspection and behavioral observation. The mink kits were selected from litters produced through regular breeding practices followed at the Fur Farm and were consistent with procedures employed by the commercial mink ranching industry.

Hedlund White Mink

Six female Hedlund white mink (HWM) and two male HWM were purchased in the fall of 1987 from Mr. Gale Evans (Melvin, MI), a commercial fur farmer. Three of the females and one of the males were born in the spring of 1987. The other HWM were older "proven breeders". None of the animals were related. Upon and after arrival, observation revealed that the animals were behaviorally deaf. There were no Preyer's or startle responses to very loud acoustic stimuli. During the study, care was taken to avoid any inbreeding or close line breeding.

Husbandry and Care of the Mink

All daily care of the mink was provided by the staff of the MSU Experimental Fur Farm. The animals were housed in cages which were located outside in open-sided buildings. The animals were exposed to ambient temperature, humidity and photoperiod. The cages were made of wire and suspended above the ground. Attached nest boxes were made of wood and were filled with excelsior (wood wool) during the winter and whelping period to provide warmth and protection. As recommended by the supplier of the HWM, the animals were housed on the outer sides of the buildings to insure that they had exposure to adequate light as the photoperiod began to increase. Drinking water was provided ad libitum by cups attached to the cages. These were filled automatically during the spring, summer and fall and were hand-filled in the winter. The mink were fed once a day using a basal diet formulated to meet the nutrient requirements of the mink (NRC, 1982) and containing :

poultry by-products
ocean fish scrap
commercial mink cereal
mineral and vitamin mix
beef by-products
beef liver
cooked eggs.

The animals were bred yearly in the months of February and March. The females were taken to the males' cages, allowed to copulate, and then were checked for the presence of viable sperm by examining vaginal aspirates. If a successful breeding took place, attempts were made to re-breed the animal the next day. If a female was not receptive to the male, attempts were made to mate the female every fourth day until a successful mating was attained. Most of the HWM females were receptive later than the SDM. Breeding usually began about March 7 for the HWM compared to the last week of February for the SDM.

Most of the females whelped early in May; litters were checked on the first day. After that, the litters were left alone as much as possible to minimize stress to the females. Standard practices were followed in raising the kits, including leaving the litter with the female until about six weeks of age. After the litter was separated from the female, the kits were left together for a few weeks. The kits were later housed in pairs until they were housed separately after reaching puberty.

EVALUATION OF HEARING

Behavioral Assessment of Hearing

Behavioral assessment of hearing was rather easy to conduct on the HWM. The mink were observed daily by the animal care staff and occasionally by other people. As mink are aggressive by nature, any attempts to approach them or their cage would normally result in a pronounced attack. When confronted by people, the SDM would be alert and ready to attack while the HWM often would sleep through close observation. If one could carefully open the cage without vibrating it and alerting the animal, the Hedlunds could be picked up without the aggressiveness that the SDM usually displayed.

Electrophysiologic Evaluation of the Mink: Anesthesia

Due to the aggressive nature of the mink and the necessity for no muscular tension to successfully conduct an ABR, the mink had to be anesthetized. The following formulation was used and was found to be safe and effective even for one-week-old mink kits:

acepromazine	0.055 mg / kg body weight
atropine	0.11 mg / kg body weight
ketamine	33 mg / kg body weight
xylazine	2.2 mg / kg body weight

The anesthesia was mixed at concentrations that could be easily administered to the size of the kits. When the kits were very young, the anesthesia was diluted to an overall concentration of 0.5 ml/kg of body weight. As the kits grew, the concentration was changed to 1.0 ml/kg body weight. It was given intraperitoneally in very young animals and intramuscularly in older animals that had sufficient muscle mass. The anesthetic formulation provided approximately 30 to 45 minutes of time to conduct the ABR. As the animals aged, the length of "usable" anesthesia shortened and thus, necessitated additional injections to conduct the testing. While under anesthesia, the animals were maintained on a warm surface to help reduce loss of body heat. The examinations were carried out in a quiet environment with minimal external noise.

RESEARCH DESIGN

Experiment A - Onset of Hearing in the Mink

Initially it was determined that to meet the objectives of the study, two questions had to be answered about hearing in the mink; (1) at what age do standard dark mink begin to hear and (2) do Hedlund white mink ever hear? To answer these questions, a preliminary experiment was designed to test both SDM and HWM at weekly intervals using ABR's to assess their capacity for hearing. From one through seven weeks of age, two age-matched mink kits of each color variety were removed from their nest boxes and transported to the Kresge Hearing Research Institute. Prior to anesthesia, the kits were observed for any behavioral response (Preyer's or startle reflex) to acoustic stimuli. They were then anesthetized and maintained on a hot water pad to protect against hypothermia. ABR testing was conducted using click (broadband with peak at approximately 4 to 6 Khz), 10 Khz, 8 Khz, 4 Khz and 2 Khz stimuli. The kits were then euthanized using a lethal intraperitoneal injection of T-61 (Hoechst-Roussel, Inc.; Summerville, New Jersey) and were systemically perfused. The cochleas were collected and prepared for histologic examination.

Experiment B - Electrophysiologic Studies of the Mink

The findings of Experiment A prompted more questions concerning the "normal" range of hearing of the SDM and the age at which the HWM lose the ability to hear. As both the SDM and the HWM kits exhibited ABR's between four and five weeks of age, a preliminary project was conducted to more closely identify the age of the onset of hearing. Two HWM kits were tested on days 33, 36, 38 and 43. Later, seven HWM kits from three litters were anesthetized, tested, allowed to recover and were tested again every other day between 26 and 42 days of age. Two SDM were also tested at similar timepoints using the same protocol as that used on the HWM.

Prior to ABR testing, the mink were examined for obvious outer or middle ear anomalies. ABR's were then used to test the function of the cochlea and the brainstem in the SDM and HWM. There are several advantages of the ABR over other types of auditory testing. The most notable is the non-invasiveness of the procedure. The ABR unit in the initial studies conducted at the Kresge Hearing Research Institute (Ann Arbor, MI) used only pure tone frequencies up to 10 Khz; the ABR unit used in later studies could only test pure tones up to 8 Khz. The ABR unit used in the later studies at the Michigan State University Veterinary Clinical Center (East Lansing, MI) had a click generator that stimulated at the 2 - 3 Khz range. This range, according to Powell and Zielinski (1989), may only stimulate a limited range of hearing which may exceed 40 Khz in the mink although the authors concluded that the prime sensitivity of the mink cochlea is probably between 1 and 16 Khz.

The ABR's for the initial experiment (A) were conducted at the University of Michigan's Kresge Hearing Research Institute. The equipment included a Tracor Northern audiosomatosensory stimulator (model number TN-3001) click generator. Intradermal needle (25g) electrodes collected the responses which were directed to a Grass P-15 amplifier (Quincy, MA). Filters were set at 300 and 3000 Hz; the responses were amplified 1000 times. A PC-AT computer collected and averaged 256 responses with a resolution of 25 microseconds.

The later studies in Experiment B were completed at the Veterinary Clinical Center at Michigan State University using a Bio-Logic Brain Atlas II evoked potential unit (Bio-Logic Systems Corporation; Mundelein, IL).

Experiment B involved examination for ABR's in the Hedlund white mink before, during and after the onset of hearing (as determined in Experiment A). The following parameters used in conducting the electrophysiologic examination on the mink were based primarily on those established by the American Electroencephalographic Society (Anonymous, 1986) and by Kelly et al. (1989). Due to the constraints of the equipment, anesthesia and the nature of mink, some variations were made in the number of sweeps and frequencies examined during the ABR's. The frequency tested was a click (with a central resonance point around 2 to 3 Khz) or pure tone pips at 8 Khz and with time permitting, 4 Khz, 2 Khz, 1 Khz and 0.5 Khz.

The stimulus parameters included:

monaural testing of left ear
alternating phase current
pulse of 10 milliseconds
rate of 10.1 stimuli per second
520 to 1040 sweeps
right ear masked with white noise.

Using the 10-20 electrode placement system, the platinum subdermal needle electrodes were placed on the left mastoid (A1), the right mastoid (A2), the vertex (Cz) and the subcutaneous region between the eyes (ground).

The montage for recording used a two channel system on the Bio-Logic Brain Atlas II unit including channel one (Cz to A1) for ipsilateral testing and channel two (Cz to A2) for contralateral testing. Once the responses were received, they were amplified 100,000 times by the pre-amplifier. Averaged responses for each frequency and intensity were either displayed on the monitor within the Bio-Logic unit or printed out using a Hewlett Packard Jet Paint Plotter (Hewlett Packard, Inc.; Palo Alto, CA).

Statistical Evaluation

All conclusions on the ABRs were made from eight mature "normal" standard dark mink. Results were defined as abnormal if they were more than two standard deviations from the mean of the control animals. For the analysis of

different samples of two different groups, sample means and standard deviations were compared using confidence limits or confidence intervals. Confidence intervals were not used when comparing an individual's response to a control sample.

Results of Experiment A

ABR's were not observed in either the SDM or HWM kits until the kits were five weeks of age. Testing at pure tone frequencies of 0.5, 1, 2, 4, 8 and 10 KHz, the SDM kits demonstrated the highest sensitivity (lowest threshold) at the highest frequencies. Sensitivities decreased as the frequencies decreased; the SDM had no ABR below a stimulus of 2 KHz. By six weeks of age, near-adult sensitivity values were obtained at 10 KHz and initial responses were observed below 2 KHz. Between weeks 6 and 7, the SDM's sensitivities improved by 20 to 30 dB SPL at the lower pure tones (below 2 KHz).

The HWM kits showed responses to 8 and 10 KHz pure tones delivered at very high intensities ranging from 100 to 115 dB SPL. No responses were seen below 8 KHz at week five. At six weeks, one of the kits demonstrated threshold improvement by 20 to 30 dB at 10 KHz and 15 to 20 dB at 8 KHz. Responses were seen at 4 KHz using the highest intensity level (115 dB SPL). No ABR was elicited in the other HWM kit. At week seven, neither kit had a brainstem response to the acoustic stimuli.

Results of Experiment B

The latencies of waves 1, 3 and 5 of the eight control SDM stimulated with a click at 90 dB intensity are listed in Table 1. That intensity level (the highest available on the Bio-Logic unit) was chosen as the "comparative normal" as very often that was the only level to which the HWM showed any response. The mean, standard deviation and range (two standard deviations from the mean) for waves 1, 3 and 5 of the SDM were determined and are shown in Table 1.

Four timepoints (26, 34, 38 and 41 days of age) were used to examine the development of the ABR in the SDM. Although only two animals were included in this part of the study, it was found that both of the SDM kits tested could hear by 26 days of age (as compared to the SDM kits tested in Experiment A in which ABR's were not detected until between weeks four and five). As can be seen in Figures 13-17 and Tables 1 and 2, the latencies of the ABR waves generally decreased with age when the animal was stimulated with a 90 dB click stimulus. All the brainstem responses were equally reduced, so amplitudes can be relatively compared.

Table 1. Latencies (in msec) of ABR waves of SDM used as controls for ABR values. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
C-A	41	0.74	2.86	5.06
C-B	41	0.78	2.86	5.22
C-C	ADULT	0.95	2.74	4.76
C-D	ADULT	0.84	2.42	4.38
C-E	ADULT	1.08	2.20	4.46
C-F	ADULT	1.20	2.46	3.64
C-G	ADULT	1.06	2.14	4.90
C-H	ADULT	0.76	2.56	4.50
MEAN		0.93	2.53	4.62
SD		0.17	0.28	0.49
1 SD	MAX	1.01	2.81	5.11
	MIN	0.76	2.25	4.13
2 SD	MAX	1.27	3.09	5.60
	MIN	0.59	1.97	3.64

Table 2. ABR latencies (in msec) of SDM over time. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
C-A	26	1.05	3.38	5.09
	34	0.94	3.12	4.34
	38	0.94	3.34	4.36
	41	0.74	2.86	5.06
C-B	26	1.02	2.36	4.64
	34	1.00	2.32	4.66
	41	0.78	2.86	5.22

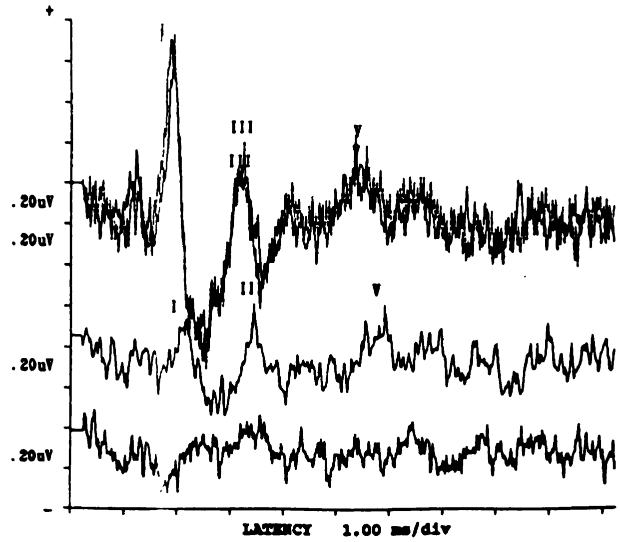


Figure 13. SDM ABR at 26 days old. Click at 90 dB.

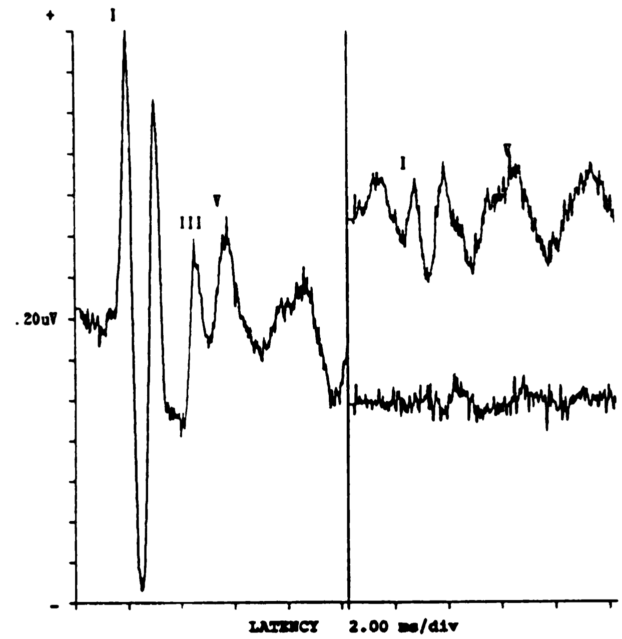


Figure 14. SDM ABR at 34 days old. Click at 90 dB.

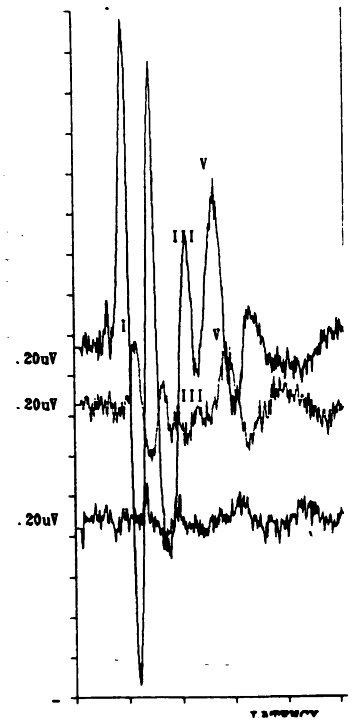


Figure 15. SDM ABR at 38 days old.
Click at 90 dB.

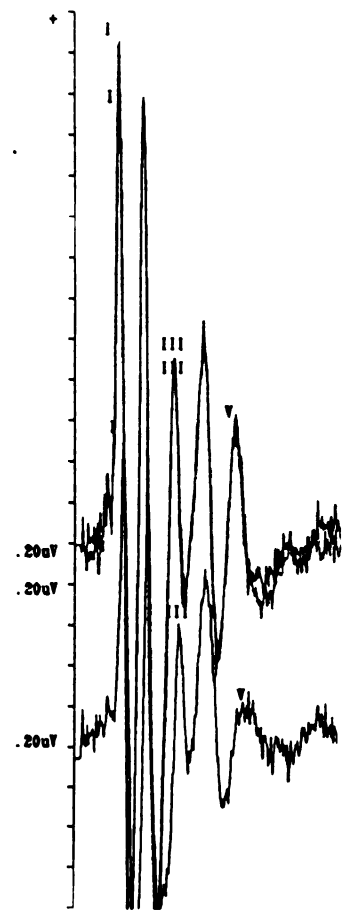


Figure 16. SDM ABR at 41 days old.
Click at 90 dB.

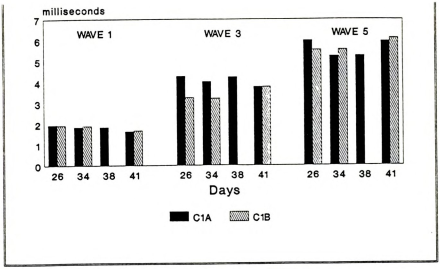


Figure 17. Changes of two SDM ABR latencies (in msec) over time.

As indicated by the results obtained in Experiment B, the overall latencies of the waves for the SDM decreased as did the threshold levels. As shown in Figure 18 and Table 3, the two SDM tested at four different timepoints demonstrated a decrease in intensity threshold of the click stimulus as they increased in age.

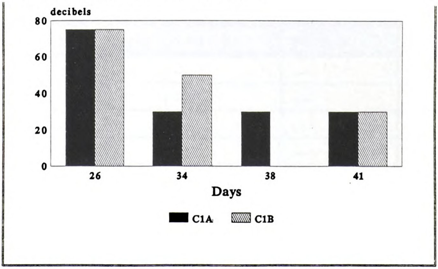


Figure 18. Changes of click threshold in SDM over time.

Table 3. Threshold levels (in dB) of intensity of click stimulus in the SDM over time.

AGE (days)	SDM C-A	SDM C-B
26	75	75
34	30	50
38	30	--
41	30	30

The preliminary trial was conducted with two HWM kits to examine in more detail the actual age when they begin to hear. The animals were tested using ABR's at 33, 36, 38 and 41 days of age. As shown in Table 4, only one HWM kit elicited a wave 1 response at 38 days of age, however, the latency of that wave was well out of the two standard deviation range of the control SDM.

Table 4. Latencies (in msec) of HWM ABR waves for Experiment B over time. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM B1	33	--	--	--
	36	--	--	--
	38	--	--	--
	41	--	--	--
HWM B2	33	--	--	--
	36	--	--	--
	38	3.10	--	--
	41	--	--	--

The latencies of waves 1, 3 and 5 brainstem responses to a 90 dB click stimulus of seven HWM examined during the "critical periods" are listed. In Tables 5 through 8, the latencies are listed by individual animal. No responses were seen in any kit younger than 32 days old. In Tables 9 through 17, the data are grouped according to the age of the kits. Attempts were made to examine each kit at two day intervals, however hardware or software problems occasionally occurred and results were not available (NA) for certain days.

Table 5. Latencies (in msec) of ABR waves of HWM litter 2 over time. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM 2A	32	--	--	--
	34	--	--	--
	36	--	--	--
	38	1.40	--	--
	40	1.33	3.56	4.68
	42	1.28	3.39	5.62
	44	1.34	3.52	4.58
	46	1.30	3.92	5.40
	55	--	--	--
HWM 2B	32	--	--	--
	34	3.00	--	--
	36	--	--	--
	38	1.20	--	--
	40	NA		
	42	2.30	--	--
	46	--	--	--
HWM 2C	32	--	--	--
	34	--	--	--
	36	--	--	--
	38	NA		
	40	1.31	3.48	5.98
	42	1.25	3.17	5.42
	44	1.05	3.11	5.40
	46	2.30	--	--
	55	--	--	--

Table 6. Latencies (in msec) of ABR waves of HWM litter 3. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 2	WAVE 3	WAVE 5
3A	26	---	--	---	---
	30	---	--	---	---
	32	---	--	---	---
	34	2.24	3.05	---	---
	36	---	--	---	---
	38	2.55	--	---	---
	40	---	--	---	---
	42	---	--	---	---
	44	---	--	---	---
3B	26	---	--	---	---
	30	---	--	---	---
	32	---	--	---	---
	34	2.31	--	---	---
	36	1.80	--	---	---
	38	2.46	--	---	---
	40	1.54	--	3.46	4.73
	42	1.05	--	2.99	4.28
	44	NA			
	55	---	--	---	---

Table 7. Latencies (in msec) of ABR waves of HWM litter 4. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 2	WAVE 3	WAVE 5
4A	26	--	--	--	--
	30	--	--	--	--
	32	--	--	--	--
	34	1.80	3.20	--	--
	36	--	--	--	--
	38	--	--	--	--
	40	--	--	--	--
	42	--	--	--	--
4B	26	--	--	--	--
	30	NA			
	32	--	--	--	--
	34	--	--	--	--
	36	1.70	2.72	--	--
	38	1.35	--	3.68	4.72
	40	--	--	--	--
	42	--	--	--	--

Table 8. Latencies (in msec) of ABR waves of miscellaneous HWM. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 2	WAVE 3	WAVE 5
E1	26	--	--	--	--
E2	30	--	--	--	--
E3	30	--	--	--	--
E4	32	--	--	--	--
E5	32	1.95	2.80	--	--
E6	34	--	--	--	--
E7	34	1.90	2.56	--	--
E8	36	1.50	2.44	--	--
E9	38	1.50	--	3.30	4.72
E10	40	--	--	--	--
E11	42	--	--	--	--
E12	42	--	--	--	--

Table 9. Latencies (in msec) of ABR waves of SDM and HWM at 26 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
SDM C1A	26	1.05	3.38	5.09
SDM C1B		1.01	2.34	4.66
HWM 2A		--	--	--
HWM 2B		--	--	--
HWM 2C		--	--	--
HWM 3A		--	--	--
HWM 3B		--	--	--
HWM 4A		--	--	--
HWM 4B		--	--	--
HWM E1		--	--	--

Table 10. Latencies (in msec) of ABR waves of HWM at 30 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM 2A	30	--	--	--
HWM 2B		--	--	--
HWM 2C		--	--	--
HWM 3A		--	--	--
HWM 3B		--	--	--
HWM 4A		--	--	--
HWM 4B		--	--	--
HWM E2		--	--	--
HWM E3		--	--	--

Table 11. Latencies (in msec) of ABR waves of HWM at 32 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM 2A	32	--	--	--
HWM 2B		--	--	--
HWM 2C		--	--	--
HWM 3A		--	--	--
HWM 3B		--	--	--
HWM 4A		--	--	--
HWM 4B		--	--	--
HWM E5		--	--	--
HWM E4		2.80	--	--

Table 12. Latencies (in msec) of ABR waves of SDM and HWM at 33 and 34 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM B1	33	--	--	--
HWM B2		--	--	--
SDMC1A	34	0.94	3.12	4.34
SDMC1B		1.04	3.56	4.80
HWM 2A		--	--	--
HWM 2B		2.00	--	--
HWM 2C		--	--	--
HWM 3A		3.05	--	--
HWM 3B		2.31	--	--
HWM 4A		3.20	--	--
HWM 4B		--	--	--
HWM E6		--	--	--
HWM E7		2.56	--	--

Table 13. Latencies (in msec) of ABR waves of HWM at 36 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM 2A	36	--	--	--
HWM 2B		--	--	--
HWM 2C		--	--	--
HWM 3A		--	--	--
HWM 3B		--	--	--
HWM 4A		--	--	--
HWM 4B		2.72	--	--
HWM E8		2.44	--	--
HWM B1		--	--	--
HWM B2		--	--	--

Table 14. Latencies (in msec) of ABR waves of SDM and HWM at 38 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
SDMC1A	38	0.94	3.34	4.36
HWM 2A		1.40	--	--
HWM 2B		1.20	--	--
HWM 2C		NA		
HWM 3A		2.55	--	--
HWM 3B		2.49	--	--
HWM 4A		--	--	--
HWM 4B		1.35	3.68	4.72
HWM E9		2.22	3.30	4.72
HWM B1		--	--	--
HWM B2		3.10	--	--

Table 15. Latencies (in msec) of ABR waves of HWM at 40 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM 2A	40	1.33	3.56	4.68
HWM 2B		NA		
HWM 2C		1.31	3.48	5.98
HWM 3A		--	--	--
HWM 3B		1.31	3.46	4.73
HWM 4A		--	--	--
HWM 4B		--	--	--
HWM E10		--	--	--

Table 16. Latencies (in msec) of ABR waves of SDM and HWM at 41 and 42 days. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
SDMC1A	41	0.74	2.86	5.06
SDMC1B		0.78	2.86	5.22
HWM 2A	42	1.28	3.39	5.62
HWM 2B		2.30	--	--
HWM 2C		1.25	3.17	5.42
HWM 3A		--	--	--
HWM 3B		1.02	2.99	4.28
HWM 4A		--	--	--
HWM 4B		--	--	--
HWM E11		--	--	--
HWM E12		--	--	--

Table 17. Latencies (in msec) of ABR waves of HWM at 43 days and over. Click at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
HWM B1	43	--	--	--
HWM B2		--	--	--
HWM 2A	44	1.34	3.52	4.58
HWM 2C		1.05	3.11	5.40
HWM 3A		--	--	--
HWM 3B		--	--	--
HWM 2A	46	1.30	3.92	5.40
HWM 2B		--	--	--
HWM 2C		2.30	--	--
HWM 2A	55	--	--	--
HWM 2B		--	--	--
HWM 2C		--	--	--

As can be seen in Figure 19, the HWM did not exhibit any brainstem response until 32 days of age. As the kits aged, more demonstrated ABR's until 38 days. At that point, the number of HWM kits that could hear declined until none responded at 55 days of age. There was a great deal of individual variation with two of the animals demonstrating only wave 1 at one timepoint, while four of the seven animals developed all five waves during the test period. Three of the HWM had persistent responses that, for a short time, demonstrated delayed waves 1 and 3 latencies and near normal wave 5 latencies followed by loss of a recordable response. By 55 days of age, none of the HWM kits were demonstrating any behavioral or brainstem responses.

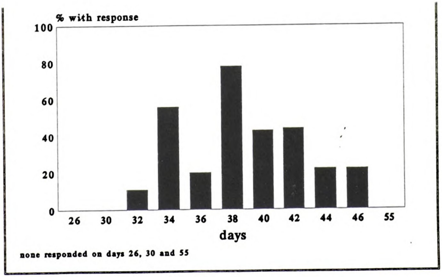


Figure 19. Percentage of HWM kits with Wave 1 ABR. Click at 90 dB.

Of the 12 HWM kits tested at the same timepoints as the seven HWM kits used in Experiment B, responses were not evident until 32 days of age, when one animal had a wave 1 response (Table 8). At day 38, this animal responded with all five waves, demonstrating comparable results to the seven kits in Experiment B where the "complete" ABR was seen for the first time also at 38 days.

Only one pure tone frequency was evaluated with any consistency in Experiment B. Because of equipment restrictions and the limited duration of the anesthesia, 8 KHz was often the only pure tone examined. Generally, very few of the HWM ever demonstrated an ABR to 8 KHz. Overall, the SDM displayed decreasing latencies over time comparable to latencies seen with the click stimulus (Table 18). Thresholds of responses to the 8 KHz stimuli were similar to those seen for the click stimuli.

Table 18. Latencies (in msec) of ABR waves of SDM over time. Stimulus 8 KHz at 90 dB.

MINK	AGE (days)	WAVE 1	WAVE 3	WAVE 5
C1A	26	--	--	--
	34	1.82	4.24	5.28
	38	1.34	3.00	4.96
	41	1.22	3.66	4.38
C1B	26	--	--	--
	34	1.50	3.28	5.20
	38	NA (a)		
	41	1.20	3.66	4.54

a : equipment failure

The following figures (22 through 40) are brainstem recordings collected from both SDM and HWM used in Experiment B. The responses have been reduced 50% so that relative comparisons of amplitude and latency may be made. The waveforms of interest are the top responses; these responses (to a 90 dB click stimuli) were duplicated and overlap each other.

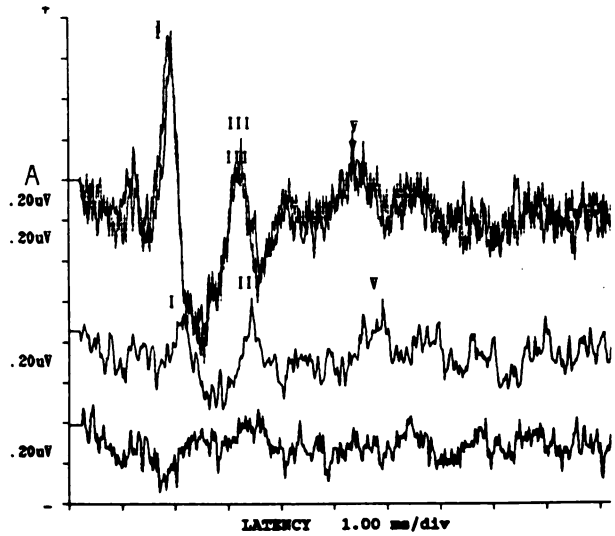


Figure 20. SDM (C1B) ABR at 26 days old. A: click at 90 dB.

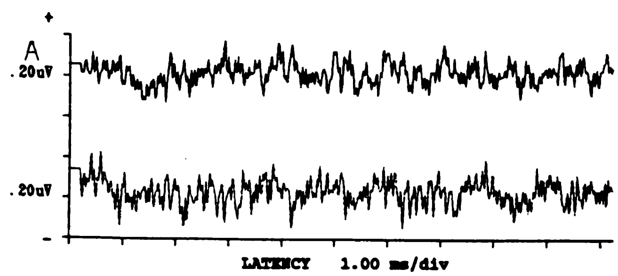


Figure 21. HWM (2A) ABR at 26 days old. A: click at 90 dB.

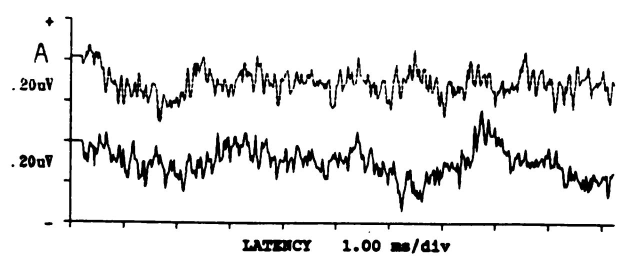


Figure 22. HWM (4B) ABR at 26 days old. A: click at 90 dB.

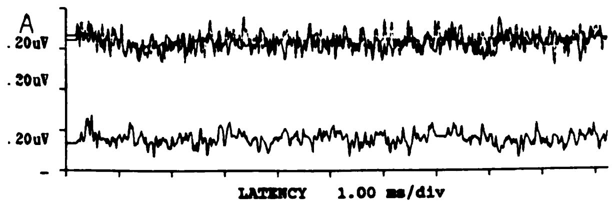


Figure 23. HWM (2B) ABR at 30 days old. A: click at 90 dB.

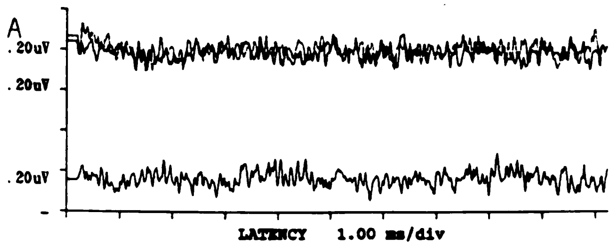


Figure 24. HWM (4B) ABR at 30 days old. A: click at 90 dB.

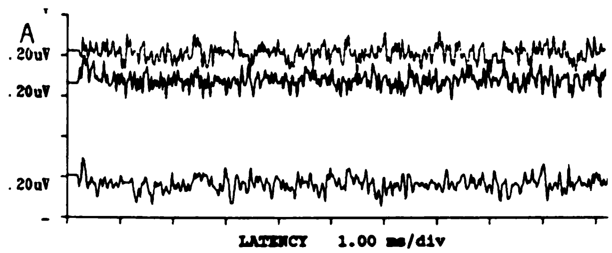


Figure 25. HWM (2B) ABR at 32 days old. A: click at 90 dB.

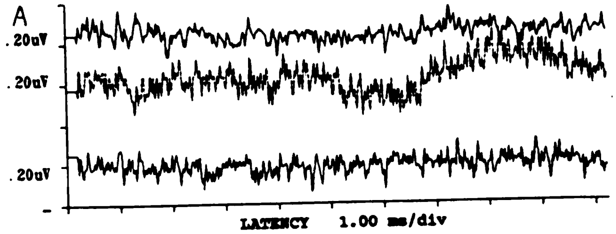


Figure 26. HWM (B1) ABR at 33 days old. A: click at 90 dB.

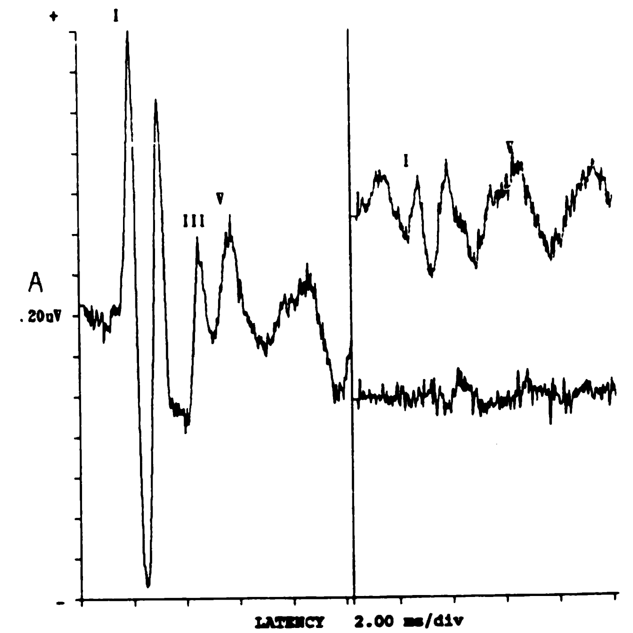


Figure 27. SDM (C1B) ABR at 34 days old. A: click at 90 dB.

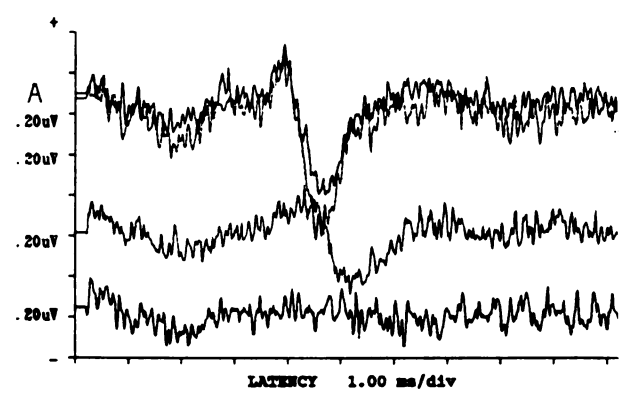


Figure 28. HWM (2B) ABR at 34 days old. A: click at 90 dB.

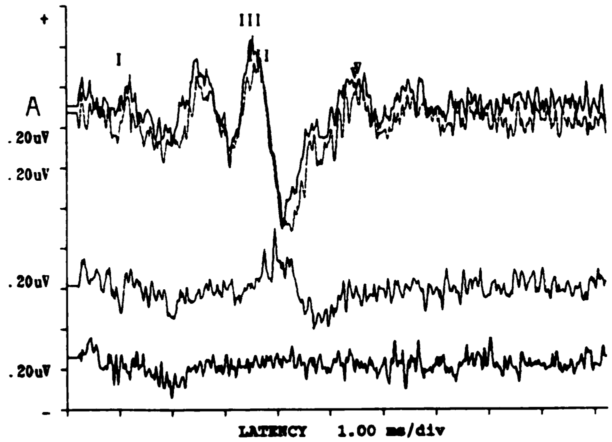


Figure 29. HWM (4B) ABR at 34 days old. A: click at 90 dB.

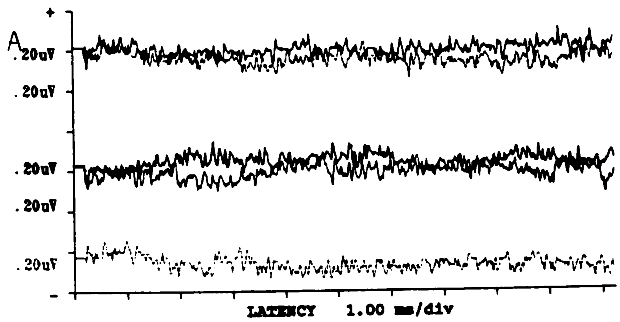


Figure 30. HWM (B1) ABR at 36 days old. A: click at 90 dB.

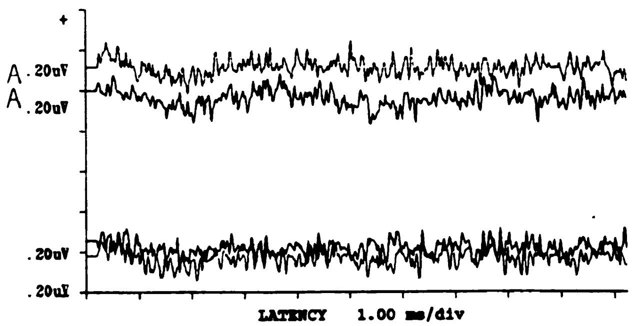


Figure 31. HWM (2B) ABR at 36 days old. A: click at 90 dB.

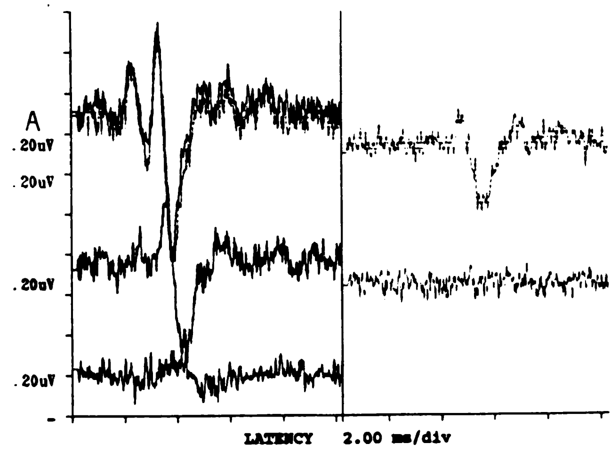


Figure 32. HWM (4B) ABR at 36 days old. A: click at 90 dB.

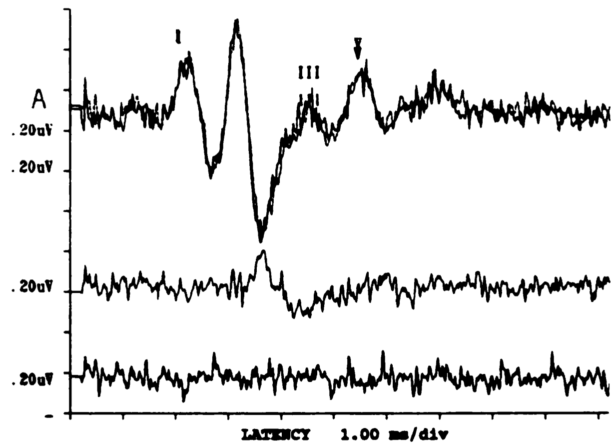


Figure 33. HWM (4B) ABR at 38 days old. A: click at 90 dB.

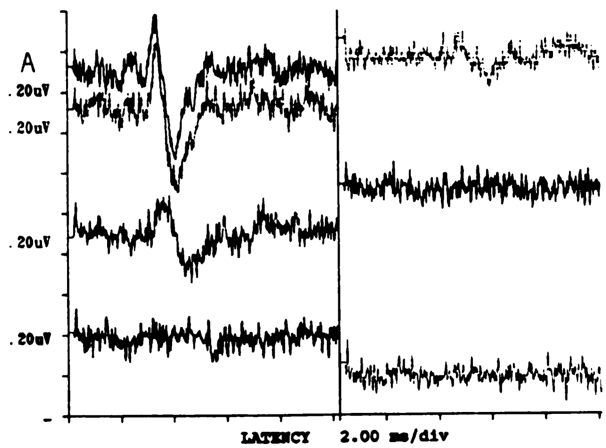


Figure 34. HWM (2B) ABR at 38 days old. Click at 90 dB.

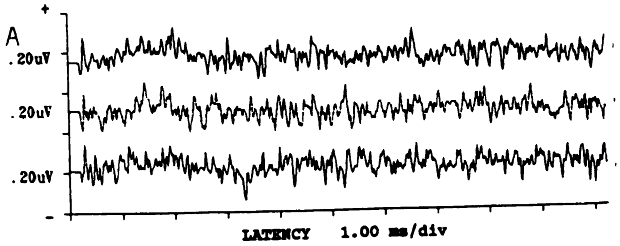


Figure 35. HWM (4B) ABR at 40 days old. A: click at 90 dB.

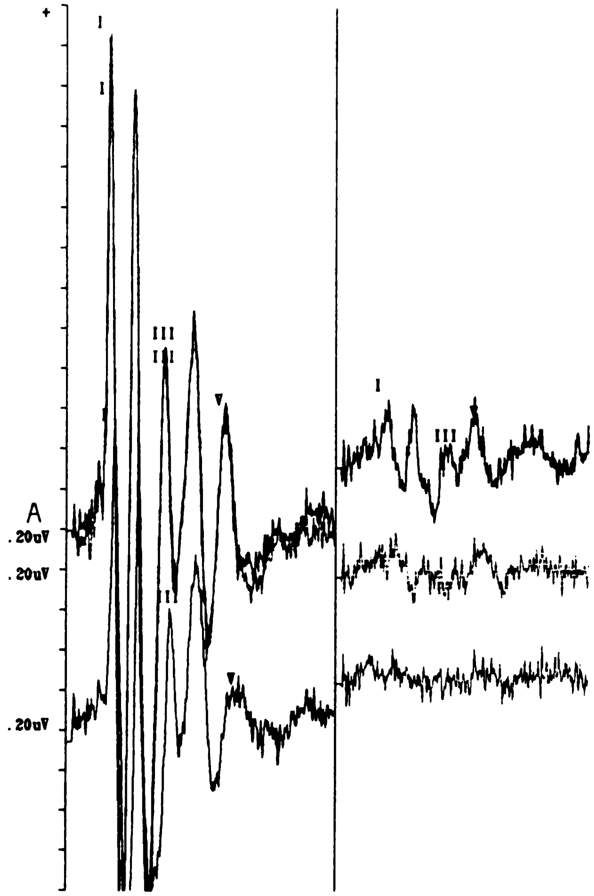


Figure 36. SDM (C1B) ABR at 41 days old. A: click at 90 dB.

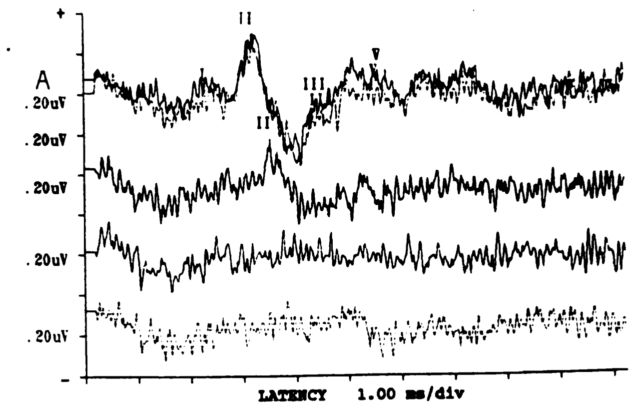


Figure 37. HWM (2B) ABR at 42 days old. A: click at 90 dB.

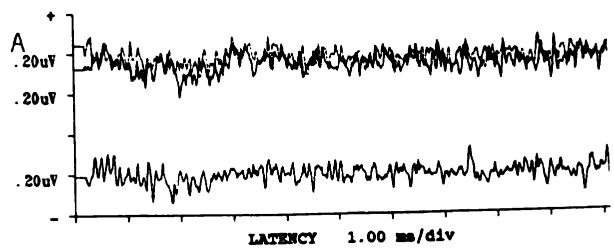


Figure 38. HWM (4B) ABR at 42 days old. A: click at 90 dB.

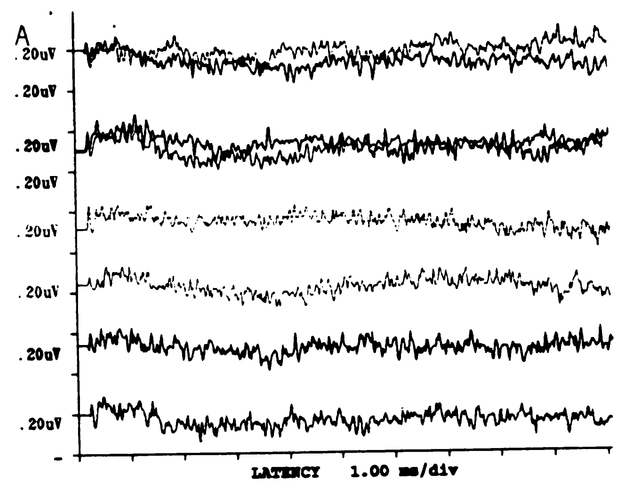


Figure 39. HWM (B1) ABR at 43 days old. A: click at 90 dB.

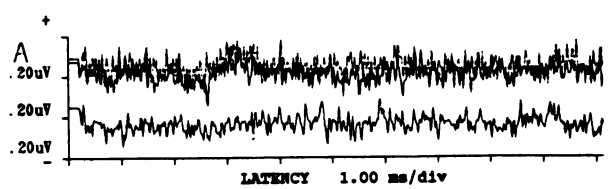


Figure 40. HWM (2B) ABR at 46 days old. A: click at 90 dB.

Discussion of the ABR Results

In the first two experiments (A and B), standard dark mink were tested and evaluated over time using auditory brainstem evoked response. The SDM exhibited ABR's whose morphology and latencies were consistent with those produced in other mammals. A study published by Bullock (1986) compared the ABR in a number of species ranging from perch to penguins to the human. He found that there was a similarity in far-field responses (including the ABR) in the number of waves produced and the latencies of the peaks. The non-mammalian vertebrates demonstrated a "strong commonality in the form of the early brainstem responses".

The "normal" mink responses appeared very similar to those found in the ferret (Mustela putorius). Kelly et al. (1989) described four prominent vertex positive peaks in the ferret. A fifth was often observed, but it was smaller in amplitude and more variable in latency. Mair and Laukli (1987) described a similar numbering system of waves in the cat, labelling them from 1 to 4 instead of 1 to 5 as described originally by Jewett in 1963. Their rationale was that as the human's eighth cranial nerve is almost one order of magnitude longer than the cat's, wave 2 in the human ABR was probably generated in the intracranial portion of the auditory nerve. Mair and Laukli concluded that wave 4 in the cat was probably the same as wave 5 in the human being, and so the generator site for each was probably the inferior colliculus.

Kelly *et al*. (1989) examined sixteen normally pigmented ferrets and found that at an intensity level of 60 dB SPL, the frequency range of the ferret varied from 36 Hz to 40 KHz. Prime sensitivity ranged from 8-12 KHz. This information is comparable to that for the mink, as published by Powell and Zielinski (1989) who stated that the frequency range in the mink extended up to 44 KHz and centered (with the highest sensitivity) around 1 to 16 KHz. Kelly *et al*. reported that the ferret's auditory system is comparably late in developing function, which also compares to information published about the mink and that found in this study. According to the authors, the onset of hearing in the ferret starts at approximately one month of age which is about the same as in mink as reported by Flottorp and Foss (1979) and found in Experiments A and B of this study. Although the auditory system was "late" in getting started, near-adult wave latencies and wave morphology were found in ferrets between six and seven weeks of age as in the SDM. Using a protocol similar to that used in Experiments A and B, Kelly *et al*. found that the ferret's mean latencies (in msec) to a click stimulus at 104 dB SPL were (wave 1) 0.96, (wave 2) 1.83. (wave 3) 2.75 and (wave 4) 3.62. The latencies found in the control SDM in this study were (wave 1) 0.93, (wave 3) 3.38 and (wave 5) 5.09 milliseconds. Although actual comparisons of ABR latencies cannot be accurately made due to differences in instrumentation, protocol and technical performance, a relative comparison may be of some value.

Kay et al. (1984) described the presence of four waves in the dog within the first five milliseconds following acoustic stimulation. Testing puppies of several breeds and using click stimuli at 5 to 25 dB NHL (based on the "normal" hearing level in 20 adult humans equaling approximately 38 dB SPL), they found no ABR until the pups were between two and three weeks of age (no response at two weeks, positive response at three weeks). By the time the puppies were seven weeks old, they showed adult ABR wave latencies (in msec) with means of wave 1 equal to 1.25 and wave 4 equal to 3.76. Marshall (1986) tested Dalmatians (adults and puppies) using ABR. In his study, if there was no waveform or only if the wave 4 - 5 complex was present, it was not included as a positive response. Therefore, the presence of the wave 4 - 5 complex could only be indicative of contralateral responses in the presence of ipsilateral cochlear pathology. Of the animals tested, Marshall found that 13 of 18 adult Dalmatians were either unilaterally or bilaterally deaf while 13 of 28 puppies were uni- or bilaterally deaf. All of the wave latencies in the dogs were within "normal limits", except for unilaterally deaf animals that had longer wave 5 and wave 6 latencies.

According to Marshall,

> "Adult Dalmatians responding only unilaterally had an increase of mean latencies for the 5th and 6th waves, probably representing caudal midbrain (5th wave) and thalamic (6th wave) activities. These increased latencies may represent evidence of brain stem auditory pathway degenerative changes (transneuronal degeneration) resulting from unilateral rather than

> normal bilateral afferent activity. These latency differences were not observed in the pups with unilateral hearing. Perhaps recording of the middle latency auditory-evoked potentials in such dogs would provide further evidence of changes in the rostral parts of the auditory system."

Studies on the ferret by D. Moore (1990) demonstrated that after bilateral cochlear removal, changes were found in the brainstem nuclei that were different than those found in unilateral conductive or cochlear loss.

Early work published by Hilding et al. (1967) and Sugiura and Hilding (1970a) indicated a failure of the Hedlund mink to respond behaviorally to hand claps or loud noise or electrophysiologically using round window recordings to respond to click stimuli. Dark ranch mink demonstrated Preyer's reflex by three weeks of age when exposed to whistles and loud noises. A definite N1N2 round window response was apparent by four weeks of age. At three weeks of age, the N1N2 response was only discernable after summation by a computer. Flottorp and Foss (1979) were the first to detect responses in both the normal (dark ranch) and the Hedlund white mink to acoustic stimulation. Using behavioral responses to evaluate hearing in both color varieties, they tested the animals using white noise, noise centered around 750 Hz and pure tones at 250, 500, 750, 1000, 2000 and 4000 Hz. The stimuli were 1 to 2 seconds in duration and ranged in intensity from 104 to 120 dB SPL. The dark mink were the first to respond at 29 days of age. The HWM followed at an average of 31 days of age. In Experiment A, the SDM showed a response for the first time between the fourth and fifth week; the SDM in Experiment B (used as normal controls) were tested for the first time at 26 days of age. Both kits demonstrated ABR's at that time. The differences in the age of response may be due to the use of click stimuli in Experiment B and pure tone stimuli in Experiment A. The click stimulates a large part of the cochlear duct, compared to pure tones which only affect a limited location in the frequency-specific cochlear duct. The HWM in Experiment A also showed discernable ABR's at five weeks of age. However, the results from a preliminary part of Experiment B failed to indicate any ABR's in HWM until one of the two kits was 38-days-old. Other data from Experiment B revealed that none of the kits tested (over time) demonstrated any brainstem response until 34 days old where only wave 1 was elicited in each of the kits tested. Flottorp and Foss found seven of eight HWM kits displayed a behavioral response to auditory stimuli between days 34 to 38; the eighth kit never showed any response at any point during the testing period which was terminated when the kits were 41 days old. They reported that hearing remained for approximately seven days (range of 2 to 10 days) in the HWM kits before degenerating to no behavioral response. This "window" was slightly broader in Experiment B. In this study, the ABR wave 1 was evident in HWM kits from 32 to 46 days of age. By 55 days old, all HWM kits tested in Experiment B were assumed deaf, as no ABR's could be elicited.

Flottorp and Foss (1979) reported the presence of behavioral responses to click stimuli in 17 of 18 HWM whereas only 3 out of 18 responded to a pure tone of 4 KHz. They concluded that "hearing at this high frequency seems to be acquired relatively late in standard (dark) mink and probably not at all in many Hedlund mink". These authors also reported that deterioration occurred tonotopically following "the inverse order of inception of hearing". In Experiment B, it was not possible to test all the animals with various acoustic stimuli. Because of the restrictions of equipment (no higher than 8 KHz pure tone pips), low accessibility of instrumentation (due to priority of clinical use) and limitations in "usable" level of anesthesia, only the click stimulus was used consistently in each of the animals tested. The other primary difference in the testing regime was that the auditory stimulus used by Flottorp and Foss was approximately 1 to 2 seconds in duration compared to 10 milliseconds for the ABR. However, the brainstem response should be a more sensitive way to detect the function of the auditory system because a positive response is not dependent on a behavioral reaction.

Because of the individual variation in the brainstem response of the HWM, it was necessary to evaluate each of them separately. Of the seven HWM kits tested in Experiment B, only three demonstrated a wave 1 response to a 90 dB click beginning on day 34 (Table 5-7). One of the kits (mink 4A, Table 12) responded only on day 34. The other two kits (mink 2B and 3A) did not respond on day 36, but did again respond on day 38. By day 42, HWM kit 2B showed an increase in the wave 1 latency when compared to the previous tests and then wave 1 disappeared altogether by 46 days of age. Four of the other kits (2A, 2C, 3B, 4B) exhibited waves 1,3 and 5. In three of the four, the presence of a "complete" ABR was preceded by a day (or more) of just the presence of wave 1. One of the four (2C) showed the reverse, as the full ABR was followed by a test day revealing only wave 1.

As can be seen in Table 20, the means of the latencies of waves 1 and 3 were generally out of the range of the accepted two standard deviations from the control means. Because of the presence of wave 1 (when other waves were not present), a major question was raised concerning the pathology of the HWM. Initially, the wave was considered to be too long in latency to be wave 1. One source (Hulce, 1991) felt that it should be discarded as an artifact, as the exact generator site was impossible to determine. Although the latencies for the HWM were often much slower than the control latencies, there was little else that could have generated that waveform. After consultation with audiologists at Kresge Hearing Research Institute and the Michigan State University Department of Audiology, it was concluded that the waveform was probably a delayed wave 1. The delay was most likely due to cochlear degeneration that was not yet severe enough to cause complete failure of the cochlea to transduce mechanical energy into neural energy. As will become apparent later in the discussion concerning morphologic changes, the cochlear changes occur in the cat (blue-eyed) and dog (Dalmatian) locally, beginning at the basal portion and moving up toward the apical turn of the cochlea. Therefore, there may have been enough cochlear function to generate a potential in the HWM kits, but it may have been only the apical end. This would partially explain the latency delay which would be due to the physiologic increased travel time for the action potential to move from the cochlear apex to the far-field recording site.

Table 19. Mean latencies (in msec) of ABR waves in HWM over time and SDM controls.

DAY	W1 #	W1 AVE.	W1 SD.	W3 #	W3 AVE.	W3 SD.	W5 #	W5 AVE.	W5 SD.
HWM									
26	0/8			0/8			0/8		
30	0/9			0/9			0/9		
32	1/9	2.80 a		0/9			0/9		
34	5/9	2.62 a	0.5	0/9			0/9		
36	2/10	2.58 a	0.14	0/9			0/9		
38	7/9	2.04 a	0.73	2/9	3.64 a	0.06	2/9	4.72	--
40	3/7	1.32 a	0.01	3/7	3.50 a	0.05	3/7	5.13	0.7
42b	4/9	1.46 a	0.57	3/9	3.18 a	0.20	3/9	5.11	0.7
43	0/3			0/3			0/3		
44	2/7	1.19	0.21	2/7	3.32 a	0.29	2/7	4.99	0.6
46	2/7	1.80 a	0.71	1/7	3.92 a	--	1/7	5.40	--
55	0/7			0/7			0/7		
SDM	8/8	0.93	0.17	8/8	2.53	0.28	8/8	4.62	0.5

represents the number of animals with ABR's over the number of animals tested. (a: latency greater than two standard deviations from control latencies; b: HWM showing no ABR were not tested after 42 days of age)

To more clearly determine if wave 1 was actually being generated, it would be necessary to conduct additional electrophysiologic testing. According to Glasscock et al. (1987), it is often very difficult to get clearly defined wave 1's on the ABR. To circumvent this, it was suggested that "dual recordings" of both the eighth nerve action potentials and the ABR be collected. A dual recording system would be necessary to collect information from an electrode on the cochlear promontory and an electrode on the mastoid. The disadvantage of this procedure for the detection of the eighth cranial nerve action potential detection is that it is invasive since electrode placement directly on the cochlea is required.

Electrocochleography would be another option, but again invasive procedures would be required. Cochlear microphonics (CM) can be conducted by using an "insert" electrode (available for the human) that fits in the external auditory meatus, but it must be remembered that CM are only present when the OHC's are normal. There is presently no easy way to directly measure either the integrity of the cochlea (via electrocochleography) or the eighth cranial nerve (via action potentials) without invasive methods.

The reason why only wave 1 (or 2) appeared in several of the kits is not clear. Sims (1987) reported that waves 1 and 2 were first seen during postnatal maturation of the human being which also may be the case in the mink.

Schweitzer (1987) also reported the presence of waves 1 and 2 in the 18-day- old hamster. By 22 days of age, all five waves were seen. However, the SDM used in this study presented an "all or none" ABR when tested at four and five weeks of age. The SDM may not have been tested early enough or at a close enough time interval (age) to determine if their hearing develops with the presence of wave 1 followed by the rest of the ABR within a given time frame.

An obvious question revolves around the failure of some of the HWM kits to ever exhibit an ABR beyond wave 1. It must be remembered that the ABR is dependent on both the cochlea and the neural pathways. Both must be functioning to generate a complete ABR. The neural transmission in the HWM may not have been strong enough or had enough "synchrony" to generate a detectable response along the complete auditory pathway. Some of the HWM kits may not have had a sufficient number of cochlear neurons innervated at the same time to be detected as a brainstem response using far-field recordings.

Failure to demonstrate an ABR response beyond wave 2 can be indicative of brainstem pathology. According to Glasscock et al. (1987), the presence of only waves 1 and 2 can "indicate problems in the brainstem neural conduction system". Problems such as an acoustic tumor would restrict the passage of the neural impulse to higher centers. This was obviously not the cause of the variability observed in the HWM kits. The American Electroencephalographic Society

(Anonymous, 1986) determined criteria for diagnosing retrocochlear dysfunction in part including the absence of all waves following waves 1 or 3. A more plausible explanation for the absence of the later waves might be degenerative changes occurring in the brainstem along the auditory pathway. Glasscock et al. (1987) described human patients with multiple sclerosis (demyelinating disease) having only wave 1 in their ABR's. A number of investigators examining the blue-eyed white cat found changes within the brainstem auditory centers. Elverland and Mair (1980) described the presence of myelin degeneration occurring before neuronal loss in the spiral ganglion. They noted that demyelination occurred in the type 1 neurons (which contact the IHC) resulting in eventual degeneration of neurons and an increase in extracellular interstitial spaces in the spiral ganglion. Schwartz and Higa (1982) reported that the changes in the spiral ganglion occurred after lesions were noted in the medial superior olive. These changes were found to occur about the same time that degenerative changes were occurring in the cat's organ of Corti. Degenerative changes in the spiral ganglion did not become apparent until several months after lesions were noted in the superior olive and the organ of Corti. They concluded that the degree of changes in the spiral ganglion could not be used as a guide to estimate damage in the central pathways as the neuronal degeneration within the spiral ganglion probably resulted from ascending atrophy.

Gerwitz (1991) described similar findings in the Dalmatian dog. Neuronal degeneration in the brain was found at about the same time that changes were noted in the organ of Corti. It would probably be safe to assume similar lesions may occur in the HWM. This would explain the absence of ABR waves beyond the first wave. It is most likely that concurrent degenerative changes occur in HWM in both the brainstem auditory pathway, as well as changes in the cochlea. An experiment in which both the eighth cranial nerve and the central auditory neural centers are examined in the HWM should be conducted before any definite conclusions are made.

The most perplexing problem with interpreting the ABR's of the HWM comes in evaluating the wave 5 latencies. When analyzing the latency means, although both waves 1 and 3 were delayed, none of the wave 5 latency means were delayed when compared to the normal limits established using the guidelines set by the Electroencephalographic Society. The fifth wave is described as the most robust and dependable to use for analysis in many species. Prosser and Arlan (1987) used wave 5 latencies as a prediction of hearing loss caused from retrocochlear lesions (especially tumors). Glasscock et al. (1987) felt that a prolonged wave 5 latency was one of the best ways to detect eighth nerve dysfunction. If only wave 5 latencies had been used to evaluate the auditory system of the HWM, "false positive" results may have led to the conclusion that the HWM auditory system was normal.

That not being the case, the question then arises: Why do the HWM, while showing delayed wave 1 and 3 latencies, have "normal" wave 5 latencies? Occasionally, while calculating an average for a group of numbers, a large range with few numbers can skew the data. Although very few of the HWM had an apparent wave 5, only one of the individuals was out of the accepted range. This value was only off by 0.02 milliseconds. A possible explanation for the normal wave 5 latencies in the HWM could be binaural interaction of both the ipsilateral and contralateral ears receiving the acoustic stimulus and then transmitting it along the brainstem pathways. Although the ipsilateral ear was "defective", as illustrated by the delayed latencies of waves 1 and 3, the contralateral ear may have somehow detected the signal and relayed it to the region of the inferior colliculus at a normal speed. This explanation is very weak in that even though only one ear was tested , the contralateral ear was masked with continuous white noise. Although the assumption of bilateral effect was made (with both ears being affected to the same degree and in the same way), that assumption could possibly be correct if the sensitivity of the contralateral ear was able to overcome the masking noise and detect the click stimulus. Testing a number of HWM kits in both ears may help explain the normal latency of wave 5.

ASSESSMENT OF MINK COCHLEAR MORPHOLOGY

Research Design: Experiment C - Morphologic Changes Over Time

Twelve HWM and two SDM were used in Experiment C. These animals were tested at the same timepoints as the animals in Experiment B. Following electrophysiologic evaluation, the animals were euthanized and their cochleas examined microscopically to allow comparison between changes in the HWM and those reported in other species with similar pigment-associated hearing loss.

Collection of Cochleas

In order to insure histologic samples that had minimal autolytic changes, the cochleas were harvested using systemic and perilymphatic perfusion methods. The mink were deeply anesthetized using the formulation described under the electrophysiologic methods. Once a deep plane of anesthesia was verified, systemic perfusion was completed. The steps used, as outlined below, are similar to those described by Hawkins and Johnsson (1975) and instruction received from personnel at the Kresge Hearing Research Institute, University of Michigan. The steps were:

1. opened thoracic cavity with midline incision extending from the thoracic inlet to the xiphoid process
2. used rib cutters to expose the heart and lungs
3. used vascular clamps to clamp off the posterior vena cava and descending aorta close to the heart
4. an incision was made in the left ventricle near the apex of the heart (The incision was long enough to allow passage of perfusion tip and tubing or a catheter up into the ascending aorta. If a needle was used, an incision was not necessary)
5. a small incision was made in the right atrium to release some of the pressure and allow the blood to exit the system
6. using a perfusion pump or gravity flow system, the rate of perfusion was regulated to provide a moderate flow (not too slow to restrict proper perfusion or too fast to damage tissue)
7. the vascular system was pre - washed with physiologic saline or phosphate buffer to remove all the blood. The flow continued until clear fluid exited the right atrium
8. perfusion with the fixative was then started and continued until the viscera and tissues began to "harden" and show signs of fixation. Fixatives used included paraformaldehyde, formaldehyde or glutaraldehyde
9. after systemic perfusion was completed, the cochleas were removed and perilymphatic perfusion methods started.

To remove the cochlea from the animal, the tympanic bullae was located and the excess tissue removed. The bullae was opened using small Rongeur forceps to expose the middle ear. At this point, the middle ear was examined to verify that it was air-filled and did not contain any serous or mucoid exudate. Once the temporal bone was exposed (by removing excessive tympanic bullae), the round window and the cochlear promontory were easily visualized. The petrous temporal bone containing the cochlea was removed from the skull and further "processed" using the following procedures.

1. isolation of the cochlea and disarticulation from the surrounding bone
2. location of the round window at the basal end of the cochlea
3. probing (carefully) with a fine point to "open" the window by removing the membrane
4. locating the oval window
5. careful removal of the footplate of the stapes with a fine forceps or rupture of the membrane to "open" the window
6. introduction of the fixative using a fine pipette into the round window, allowing it to perfuse through the perilymphatic spaces in the scalas vestibuli and tympani and exiting through the oval window
7. opening the tip of the apex by using a sharp probe or Rongeur forceps
8. during perfusion, care was taken to not introduce any air bubbles into the cochlea
9. after the perilymphatic perfusion was completed, the cochlea was submerged into fixative and stored until further processing for histologic examination.

Embedding Protocol

Once the cochleas were collected and perfused, they were allowed to fix further in 2% glutaraldehyde in 0.1 M phosphate buffer for one hour at 4 degrees Celsius (C). After fixation, they were held at 4 degrees C. Before further processing, they were post-fixed in 1% osmium tetroxide in 0.1 M phosphate buffer for one hour at 4 degrees C. Then the cochleas were rinsed several times with 0.1 M phosphate buffer before continuing. Once the cochleas were fixed, they had to be decalcified to allow sectioning of the otic capsule and inner membranous structures to acquire mid-modiolar cochlear sections. Using "slow" decalcification methods, they were kept in 4% ethylenediamine tetracetate (EDTA) in distilled water for several weeks. The solutions were changed daily and the cochleas stored on rotors to help decrease the time required for removal of the calcium and soften the otic capsule. Removal of the EDTA solutions was completed by rinsing the cochleas in distilled water for at least 48 hours.

Once the cochleas were decalcified, they were processed using a protocol established at Kresge Hearing Research Institute. The resin used was Embed-812 (Ted Pella, Inc.; Redding, CA) and the process was as follows:

1. after dehydration to 100% ethanol, the tissues were placed in propylene oxide for 2, 10 minute periods
2. next, they were placed in 1 : 1 propylene oxide: resin for at least 4 hours (best overnight)
3. they were then placed in 1 : 4 propylene oxide: resin for at least 4 hours (best overnight)
4. they were placed in 100% resin for about 4 hours
5. the cochleas were then transferred to molds which had a small amount of new resin placed in the bottom and allowed to "set" for about 15 to 20 minutes in an oven at 60 degrees C
6. the cochleas were then oriented in the molds and allowed to polymerize in an oven at 60 degrees C for at least 36 hours
7. at each stage of the processing, it was necessary to try to pipette the solution (being careful not to introduce any air bubbles) through the perilymphatic spaces to facilitate perfusion completely through the cochlea.

Initially the blocks were trimmed with a razor blade and oriented to allow sectioning of the length of the cochlea. Using 6 mm thick glass knives and an LKB Microtome III ultratome (Deerfield, IL), three to six micron-thick sections were taken until the desired mid-modiolar orientation was achieved. One to two micron thick sections were then collected, transferred to a drop of 50% ethanol on a glass slide and allowed to heat for a few minutes on a 60 degrees C hotplate. After the fluid evaporated and the tissue was firmly attached to the slide, the cochlear sections were stained with 1% toluidine blue. The slides were then coverslipped using a permanent mounting medium. The slides were examined microscopically and photomicrographs taken through a light microscope.

Evaluation of Intercanthal Distance

An attempt to make comparisons of the physical characteristics described by Waardenburg (1951) for human beings with inherited hearing deficits with those found in HWM was made using measurements of intercanthal distances in the mink. Calipers were used to measure the distance (in mm) between the medial canthi of each eye in nine-day-old SDM and HWM kits. In order to accommodate differences in size of the kits, body weights were used to establish a ratio of intercanthal distance (in mm) and body weight of the kit (in g).

Results of Experiment C

Comparison of Intercanthal Distance

Comparison of the physical characteristics described by Waardenburg (1951) for humans with inherited hearing deficits with those found in HWM revealed that there were essentially no differences in the intercanthal distances between SDM and HWM. By comparing the 18 HWM and 14 SDM kits at nine days of age, the ICD/BW ratios were very similar as the means were 0.406 (SD = 0.086) for the HWM and 0.473 (SD = 0.059) for the SDM. Results for the individual mink are presented in Table 19.

The other characteristics defined by Waardenburg were much more difficult to evaluate. Using simple observation, it was not possible to discern the presence of a broad nasal root and hypertrichosis in the HWM. Partial albinism or a white forelock was obviously not apparent, as the HWM were completely white in color. Heterochromia iridum was not observed in any of the HWM in the herd; the iridal color in all of the mink was dark. The only other characteristic Waardenburg described in humans was partial or complete deafness which was demonstrated in all of the HWM.

Table 20. Intercanthal distance (mm)/body weight (g) ratios of nine-day-old HWM.

TYPE	LITTER	SEX	ICD (mm)	BW (g)	RATIO
HWM	FH902	F	11.55	45.8	0.252
		F	13.50	36.3	0.372
		F	14.56	41.3	0.353
		M	14.55	50.9	0.286
	EH412	M	14.56	39.9	0.365
		M	13.52	35.2	0.384
		M	12.50	37.4	0.334
		M	14.54	36.1	0.403
		M	13.51	36.6	0.369
		M	13.52	35.7	0.379
	EH402	M	12.46	30.2	0.413
		M	11.42	18.8	0.607
		F	11.45	21.2	0.54
		F	11.46	22.6	0.507
		F	12.49	28.2	0.443
		F	12.50	30.5	0.410
		M	13.52	31.7	0.427
		F	13.50	28.7	0.47

Table 21. Intercanthal distance (mm)/body weight (g) ratios of nine-day-old SDM.

TYPE	LITTER	SEX	ICD (mm)	BW (g)	RATIO
SDM	F832	M	9.50	18.6	0.511
		M	13.56	33.8	0.401
		M	13.52	29.7	0.455
		M	13.52	27.0	0.501
		F	10.51	25.0	0.420
		F	13.52	26.4	0.512
		M	14.54	27.4	0.531
		F	14.55	24.8	0.587
	Z906	M	11.44	28.4	0.403
		F	12.46	24.5	0.509
		F	12.50	32.0	0.391
		M	13.52	32.0	0.423
		F	12.48	25.6	0.488
		M	13.58	27.4	0.496

Cochlear Morphologic Findings

The results of the morphologic examination of the HWM at varying timepoints were limited. Although only ten cochleas proved to be useful in evaluating the morphologic changes observed in the HWM, the changes were consistent with those reported in the HWM as well as the white cat and Dalmatian dog. It was not possible to visually examine the saccule of the vestibular system in the mink because of the priority for observation of the cochlear changes.

The mink has approximately 3.5 turns to the cochlea. The hook at the basal end and the helicotrema account for an additional half turn; the full turns are referred to as the basal, middle and apical turns. The SDM cochleas appeared comparable to other mammalian inner ears, so the morphologic descriptions presented here refer only to changes found in the HWM over the course of time. Morphologic examination was restricted to the light microscopic level, as the quality of sections did not allow any higher level inspection. Although some artifactual changes existed, pathologic changes along the entire cochlear duct were evident in a HWM kit at 30 days of age, as shown in Figure 41. The apical, middle and basal turns are depicted using mid-modiolar sections. There appeared to be a start in the collapse of Reissner's membrane (and the cochlear duct) in the basal turn of the kit. Although the tectorial membrane was pulled back slightly, the change became progressively more pronounced as can be seen in Figure 42. These photographs show the middle turn of a 30-day-old and a six-month-old HWM kit. In the older kit (Figure 42), Reissner's membrane was completely collapsed, the support cells had degenerated beyond the point of recognition and the sensory cells were still visible but were very abnormal in appearance. The tectorial membrane had retracted and "rolled up" in the inner sulcus and was covered with the collapsed Reissner's membrane.

Figure 41. Various turns of the cochlear duct of a 30-day-old HWM kit. A : apical turn; B: middle turn; C: basal turn; R = Reissner's membrane; S = stria vascularis; T = tectorial membrane. (100 x, stained with 1% toluidine blue). Note the sagging Reissner's membrane in the basal turn.

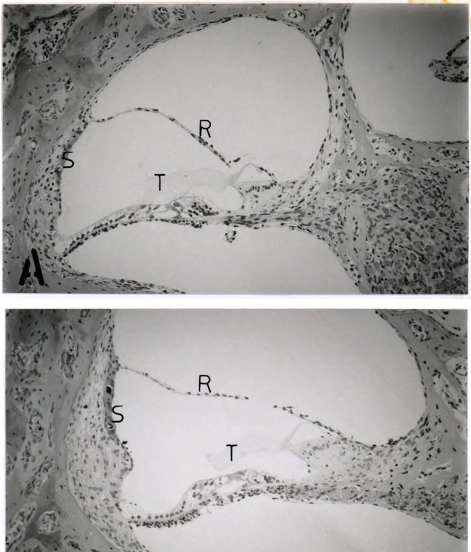
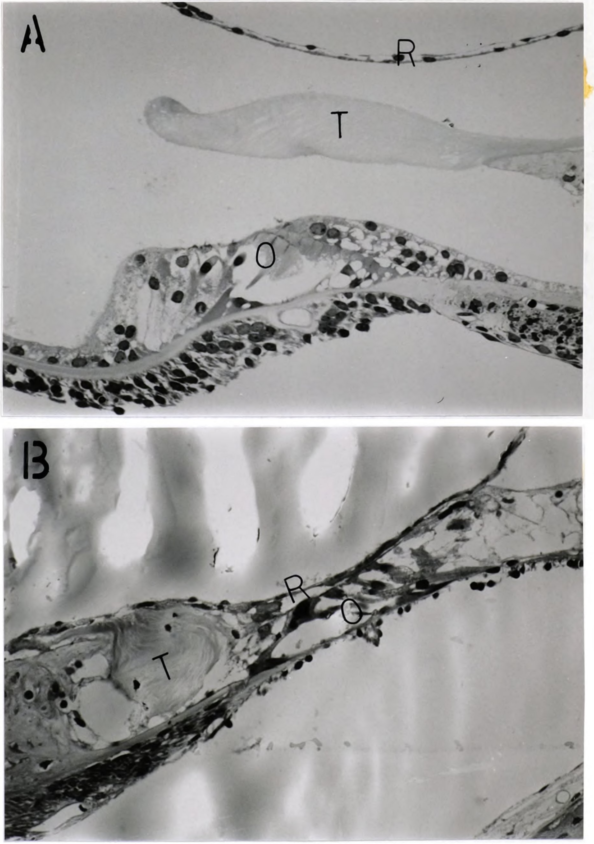

R
S
T
A
R
S
T
B
S
R
T
C

Figure 42. HWM cochlear duct changes over time. A: 30 day old; B: 180 day old. T = tectorial membrane; R = Reissner's membrane; O = organ of Corti. (250 x, stained with 1% toluidine blue). Note the loss of the cochlear duct evidenced by the collapse of Reissner's membrane as well as the degeneration in the organ of Corti and the curled tectorial membrane in the 180-day-old HWM.


A
R
T
O
B
R
O
T

The initial changes seen in the stria vascularis were apparent by day 30 in HWM kits. Using a comparison between the turns in a cochlea, the same "level" of the turn was examined and photographed at the same magnification. The area of the stria vascularis closest to the attachment of Reissner's membrane was the area of focus in Figure 43. As can be seen, the stria vascularis in the apical turn was thinner and shorter when compared to the stria vascularis in the basal turn. The marginal cells appear to be thinner and less dense, and fewer capillaries were visible in the apical stria vascularis.

Comparison of the stria vascularis of the middle turn of the HWM, using the same magnification, revealed only minimal differences (Figure 44) in the 30- and 38-day-old kits. There was, however, a dramatic change in the 180-day-old HWM. The stria vascularis of the 180-day-old SDM showed that the marginal cells were very dark and thick. The 180-day-old HWM stria appear much thinner, have an overall "shortened" row of marginal cells and a collapsed Reissner's membrane. The cells located near the otic capsule appeared to become more vacuolated as the animal aged.

Figure 43. HWM (30-day-old) cochlear stria vascularis at different sites along cochlear duct. A: apical turn; B: middle turn; C: basal turn. R = Reissner's membrane; S = stria vascularis; L = spiral ligament. (250x, stained with 1% toluidine blue). Note the lighter staining cells in the stria vascularis in the apical turn.

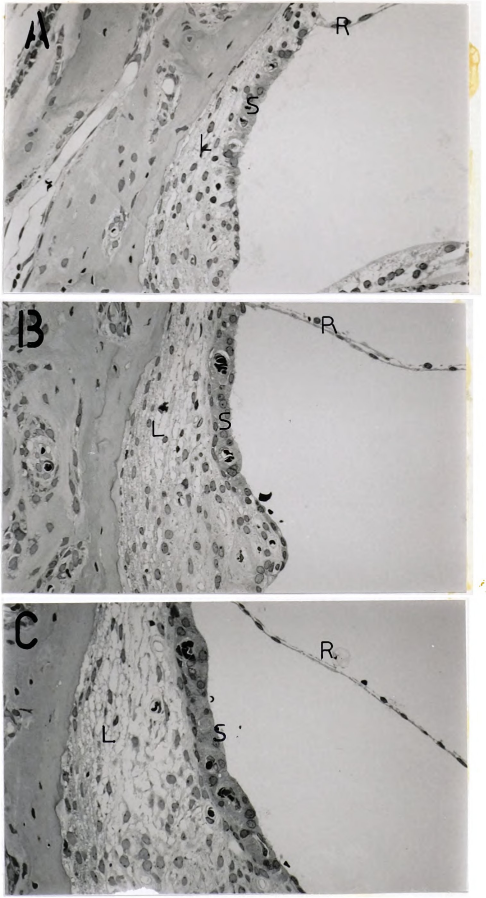
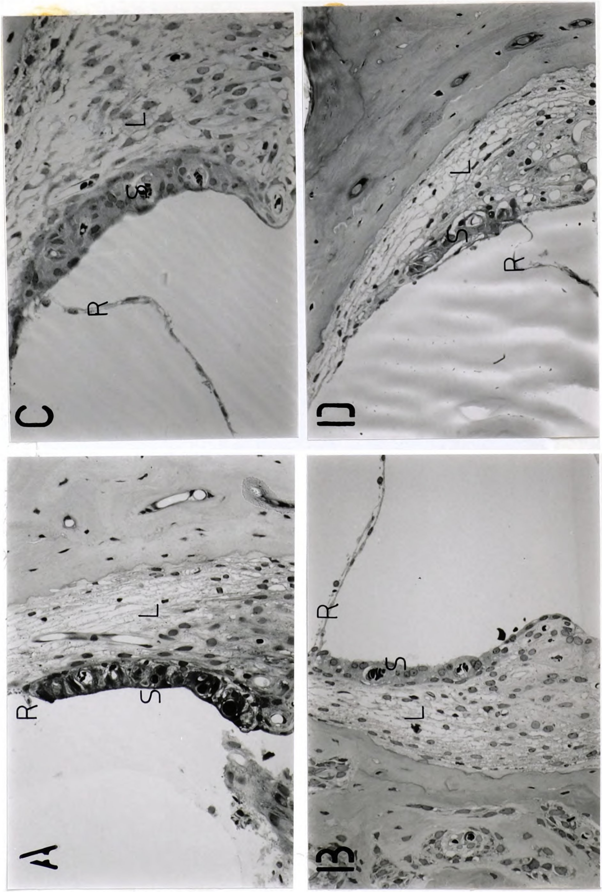

A
R
S
L
B
R
S
L
C
R
L
S

Figure 44. Temporal changes of HWM stria vascularis (middle turn). A: SDM 180 days; B: HWM 30 days; C: HWM 38 days; D: HWM 180 days. R = Reissner's membrane; S = stria vascularis; L = spiral ligament (250x, stained with 1% toluidine blue). Note the contrast between the stria vascularis of the SDM and the 180-day-old HWM; the strial cells appear much thinner, vacuolated and are generally "shortened" in appearance.


C
L
S
R
D
L
S
R
A
L
S
R
B
R
S
L

The spiral ganglion of HWM showed drastic changes over time. The morphologic differences between a 180-day-old SDM (control) and 26-, 30- and 180-day-old HWM kits' spiral ganglions located at the middle turn, mid-modiolar area are in Figure 45. The SDM kit demonstrated the "normal" neuronal population within the spiral ganglion. Differences were already apparent in both the 26- and 30-day-old HWM kits. The concentration and the density of the HWM ganglion neurons were decreased when compared to the SDM. The 180-day-old HWM showed severe atrophy and depletion in the number of spiral ganglion neurons. The nuclei were almost unrecognizable except for a few in the center that are surrounded by large perinuclear vacuoles. It is probable that the intranuclear space becomes filled with fibrous connective tissue.

Although a comparison between electrophysiologic results and morphologic changes was attempted in the HWM, success was limited due to problems in histologic processing. Both ABR's and photomicrographs of the cochlear duct from three HWM at different ages are found in Figure 46. No response was detected in either the 30-day or the 180-day old HWM, obvious differences were noted in the scala media. An ABR was detected in the 38-day-old HWM kit (using a 90 dB click stimulus), even though minor changes were observed in the cochlear duct.

Figure 45 : Temporal changes of HWM spiral ganglion (mid - modiolar section, middle turn). A: SDM 180 days; B: HWM 26 days; C: HWM 30 days; D: HWM 180 days. (250x, stained with 1% toluidine blue). Note the decrease in the number of neurons in the spiral ganglion of the 180-day-old HWM when compared to the SDM.

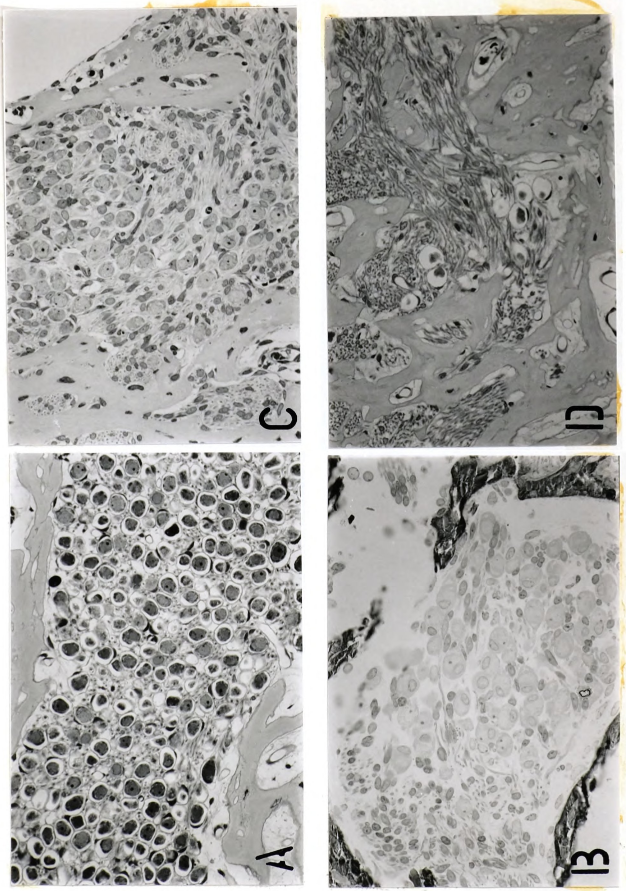
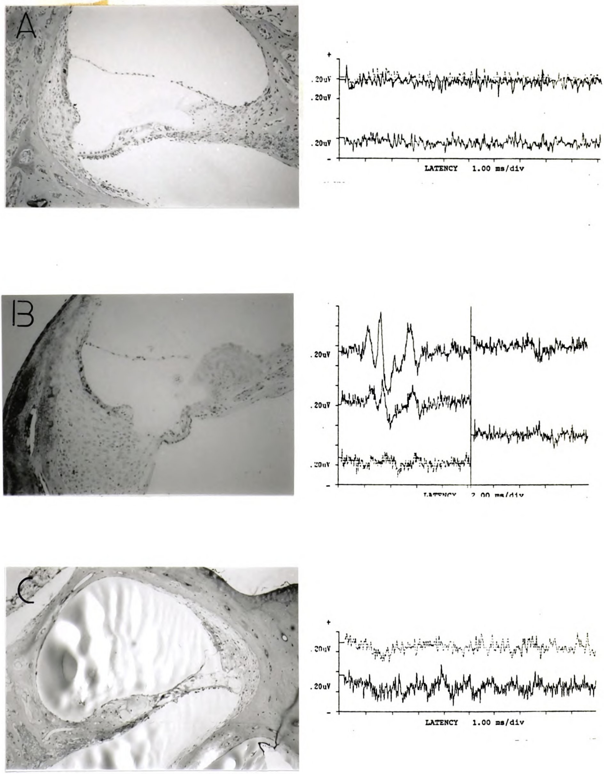

C
D
A
B

Figure 46. Electrophysiologic responses and morphologic features of the cochlear ducts in HWM over time. A: HWM 30 days; B: HWM 38 days; C: HWM 180 days. Note the presence of an ABR in the 38-day-old HWM.


A
.20uV
.20uV
.20uV
LATENCY 1.00 ms/div
B
.20uV
.20uV
.20uV
C
.20uV
.20uV
LATENCY 1.00 ms/div

Discussion of Morphologic Results

Other than the hereditary cochleosaccular deafness described in the mink, there is only one other known "syndrome" that affects the auditory system of mink. This syndrome called "screwneck" affects pastel-colored mink. The name "screwneck" describes the appearance of the animal afflicted with it. Erway and Mitchell (1973) and Deol (1980) described a recessive trait that causes a loss or complete lack of otoliths within the maculae of the vestibular system. The saccule is drastically affected, followed by loss of the otoliths in the utricle. The semicircular canals are not affected.

Comparison of the physical characteristics described by Waardenburg (1951) for human beings with inherited hearing deficits with those found in the HWM revealed that there were essentially no differences in the intercanthal distances between the SDM and HWM.

The other characteristics defined by Waardenburg were more difficult to evaluate. Using simple observation, it was not possible to discern the presence of a broad nasal root or hypertrichosis in the HWM. Partial albinism or a white forelock was obviously not apparent, as the HWM were completely white in color. Heterochromia iridum was not observed in any of the HWM; the iridal color in all of the mink was dark. The only other characteristic Waardenburg described in the human beings was partial or complete deafness which was demonstrated in all of the HWM.

As mentioned in the results, a temporal comparison of changes in the electrophysiologic and morphologic characteristics of the HWM was difficult due to the poor quality of the tissue sections. However, the results were "pieced" together and appeared to correspond with descriptions of morphologic changes found in the literature regarding the HWM mink, blue-eyed white cat and Dalmatian dog.

The lesions in the HWM were probably most consistent with Waardenburg's syndrome type 2 in humans where the primary characteristics were hearing loss or deafness without dystopia canthorum. Balkany and Pashley (1986) described 53% of a human population with type 2 having bilateral deafness. Type 1 had only 28% bilateral deafness. The HWM showed no signs of obvious dystopia canthorum and a very high incidence of deafness among the resident population.

The principle morphologic changes are quite consistent across the species in which a pigment-associated genetic deafness has been described. An overall description and comparison of morphologic changes seen in these animals corresponds with that listed earlier in the literature review of cochleosaccular degeneration of the blue-eyed white cat. Hilding (1973) described severe cochleosaccular degeneration with collapse and rupture of the saccule and collapse of the scala media of HWM. Sugiura and Hilding (1970a) described minimal changes in the lining of the endolymphatic sac and duct of HWM, but found dramatic changes in the saccule wall by 3 weeks of age. Eventually, otoconia were noted to be missing from the lumen of the saccule, the sensory epithelium degenerated and the wall thickened and ultimately folded over on itself. Saunders (1965) found the cochlear duct to be completely collapsed in 180-day-old HWM as evidenced by a thickened, hyalinized Reissner's membrane adhering to both the tectorial membrane and the stria vascularis. Hilding et al. (1967) and Sugiura and Hilding (1970a) described essentially the same findings. Experiment C of this study revealed similar findings as depicted in Figure 46.

Several researchers reported comparable findings of lesions in the organ of Corti and the tectorial membrane. Sugiura and Hilding (1970a) found the tunnel of Corti to be opened in the 3-week-old HWM kit while the cochlea appeared almost mature at that age. By 46 days of age, Foss (1969) found that the organ of Corti had decreased in size with collapse of Reissner's membrane occurring after 46 days of age in the HWM. This information corroborated that found in Experiment C, in that the cochlear duct was still apparent in the 40-day-old HWM, but was gone by 180 days of age. Hilding et al. (1967) described cytoplasmic vacuolation in the outer hair cells of 3-week-old HWM kits using transmission electron microscopy (TEM). By 5 weeks, Sugiura and Hilding (1970a) described Reissner's membrane being thinner in the HWM with no obvious endolymphatic space between the stria vascularis and Reissner's membrane. Sugiura and Hilding characterized the outer hair cells of adult HWM as having nuclear swelling and cytoplasmic vacuolation with cross-striated Friedman-type bodies in the OHC. Saunders (1965) reported the hair cells were absent in the 6-month-old HWM with only a few undifferentiated cells positioned on the basilar membrane. Most of the authors studying the HWM found that initial changes occurred in the basal cochlear turn. Essentially the same lesions were noted in the blue-eyed white cat and Dalmatian dog while either the collapse of Reissner's membrane or strial atrophy were the first differences noted. Johnsson et al (1973) noted that the various phases of degeneration "cannot be strictly applied" because of species and individual variation. Cochlear hair cell degeneration was first noted by small, light vacuoles that appeared in the upper part of the outer hair cells. Of the cochlear support cells, Igarashi et al (1972) described the order of deterioration occurring first in the inner and outer sulcal cells, Deitter and Henson cells and lastly the Claudius and Boettcher cells.

The HWM examined displayed similar findings. As early as day 30, one of the mink showed vacuolation of the support cells in the organ of Corti in the basal turn. As the animals aged, deterioration of the support cells ensued with eventual loss of recognizable support cells and only remnants of the sensory cells left in the adult HWM.

Saunders (1965) described the change in position of the tectorial membrane in the 180-day-old HWM. The primary change seen was the "rolling up" of the membrane in the inner spiral sulcus. Anderson et al.(1968) described the stages of changes of the tectorial membrane in the Dalmatian as first retracting upward toward the inner sulcus and then finally adhering to the limbus and rolled up in the inner sulcus with an overall globular appearance. Similar positional changes were noted in the HWM in Experiment C.

Although Saunders (1965) failed to find changes in the stria vascularis of 6-month-old HWM and concluded that it was "safe to rule out an inadequate vascular supply", several other researchers did find pathologic changes in the mink stria vascularis. In 1967, Hilding et al. described the stria as atrophic in adult HWM, while at 2 weeks of age they found a decrease in the mitochondria of the strial marginal cells. In 1969, Foss reported that the number of capillaries in the stria vascularis of the HWM was reduced when compared to the number in SDM. These differences became significant depending on the anatomic location along the cochlear duct. The differences were first apparent in the middle turn, followed by the basal and finally by the apical turn. Foss also observed that the diameter of the strial capillaries decreased after the kits were 46 days old. The number of capillaries in the apex of the HWM cochlea was comparable to the number in SDM cochlea. Johnsson et al. (1973) reported strial atrophy in the middle and apical turns of deaf Dalmatians and capillaries that eventually left "ghosts" of vessels within the stria. They found some puppies that had a limited apical strial atrophy with little to no hair cell loss. This could explain the difference among the cochlear turns in the presence of the stria vascularis of a 30-day-old HWM as seen in Figure 43.

Sugiura and Hilding (1970b) investigated the stria vascularis in HWM. By administering ferritin injections and then euthanizing the animals, they were able to demonstrate the presence of ferritin particles in the HWM strial capillaries. The poor circulation of the mink became even more apparent when the authors observed intravascular clotting (white clots and adhesion of platelets) and noticeable endothelial swelling which almost completely occluded some of the strial vessels. The endothelial swelling was found using TEM in two-week-old HWM kits. Sugiura and Hilding also described strial atrophy in older HWM. The atrophy was characterized as a shortening of the length of the line of marginal cells and as a thinning of the width of the stria from the endolymphatic surface to the spiral ligament. The authors found that (when measuring in the same anatomic site in the basal and middle turns) the length of the HWM stria was approximately 140 micrometers, while the stria of SDM measured approximately 250 micrometers. The thickness of the stria vascularis was about 18 micrometers in the HWM and 30 micrometers in the SDM. Although exact measurements were not made in the HWM in Experiment C, the changes in the middle turn stria vascularis are evident in Figure 43. Although the thickness of the marginal (dark) cells and the length of the stria in the SDM (180 day) and 38-day-old HWM appeared to be similar, there was an obvious change in the quality of the stria vascularis of the 180-day-old HWM, as evidenced by the partial adhesion of Reissner's membrane and loss of obvious definition of the marginal cells.

Kimura (1973) found that the normal rate of blood flow through the cochlea is slow compared to the rest of the body, as demonstrated by the flow rate in the arterioles supplying the cochlea (70 microliters/second) and the rate in epithelial arterioles (165 microliters/second). As the technique of measuring cochlear blood flow rates is well established, it would be of interest to conduct a temporal study to quantify the actual rate of blood flow in the HWM cochleas to determine if there is a decrease in flow rates as well as vascular degeneration, thereby enhancing the onset of hearing loss in the HWM.

The spiral ganglion was the last anatomic region associated with deafness in the HWM to be examined morphologically. As indicated in the literature, the spiral ganglion initially shows little to no changes. Saunders (1965) did not observe any changes in the spiral ganglion of six-month-old HWM. Both Hilding _et_ _al_. (1967) and Sugiura and Hilding (1970b) described the presence of normal afferent and efferent neural supply in two-week-old and adult HWM. In the cat, Elverland and Mair (1980) found the greatest decrease in the number of neurons in the spiral ganglion occurred in the ends of Rosenthal's canal and not in the center of the ganglion. Losses were first noted in the upper basal turn and then proceeded in either direction along the cochlear duct. Schwartz and Higa (1982) found degenerative changes in the medial superior olive of the cat that occurred before any changes were seen in the spiral ganglion. The lesions in the superior olive developed about the same time as the collapse of Reissner's membrane, strial atrophy and deterioration of the organ of Corti. Gerwitz (1991) described similar lesions in the brainstem of the Dalmatian.

As deaf dogs aged, Anderson et al. (1968) found that the number of neurons decreased and were replaced with fibrous connective tissue. This change was also observed in the HWM. As shown in Figure 45, the spiral ganglion of the 180-day-old HWM is almost completely filled with fibrous connective tissue surrounding the very few neurons whose nuclei are surrounded by large perinuclear spaces.

The morphologic changes observed in the HWM in Experiment C were very similar to those previously described in HWM. The microscopic alterations seen in the cochleas of the HWM were also comparable to those reported in Dalmatian dogs, blue-eyed white cats, humans with Waardenburg's syndrome and other animals with pigment-associated deafness.

GENERAL DISCUSSION

Based on the results of studies involving the ABR of HWM, the mink can hear between approximately 32 and 46 days of age. None of the HWM kits tested at 30-days-old had an ABR, but they had an open scala media in all turns. At 38 days of age, there was an ABR and no collapse of Reissner's membrane. No HWM over 46 days of age had a detectable ABR and older (180-day-old) mink had profound changes in both the scala media and the spiral ganglion.

The reasons for the later onset of hearing and the increased latencies of waves 1 and 3 and normal latency of wave 5 in the HWM are unknown, but there are several possible explanations. Flottorp and Foss (1979) described the vascular blood flow as greatest in the basal portion of the cochlea. They concluded that was why the basal portion of the cochlea was the first part to function. This was apparent in mink tested in Experiments A and B evidenced by their responses to high frequency stimuli. As demonstrated by Sugiura and Hilding (1970b), the overall vasculature and flow is less in the HWM (as compared to the SDM). The decreased blood flow in all areas of the HWM cochlea probably accounts for the lack of function or the delay of function in the basal (high frequency) portion.

If the HWM kits were unable to hear acoustic stimuli during specific times of their lives, the central auditory pathways may not have been activated during critical periods of development. This could account for the findings in the white cat and Dalmatian dog where concurrent anomalies have been found in the medial superior olive and the cochlear duct (Schwartz and Higa, 1982; Gerwitz, 1991). If the central areas were not stimulated at either the right time or in sufficient "amounts", the auditory neural pathway may not have developed completely. This could be evidenced by fewer neurons or a decrease in synaptic terminals. An auditory center could lose its ability to respond to some sounds or may experience loss of speed of the neural conduction system, resulting in an ABR latency delay.

Morphologic and electrophysiologic examination revealed changes in the HWM that are very similar to those found in other species. Because HWM have no evidence of dystopia canthorum and show no response to acoustic stimuli, they may be a model for Waardenburg syndrome Type 2. Foy et al. (1990) described how the Sp mouse was instrumental in identification of the gene which causes Waardenburg syndrome Type 1 in human beings. Because of the apparent 100% expressivity of the syndrome in HWM, they may similarly be useful in defining the location of the human being Waardenburg syndrome Type 2 chromosome.

FUTURE STUDIES

Based on results of the experiments conducted, there are questions still unanswered. The following questions need to be answered in future projects :

1. Continuation and expansion of ABR testing of SDM to clarify the actual age at onset of hearing in the mink. Begin the ABR testing well before day 26 of life.

2. Determine if the SDM first exhibit wave 1 (followed later by the "full" waveform) as was found in several of the HWM kits and some other species.

3. Test both the left and right ears in the HWM to determine if hearing loss or deafness is unilateral or bilateral. Although all of the animals tested failed to behaviorally respond to acoustic stimuli during the duration of the project, it is still unknown if the HWM are completely (bilaterally) deaf.

4. Examine the stria vascularis in HWM for the presence of melanocytes to determine if the pathogenesis of deafness in mink compares with deafness in the white cat and the Dalmatian dog.

5. Examine cochlear blood flow rates in the SDM and HWM and determine if there are differences in the rates in different portions of the cochlea at different ages of mink kits.

6. Conduct invasive electrophysiologic examination of nerve conduction velocities between the generator sites or by conducting in vivo nerve function tests to evaluate the amounts of neurotransmittors produced and released at each of the generator sites.

7. Do morphologic examination of the cochlear nerve and brainstem for quantification of neuronal size and number in both the SDM and HWM at different ages.

8. Determine if the type of hearing loss in the HWM is similar to Type 2 Waardenburg syndrome.

9. Administer pentoyxfilline, a compound which has the ability improve cochlear blood flow rates in the guinea pig, to HWM. This would allow for examination of the effects of the compound on the cochlear blood flow in HWM. If there was a delay in the onset of hearing loss, the drug might be useful in the treatment of human beings or pet animals afflicted with a similar type of cochleosaccular degeneration.

SUMMARY

The use of technically advanced electrophysiologic methods allowed the demonstration of hearing in the Hedlund white mink. A defined "window" of hearing followed by progressive loss was found to occur during the 24th and 42nd day of age in most HWM kits. By the 55th day of age, none of the HWM had an auditory evoked brainstem response and none had behavioral responses to acoustic stimuli. These findings corresponded with morphological alterations. The morphologic finding in cochleas of HWM included a diminished scala media as evidenced by collapse of Reissner's membrane, strial atrophy, degeneration of the organ of Corti and loss neurons in the spiral ganglion. These changes were similar to those reported in HWM, white cats and Dalmatian dogs.

The mink were found to have a comparably late onset of hearing. The additional time for development and maturation of the structures of the auditory system could allow for an informative study of developmental auditory anomalies. The consistent, naturally occurring hearing loss in the Hedlund white mink may be an animal model for the study of genetic deafness or the study of cochlear prosthetics.

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