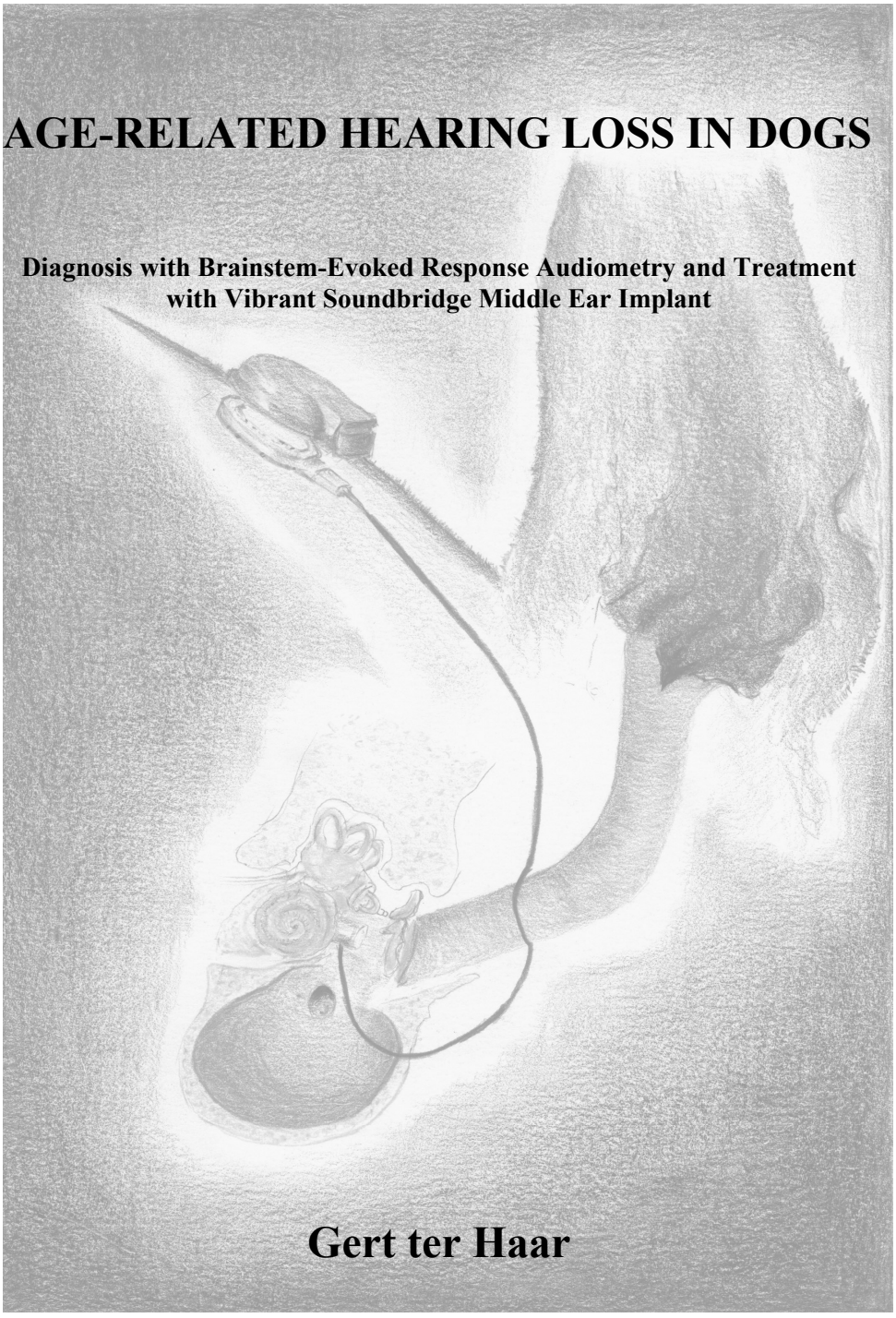
AGE-RELATED HEARING LOSS IN DOGS

Diagnosis with Brainstem-Evoked Response Audiometry and Treatment with Vibrant Soundbridge Middle Ear Implant



Gert ter Haar

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AGE-RELATED HEARING LOSS IN DOGS

Diagnosis with Brainstem-Evoked Response Audiometry and Treatment with Vibrant Soundbridge Middle Ear Implant

Leeftijdsgerelateerd Gehoorverlies bij Honden

Diagnose met Meting van Auditieve Hersenstamresponsies en Behandeling met Vibrant Soundbridge Middenoor-implantaat

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
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ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op
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door

Gerrit ter Haar

geboren op 3 februari 1972 te Eck en Wiel

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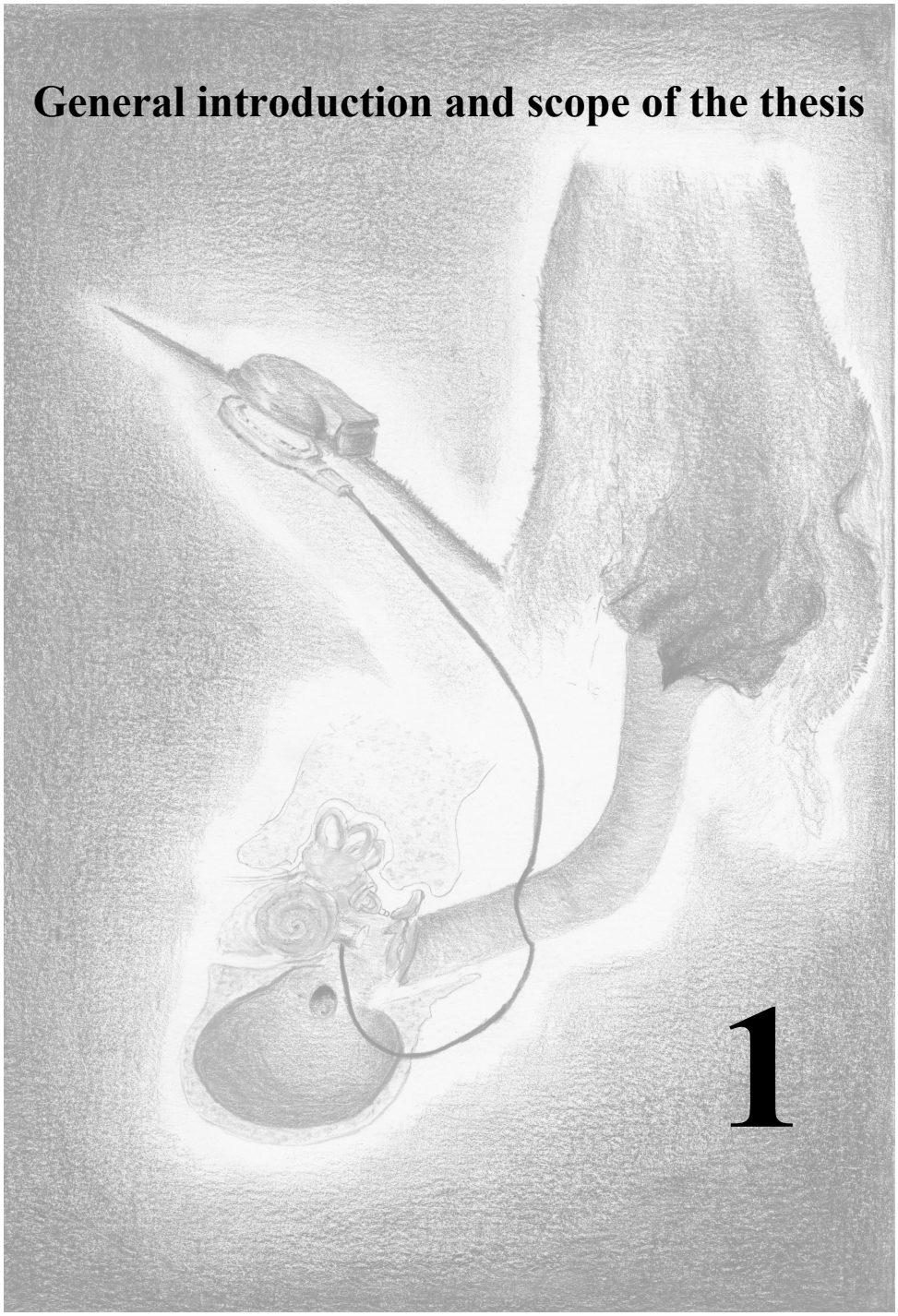
General introduction and scope of the thesis



1

Hearing is a fascinating phenomenon and a very important sensory function in humans and animals. Purves described the auditory system as one of the engineering masterpieces of the body, with an array of miniature acoustical detectors packed into a space no larger than a pea. Although humans are highly visual creatures, much of human communication, socializing, learning, and listening is mediated by the auditory system and indeed, under some circumstances, deafness can be even more debilitating than blindness.[213] It is much more difficult to establish the impact of partial hearing loss or complete deafness in dogs than in humans.

Hearing loss is a common disorder in many breeds of dogs and auditory dysfunction and its clinical consequences can vary from mild to severe. Dogs with unilateral hearing loss can have difficulty in localizing the source of a sound.[277] They are not suited as working dogs for blind and deaf people, or rescue and police work[314], but are not themselves severely handicapped. However, dogs with bilateral hearing loss are unable to anticipate dangers such as motor vehicles and they may consequently fall victim to serious or fatal injury.[277] In addition, they seem to be easily startled and have an increased tendency to bite.[215] Furthermore, deaf puppies require specialized training and are therefore usually euthanized.[175,215]

Acquired hearing loss in humans is receiving growing attention because of its detrimental effects on the affected individual's psychosocial situation, including social isolation, depression, and loss of self-esteem.[80,112,226,313] Hearing impairment has also been implicated as a cofactor in senile dementia.[80] The psychosocial effects of hearing impairment in dogs are not known, but undoubtedly hearing loss contributes to the lethargy, depression, and lack of interest in interaction with the environment that is commonly observed in old dogs.[106] Another serious behavioral side effect of acquired deafness in dogs is exaggerated barking.[106] Similar to acquired hearing loss in humans, which affects not only the quality of life of those with hearing loss but also that of their loved ones, it is to be expected that hearing disabilities in dogs strongly affect the intimate relationship with the owner, due to the interactive nature of the habitual vocal communication.[91,102,229] Timely recognition and correct diagnosis of hearing impairment are therefore mandatory for both audiological rehabilitation of the patient and counseling of the owner.

Much less is known about hearing impairment, hearing capacity, frequency range of hearing, and threshold audiograms in dogs than in humans and laboratory animals. This is due to the unpopularity of dogs as a model in hearing research, problems associated with behavioral testing, and the fact that objective hearing tests were only introduced in veterinary medicine rather recently. As in humans, hearing loss in dogs can have central or peripheral causes. Central deafness can theoretically result from a variety of retrocochlear lesions but is very rare in veterinary practice.[277] Bilateral central deafness

requires bilateral lesions of the auditory cortex or lesions of such a significant portion of the brainstem or midbrain that significant clinical signs beyond deafness are to be expected.[277]

Peripheral hearing loss in dogs is much more common and has been classified as inherited or acquired, conductive or sensorineural, and congenital or late onset.[215,265,277] The most frequently observed forms are acquired conductive hearing loss as a result of chronic otitis externa and media, congenital (inherited) sensorineural hearing loss (SNHL) and acquired SNHL including age-related hearing loss (ARHL) or presbycusis, noise-induced hearing loss (NIHL), and ototoxicity.[277] With a thorough physical examination including otoscopy, the differentiation between conductive and sensorineural hearing loss can usually be determined.[283] Advanced imaging with CT or MRI is necessary, however, to definitely rule out conduction deafness and to devise a plan for treatment.[17,225] Though essential for the diagnostic work-up of all patients with hearing disorders, these two techniques can only identify morphological abnormalities of the petrous bone, middle ear, and inner ear. Diagnosis of functional abnormalities requires hearing tests.

Hearing evaluation in humans can be relatively straightforward, using behavioral tests in which a variety of tones with different levels of loudness are presented. Albeit prone to some subjectivity, in addition to hearing a specific tone the person being tested can describe the sensation of hearing or the lack of it using this technique.[213] Behavioral studies have been performed on a very small scale in dogs[98] and although the sensation of hearing cannot be determined in this species, studies have shown that dogs can hear frequencies up to 45 kHz, which is considerably higher than heard by humans.[98,199] Training dogs to respond reliably and repetitively to different auditory stimuli is time-consuming, even for research purposes, and is therefore not applicable to veterinary practice, where client-owned patients with hearing disabilities have to be examined.

Hearing can be assessed with greater objectivity by several methods, including impedance audiometry, evoked response audiometry, and electrocochleography.[245] Brainstem evoked response audiometry (BERA), initially developed for hearing assessment in very young children when behavioral tests could not be performed, is the technique most commonly used in veterinary medicine. With this technique, the consistent changes in electrical activity in the brainstem following auditory stimulation can be recorded from scalp electrodes.[115] Although clinically relevant information can be obtained with BERA, this technique only evaluates the functional integrity of the peripheral auditory system but not sound awareness. It must be borne in mind that hearing is a much more complex phenomenon than can be appreciated by hearing assessment with BERA.

Conductive hearing loss in dogs is usually the result of chronic otitis externa and media and is therefore amenable to treatment. The diagnostic work-up and treatment of these patients and the use of BERA for diagnosis of the associated conductive hearing loss and for evaluation of the effects of medical treatment and surgery on hearing have been reviewed in the veterinary literature.[52,90,160,161,223,248,315]

Congenital sensorineural deafness is the most extensively studied form of deafness in dogs (especially Dalmatians) and many studies have been published in the past 20 years on the etiopathogenesis of this disease and its diagnosis with BERA.[104,173,215,216,265,278,279] The acoustic signal used to diagnose congenital deafness usually consists of a click, which is a short sound containing many frequency components, thus stimulating a large part of the cochlea. Brainstem evoked response audiometry using click stimulation is useful for differentiating sensorineural from conduction deafness and for demonstrating complete deafness as is the case in the congenital inherited form in dogs. However, frequency-specific information is needed to assess the extent of sensorineural deafness and its possible origin, such as NIHL, ototoxicity, and ARHL, each of which can be partial and frequency specific. Acquired SNHL in dogs has received little attention in the veterinary literature, with the exception of that due to ototoxicity. There have been several reports on ototoxicity in dogs, demonstrating the effect on hearing of commonly used ototoxic agents and stressing the importance of early detection using BERA.[75,158,204,276,277,294] There have been no reports on NIHL in the veterinary literature and but few on ARHL[131,132,240], nor have there been any reports of frequency-specific thresholds for these forms of acquired hearing loss in dogs.

The aims of this thesis were to develop BERA into an objective and complete hearing test for dogs, to determine frequency-specific thresholds over the entire audible frequency range, to collect reference values for BERA characteristics in normal dogs, and to investigate and document the audiometric and histological characteristics of ARHL in this species. In addition, the clinical feasibility and applicability of treating this typical form of hearing loss in dogs with a middle ear implant as a hearing aid was examined.

Chapter 2 describes the embryology, the macroscopic, microscopic, and computer-tomographic anatomy, and the physiology of the dog's ear. This is essential background information for understanding the results presented in this thesis and as a reference for the description of the surgical implantation technique.

Chapter 3 reviews the etiopathogenesis and diagnosis of acquired SNHL in dogs, with emphasis on NIHL and ARHL, and the technique of

BERA. The current status of knowledge about acquired SNHL is presented, including the first four original articles of this thesis, together with discussion of treatment options for SNHL and future directions in hearing research.

Chapter 4 describes the modified BERA technique for frequency-specific tone burst stimulation of the cochlea in a group of 10 young to middle-aged dogs. The results provide a normative database with stimulus variables necessary to evaluate frequency-specific hearing losses in dogs. A pure-tone threshold audiogram was constructed from these variables for this group.

Chapter 5 presents a longitudinal study on ARHL demonstrating the hearing loss in the group of 10 dogs by yearly testing for 7 years after their use in evaluation of the BERA technique described in chapter 4. The hearing loss in these dogs over this interval was primarily due to presbycusis. The BERA results in this group are compared with those in a group of 10 very young dogs and a group of 10 geriatric dogs (cross-sectional study), demonstrating severe hearing impairment in the geriatric dogs.

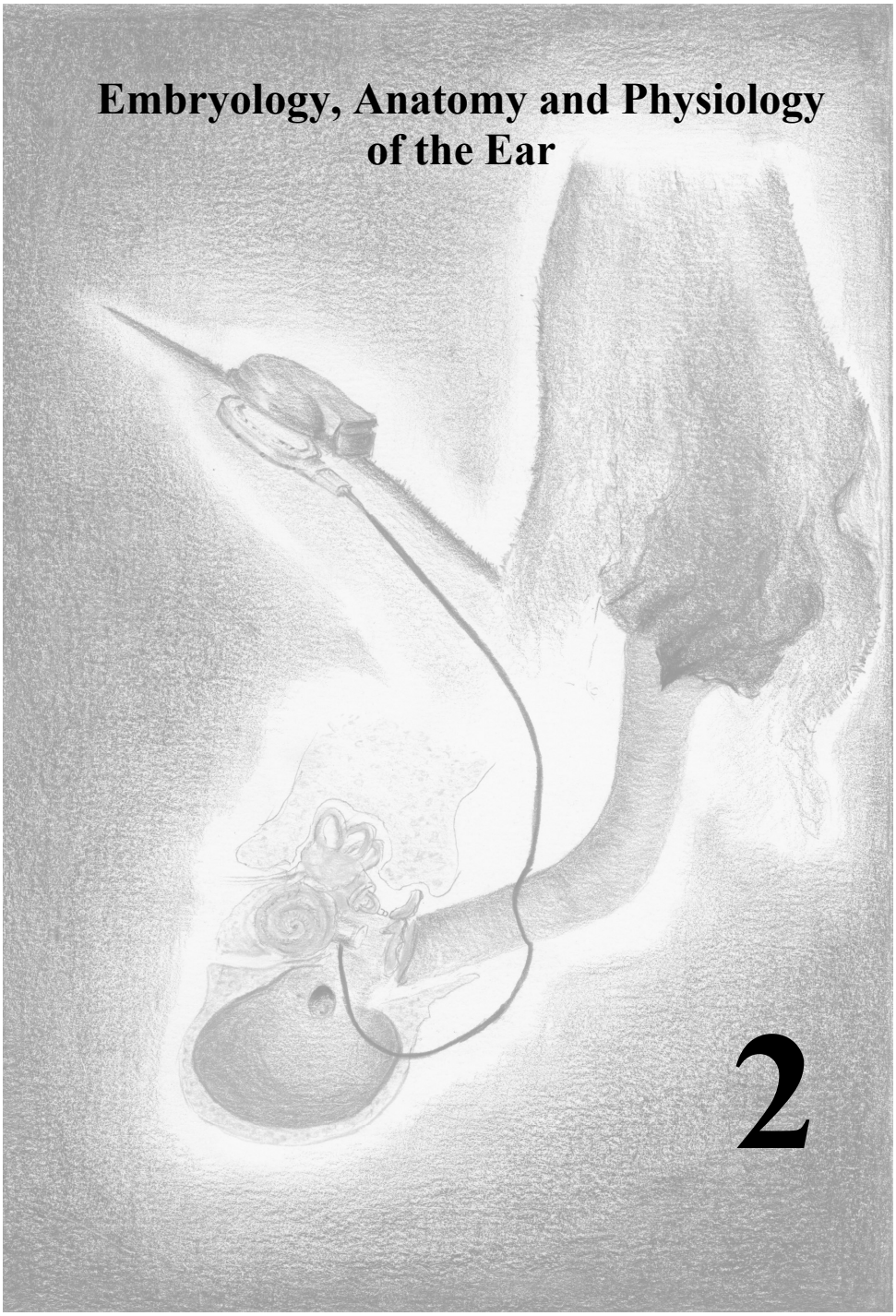
Chapter 6 presents the histological findings in 10 geriatric dogs with severe sensorineural ARHL as demonstrated by BERA. These findings include the inner and outer hair cell counts, ganglion cell counts, pathological characteristics of these cells, and the cross-sectional area of the stria vascularis. The correlation of BERA-derived thresholds with the histological findings is discussed.

Chapter 7 presents a study of the surgical feasibility of implantating the Vibrant Soundbridge middle ear implant in dogs using a modified lateral bulla osteotomy to approach the middle ear cavity. This approach facilitates successful placement of the transducer of the VSB in the round window niche.

Chapter 8 presents the early clinical results of implantation of the Vibrant Soundbridge middle ear implant in three dogs using the technique described in chapter 7 for treatment of ARHL. Residual hearing is not affected by the implant procedure and correct functioning of the implants can be demonstrated by auditory steady-state evoked potentials.

Chapters 9 and 10 present the summarizing discussion and conclusions of this thesis in the English and Dutch languages, respectively.

Embryology, Anatomy and Physiology of the Ear



2

Introduction

During the evolution of vertebrates, the ear evolved as the sense organ for balance, orientation, and hearing. In modern mammals it consists of three embryologically and functionally separate parts, the inner ear, the middle ear, and the outer ear.[68,126,129,187] The inner ear contains the mechanoreceptors involved in the senses of equilibrium and hearing, the sense of equilibrium being phylogenetically older. The organ of equilibrium is very common throughout the animal kingdom and has the same fundamental structure in all vertebrates.[82] Hearing is much less widespread. Although it is present in most vertebrates, it is present in only a small number of invertebrate species, mostly spiders and insects. The organ of hearing has undergone considerable evolution in vertebrates to reach its most complex level of development in mammals.[82,126] The middle ear and outer ear have evolved to aid in the transmission of sound waves to the receptors in the inner ear. Amphibians and reptiles were the first to evolve the middle ear as an additional chamber. In fish and aquatic amphibians another sense organ using the same mechanoreceptors evolved, the lateral line organ, which is involved in spatial orientation and sound detection.[126] Outer ears have evolved in lizards and birds, but only as short ear canals.[82,187] Only mammals have a well-defined outer ear canal with a cartilaginous pinna or auricle covered by skin and moved by muscles.[55,126]

In this chapter the embryology and the macroscopic, microscopic, and computed tomographic anatomy of the inner ear, middle ear, and outer ear of the dog and the physiology of hearing will be discussed. The review of anatomy will be limited to description of the organ of hearing and important differences between dogs and humans. In the review of embryology, the development of both the cochlea and the vestibular organs will be discussed.

Embryology

Early in their development all vertebrate embryos form three layers or sheets of cells from which all tissues and organs in the emerging fetus are formed.[187] The outer layer is the ectoderm, which will form the epidermis, neural tissues, and some of the skeletal and connective tissues of the head. The inner layer is the endoderm and it will form the lining of the digestive tract, respiratory system, and organs associated with digestion. Between these two layers is a more loosely arrayed population of cells, the mesoderm. This germ layer will form most of the muscles and skeletal tissues, the urogenital system, and the heart and blood vessels. However, nearly all organs in the body are derived from more than one germ layer or from different subsets of the same germ layer.[187]

Inner ear

The primordium of the inner ear is first visible at the late neurula stage as a focal thickening of the surface ectoderm, called the otic placode.[82,127,187] The cells of the placode are believed to originate from both the surface ectoderm and the neuroectoderm.[82] The placode invaginates under the influence of fibroblast growth factor-3 (FGF-3) secreted by rhombomeres 5 and 6, to form the otic cup or otic pit.[129,187] After closure of the lips of the cup, the otic vesicle or otocyst is separated from the surface ectoderm.[68,127,129] The point at which the vesicles become detached from the epidermis is marked by an elevation on the dorsomedial side of the vesicle, which later elongates to produce the nonsensory endolymphatic duct and sac system.[68] Neuroblasts break away from the ventromedial wall of the otic epithelium and will form all of the neurons that innervate the inner ear, as well as the cochlea.[127,129]

A series of protrusions from the otic vesicle establish the primordia of the membranous labyrinth, consisting of the cochlea, the three semicircular canals, the utricle, and the saccule. Elongation of the otic vesicle results in formation of the dorsal vestibular and ventral cochlear regions and is regulated by the Pax2 gene; neither the cochlea nor the spiral ganglion will form in the absence of this gene.[129] First, the otic vesicle becomes divided by a deep constriction. Then the most posterior part develops into the utricle, whereas the anterior part becomes the saccule. The two parts remain connected by a thin tube that develops into the utriculosaccular duct. A long, slender diverticulum grows out from the utricular part and becomes the endolymphatic duct and endolymphatic sac. This process is also regulated by FGF-3.[129] The semicircular canals develop from the utricular part by the formation of three flattened diverticula, a process regulated by the Prx1 and Prx2 genes.[129] One end of each semicircular canal enlarges where it joins the utricle, forming an ampulla. Within this the epithelium forms a thickening, the crista ampularis, containing the mechanosensory cells (hair cells). Similar thickenings containing hair cells in the utricle and saccule are called the macula utriculi and macula sacculi, respectively.

The cochlear diverticulum arises from the anterior-ventral part of the saccule in humans and from the ventral margin of the otic vesicle in animals.[82,127,187] The cochlear duct remains in communication with the saccule by means of a narrow duct, the ductus reuniens. The cochlear duct winds as it elongates to make 2.5 turns in humans and a little more than 3 in dogs, and the epithelium on one surface of the duct thickens to form the organ of Kölliker. At a later stage this forms the organ of Corti, containing mechanosensory cells and nonsensory supporting cells.[126,127] Development into vestibular or cochlear mechanosensory cells or supporting cells is regulated by a complex mechanism that involves interaction of signaling pathways, gene expressions ($p27^{kip1}$), transcription factors (*Math 1*), and various proteins and hormones.[127]

The embryonic vestibular and cochlear ducts are surrounded by the cartilaginous otic capsule, which undergoes continuous reshaping as the membranous labyrinth grows and later forms the bony labyrinth.[82,129] The mesenchyme between the cartilaginous capsule and the epithelium of the membranous labyrinth differentiates into the connective tissue component of the membranous labyrinth. In the deep part of the cartilaginous capsule, vacuoles arise that coalesce to form the perilymphatic compartment.[82] The narrow spaces in which the membranous labyrinth develops are the vestibule (containing the utricle and the saccule), the semicircular canals, and the cochlea, all filled with perilymph. One side of the cochlear duct, which is derived from ectodermal tissue and will become the scala media, is attached to the connective tissue of the otic capsule via the spiral ligament. Differential reshaping of the cochlea results in the establishment of two chambers adjoining the cochlear duct: the scala vestibuli and scala tympani, both of mesodermal origin.[126]

Middle ear

The middle ear develops from an endodermal diverticulum, the tubotympanic recess, which extends upward and laterally from the first pharyngeal pouch.[82] The ventral part of the first pair of pharyngeal pouches is obliterated by the developing tongue. The dorsal projection expands and develops into the tympanic cavity (and mastoid antrum in humans), whereas the connection to the pharynx becomes the auditory or Eustachian tube.[129,187] The endoderm of this pouch forms the epithelial lining of the middle ear cavity and the inner layer of the tympanic membrane. The three auditory ossicles arise from mesenchyme by endochondral ossification, the malleus and incus from the dorsal part of the cartilage of the first branchial arch and the stapes from the second branchial arch.[82] The mammalian tympanic bulla grows from a ring of dermal bone that is homologous with the angular bone of birds and reptiles.[187] The tensor tympani and stapedius muscles develop from the mesenchyme of the first and the second branchial arch, respectively. Both the bones and the muscles with associated ligaments gradually become surrounded by the mucosa of the tympanic cavity during its development, as they become invaginated into the distal blind end of the endodermal tubotympanic recess.[82]

Outer ear

The first visceral groove forms the outer ear canal. The mesenchyme on both sides of the first visceral groove first forms a series of six small swellings called auricular hillocks.[129,187] They gradually merge to form the auricle; the tragus and the rostral part of the auricle arise from the first visceral arch and the rest of the outer ear arises from the second arch. The groove between the hillocks becomes the external bony auditory meatus.[187] From the dorsal end of this meatus ectodermal cells grow into the depth to form a solid epithelial plate that

becomes a canal. At the base of this canal the ectodermal lining is closely apposed to the endoderm of the first pharyngeal pouch. Mesenchyme grows in between these two layers and forms the middle fibrous layer of the tympanic membrane. Thus, the tympanic membrane originates from all three germ layers. During most of the fetal development the canal is filled with an epithelial plug which dissolves near the time of birth.[187] The outer ears arise on the ventrolateral surface of the head and their location does not change during development. Rather, the subsequent growth of the lower jaw and associated muscles greatly expands the volume of tissue ventral to the ear.[187]

Macroscopic Anatomy

The skull (cranium) is the most complex and specialized part of the skeleton, lodging the brain and the sense organs for hearing, equilibrium, sight, smell, and taste, while providing attachment for the teeth, tongue, larynx, and a host of muscles.[54] Of the bones that make up the skull, the one important for the organ of hearing is the temporal bone, which houses both the middle ear and the inner ear. The temporal bone is the area of interest for surgical approaches to the middle ear and the inner ear and is presented in detail within the context of this thesis.

Temporal bone

In dogs, the temporal bone forms a large part of the ventrolateral wall of the cranium and in young animals it can be seen to consist of a petrosal part (inner ear), a tympanic part, and a squamous part (Fig. 2.1).[4,51,54]

The squamous part of the temporal bone has a long, curved zygomatic process which forms part of the zygomatic arch. The mandibular fossa is located ventrally and together with the condyle of the mandible it forms the temporomandibular joint. The retroarticular process is a ventral extension of the squamous temporal bone and is a surgical landmark (Fig. 2.1 and Fig. 2.3).[4,54,70]

The tympanic part of the temporal bone is the ventral portion, easily identified by its largest component, the smooth, bulbous tympanic bulla, lying between the retroarticular and jugular processes (Fig. 2.2).[4,51,54]

The external bony acoustic meatus is the short canal connecting the outer ear canal to the tympanic membrane. The membrane is attached to the tympanic ring, which thus forms the proximal end of the external bony acoustic meatus. The tympanic cavity itself can also be divided into three parts. The largest and most ventral is the fundic part. The middle compartment opposite the tympanic membrane is called the tympanic cavity proper and the dorsal part where the incus, part of the stapes, and head of the malleus are situated, is called the epitympanic recess.[4,54] The middle ear cavity is lined with ciliated epithelium (see section on the microscopic anatomy of the ear).

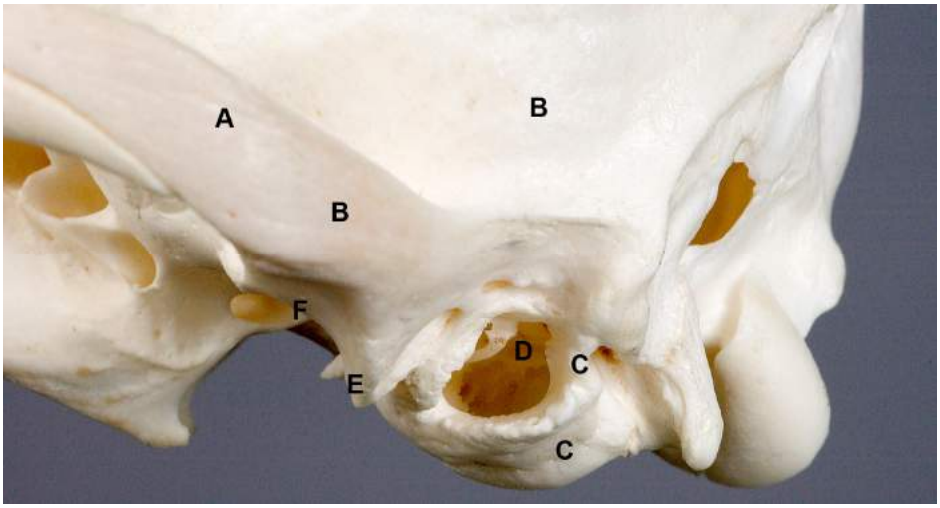


Fig. 2.1. Lateral view of the left temporal bone in association with the adjacent bones of the canine skull. A = Zygomatic process. B = Squamous part of the temporal bone. C = Tympanic part of the temporal bone. D = Petrosal part of the temporal bone. E = Retroarticular process. F = Mandibular fossa.

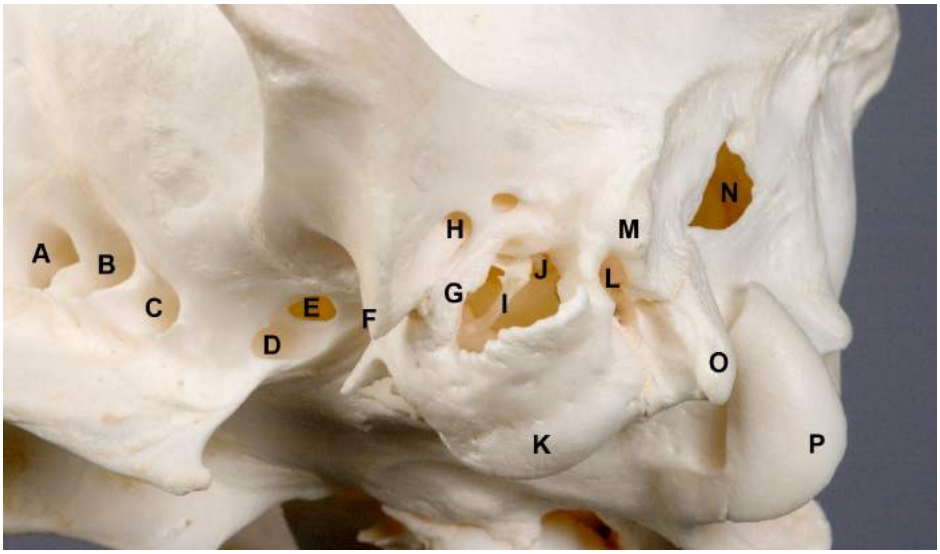


Fig. 2.2. Left lateral view of the canine skull. A = Optic canal. B = Orbital fissure. C = Rostral alar foramen. D = Caudal alar foramen. E = Oval foramen. F = Retroarticular process. G = External bony acoustic meatus. H = Retroarticular foramen. I = Handle of malleus. J = Round window. K =Tympanic bulla. L = Stylomastoid foramen. M = Mastoid process. N =Mastoid foramen. O = Jugular process. P = Occipital condyle.

The petrosal bone is one of the hardest bones of the body and on a lateral view the only part that can be seen is the mastoid process, lying between the mastoid foramen dorsally and the stylomastoid foramen ventrally.[4,51,54] The thickened ventral part serves for the attachment of the tympanohyoid cartilage.

From a more ventral perspective, the musculotubal canal is visible lateral to the foramen lacerum, which is continuous with the auditory or Eustachian tube (Fig. 2.3). The carotid canal that houses the internal carotid artery runs longitudinally through the medial wall of the osseous bulla.[4,51,54] A barrel-shaped eminence, the promontory, can be seen when the ventral part of the tympanic cavity is removed (Fig. 2.4). The round or cochlear window can be found caudolaterally in the promontory and the oval or vestibular window can be found slightly rostrodorsolaterally, occluded by the footplate of the stapes.[54,99]

A small grooved fossa facing the oval window is the open part of the canal for the facial nerve before it emerges through the stylomastoid foramen. The incus and the head of the malleus lie in the epitympanic recess. The petrosal bone also contains the labyrinth, which is divided into three parts: the cochlea, the semicircular canals, and the vestibule. The basal turn of the cochlea produces the obvious bulk of the promontory (Fig. 2.4). A small canal carries the chorda tympani nerve fibers from the facial canal to the middle ear cavity. They usually pass through a small canal in the rostrodorsal wall of the tympanic bulla and emerge through the petrotympanic fissure by a small opening medial to the retroarticular process.[4,51,54]

On the caudomedial surface of the petrosal part several important features can be distinguished (see Fig. 2.5). Dorsally the cerebellar fossa can be seen; it houses the paraflocculus of the cerebellum. Ventral to the fossa, the internal acoustic pore can be seen leading to the internal bony acoustic meatus. In the recess is the opening of the facial canal, containing the facial nerve. Ventrorostral to the internal bony acoustic meatus is the short canal through a structure called the pyramid, for the passage of the trigeminal nerve.[54] The bony external acoustic meatus serves for attachment of the outer ear, which consists of the auricle and the outer ear canal.[89,99]

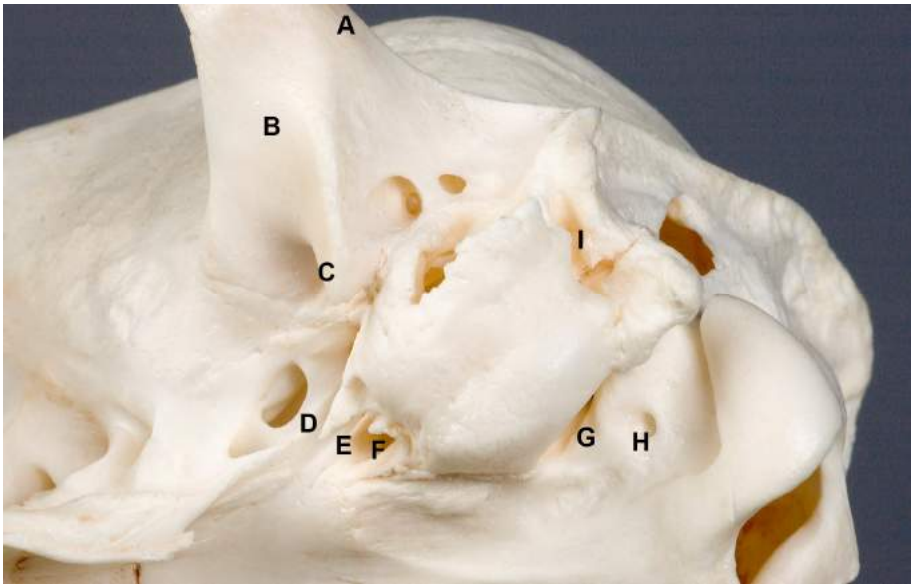


Fig. 2.3. Ventrolateral view of the caudal part of the canine skull, with the tympanic bulla in the middle of the picture. A = Zygomatic process. B = Mandibular fossa. C = Retroarticular process. D = Muscular process. E = Musculotubal canal. F = Foramen lacerum. G =Tympano-occipital fissure. H = Hypoglossal canal. I = Stylomastoid foramen.

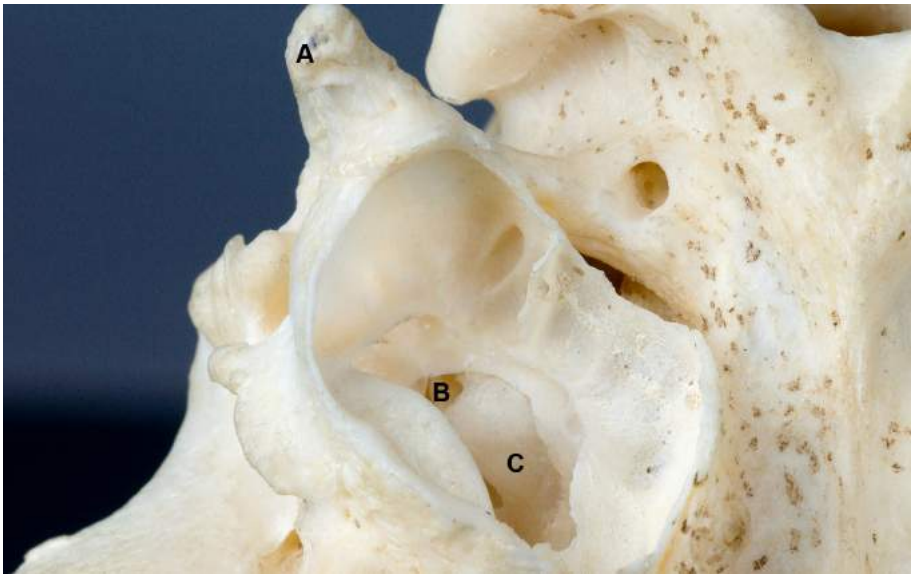


Fig. 2.4. Ventral view of the petrosal part of the right temporal bone after removal of the ventral wall of the right tympanic bulla. The jugular process points caudally, the bottom of the figure faces rostrally. A =Jugular process. B = Round window. C = Promontory.

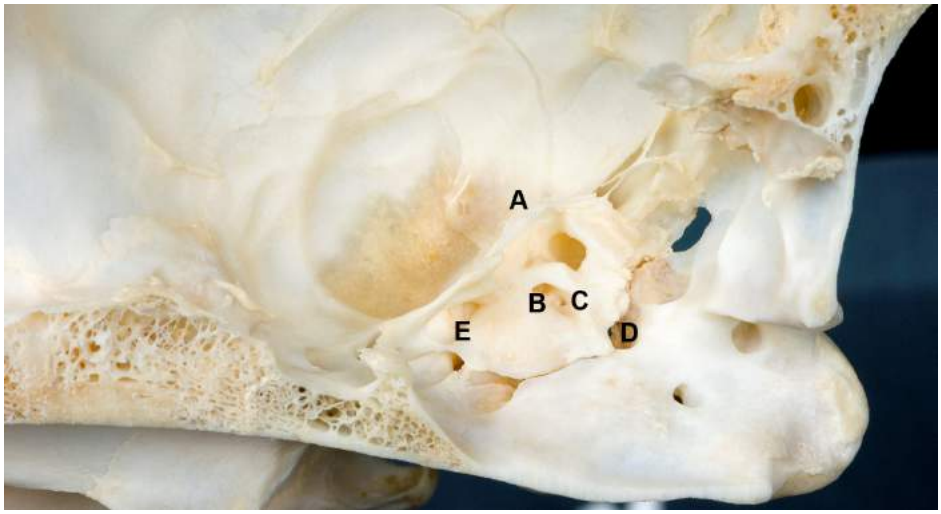


Fig. 2.5. Medial view of the right temporal bone with the adjacent bones of the canine skull. A = Cellebellar fossa. B = Canal for facial nerve. C = Internal acoustic part of internal acoustic meatus. D = Jugular foramen. E = Canal for trigeminal nerve.

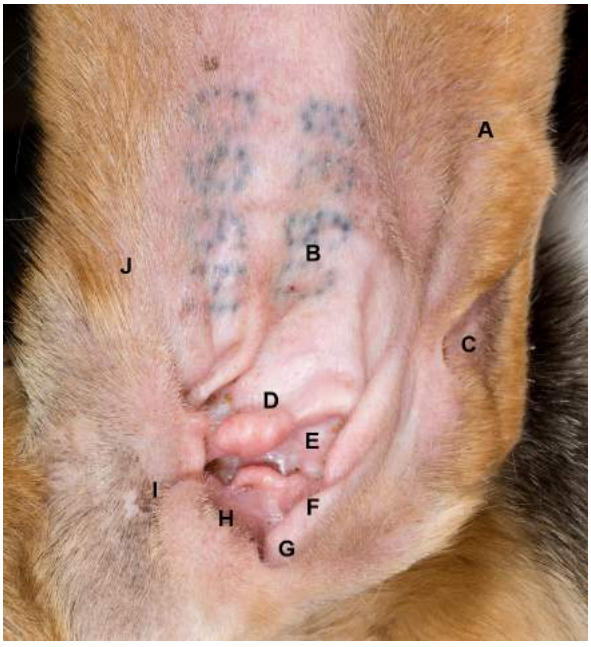


Fig. 2.6. The concave side of the left auricle of a dog with pendulous ears, showing anatomical landmarks. A = Lateral border of helix. B = Scapha. C = Marginal pouch. D = Antihelix. E = Concha. F = Antitragus. G = Intertragic incisure. H = Tragus. I = Tragohelicine incisure. J = Medial border of helix.

Outer ear

The auricle or pinna of dogs varies greatly in size and shape between breeds.[83,89,97,99] The auricular cartilage determines the appearance of the auricle, which may vary from erect to pendulous. The cartilage is completely covered by skin and can be moved in the direction of sound by muscles. In an erect ear, the conchal cavity is on the lateral or concave surface and is usually directed rostrally (Fig. 2.6). The medial or caudal surface is convex and many foramina pierce the cartilage to allow the passage of blood vessels and nerves from the convex to the concave surface (Evans, 1993b; Harvey et al., 2001).[55,97] A transverse plica or antihelix separates the concha from the more distally located and flattened scapha.[55,83,97] The free margin of the scapha is slightly folded and called the helix. The basal portion of the concha twists and rolls to form the largest part of the vertical ear canal. The annular cartilage fits within the base of this tube and forms the largest part of the horizontal ear canal.[55,89,99] The annular cartilage overlaps and attaches to the external bony acoustic meatus. The lateral boundary of the initial portion of the ear canal lying opposite the antihelix is formed by an irregular quadrangular plate of cartilage and is known as the tragus.[55,70,89,99] The antitragus lies caudal to the tragus, separated from it by the intertragic incisure, and is divided into two limbs: the medial and lateral horns (Fig. 2.6). Just distal to the styloid process of the lateral horn there is a marginal pouch.[55] Medially, the medial crus of the helix is separated from the tragus by the tragohelicine incisure. From distal to proximal the skin lining the scapha and concha is increasingly pigmented and has a decreasing amount of hair (see section on the microscopic anatomy of the ear). The other prominent transverse and longitudinal ridges on the concave side are simple skin folds and do not contain cartilage.[55] A small, boot-shaped scutiform cartilage is located in the rostroauricular muscles medial to the ear and serves as an attachment for several muscles that move the ear.[55,59,99]

Blood supply to the ear

The blood supply to the head and neck leaves the aorta through the brachiocephalic trunk and left subclavian artery.[56] The left common carotid artery is the first branch to leave the brachiocephalic trunk, while the brachiocephalic artery itself terminates in the right common carotid and the right subclavian arteries. The vertebral arteries arise from the subclavian arteries and after entering the skull they anastomose to form the basilar artery. Here, the labyrinthine artery branches off and supplies blood to the structures within the labyrinth.[56,99,239]

The common carotid arteries divide into internal and external carotid arteries. The external carotid is the main supply to the head. A condyloid artery arising from the cervical branch of the occipital artery or directly from the occipital artery supplies branches to the middle and inner ear.[56] The caudal

auricular artery arises at the base of the annular cartilage from the dorsocaudal surface of the external carotid and branches into the stylomastoid artery, which supplies the facial nerve and tympanic membrane, and a lateral, medial and intermediate auricular branch.[56,151] The lateral auricular branch is a large artery supplying the caudal surface of the auricular cartilage and it terminates by anastomosing with the intermediate auricular branch, which is the largest supplying artery. The caudoauricular muscles and the auricular cartilage receive branches from this artery. The rostroauricular muscles near the tragus are supplied by the superficial temporal artery, a terminal branch of the external carotid artery.[56]

The veins of the head follow the same general course as the arteries, although variations in their number, size, and course are more frequent than for arteries.[57] The rostral auricular vein is a small vein that begins in the interauricular musculature and skin and receives branches from the skin and auricular muscles and the base of the pinna itself and terminates in the superficial temporal vein or caudal auricular vein formed by the lateral and intermediate auricular veins.[57,99] The medial auricular vein drains into the rostral auricular vein, which joins the maxillary vein close to the caudal auricular vein. Between the rostral and caudal auricular veins the deep auricular vein enters the maxillary vein. The marginal veins, medial and lateral auricular, anastomose with each other near the tip of the auricle on the caudal or convex side. The caudal auricular vein terminates in the caudal maxillary vein, which in turn drains into the external jugular vein to terminate in the cranial vena cava via the brachycephalic vein.[57]

Lymphatic vessels

The parotid lymph center consists of the parotid lymph nodes on the rostral side at the base of the ear, which drain the superficial parts of the outer ear, the muscles of the ear, and the temporal bones.[58] The retropharyngeal lymph center consists of a medial and sometimes a lateral retropharyngeal lymph node. The afferent lymphatics of the medial node drain the deep parts of the outer ear. The afferent lymphatics of the superficial cervical lymph center drain the skin of the caudal part of the head, including part of the auricle.[58]

Microscopic Anatomy

Outer ear

The auricle consists of a single, continuous, thin plate of elastic cartilage covered with thin skin.[55,68,82,89,99] This skin possesses fine vellus hairs and associated sebaceous glands. The skin covering the auricle is continuous with the skin of the outer ear canal, just as the cartilage of the auricle is continuous with the cartilage of the vertical part of the ear canal. The outer ear canal extends medially from the auricle to the tympanic membrane. In humans, approximately the external three-fifths consist of cartilage[82,165], whereas the remaining internal two-fifths are formed by bone (the temporal bone). In dogs, the bony part constitutes only a small part of the outer ear canal. The skin lining the ear canal in both humans and dogs is thin and firmly attached in both the cartilaginous and the osseous parts. In the cartilaginous part of the ear canal, there are coarse hairs associated with large sebaceous glands, and there are also ceruminous glands, which are specialized apocrine sweat glands. Earwax or cerumen is a mixture of the secretion of the ceruminous and sebaceous glands (Fig. 2.7).[68,82] In the skin of the osseous part of the human ear canal there are no hairs or glands, except in the roof in the outermost part, where there are fine vellus hairs with associated small sebaceous glands. In dogs, there are also some loose hairs within the osseous part (Fig. 2.7).[89,99]

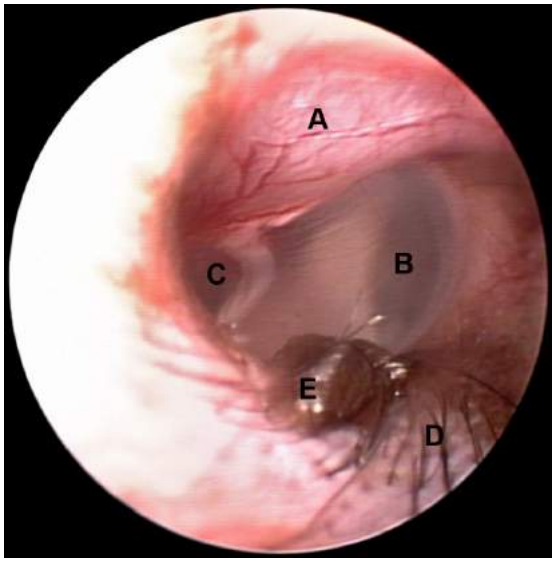


Fig. 2.7. Normal otoscopic view of the left outer ear canal and tympanic membrane of a dog A = Pars flaccida of the tympanic membrane. B = Pars tensa of the tympanic membrane. C = Malleus. D = Hairs. E = Cerumen.

Middle ear

The middle ear comprises the tympanic cavity, the tympanic membrane, the auditory ossicles, and the auditory or Eustachian tube, and in humans also the mastoid antrum and mastoid air cells.[55,68,82,89,99] The tympanic cavity is a small, irregular, air-filled cavity interposed between the tympanic membrane laterally and the inner ear medially. The air in the tympanic cavity comes from the nasopharynx via the auditory tube (and the mastoid antrum in humans).[82,165] Epithelium lines the tympanic cavity and surrounds all of the structures within it. Most of it is simple squamous epithelium but especially near the opening of the auditory tube there is ciliated, pseudostratified columnar epithelium with goblet cells.[68,82] The lamina propria consists of a thin layer of connective tissue that binds the mucosa of the tympanic cavity firmly to the underlying structures.

Tympanic membrane

The tympanic membrane**,** or eardrum, is a thin, semitransparent membrane. The greater part of it is taut and is termed the pars tensa. In dogs, the pars flaccida is a small, triangular portion lying between the lateral process of the malleus and the margins of the tympanic incisure (Fig. 2.7).[55,97] The tympanic membrane is firmly attached to the handle of the malleus. In humans, the most depressed point, opposite the distal end of the handle of the malleus, is called the umbo.[82] The handle of the malleus is embedded in the tunica propria of the tympanic membrane. Histologically, the tympanic membrane consists of three layers: an outer layer of squamous epithelium, an intermediate fibrous connective tissue layer (tunica propria), and an inner layer of respiratory epithelium. The squamous epithelium of the outer layer is continuous with the epithelium lining the outer ear canal.[68,82] On the outer surface of the tympanic membrane it originates around the location of the attachment of the handle of the malleus.[55] From this site, the umbo, there is continuing mitotic proliferation and migration across the outer surface, radiating in all directions. This migration clears the membrane of keratinized debris.[55]

Auditory ossicles

The auditory ossicles are three small bones that according to their shapes are named the hammer or malleus, the anvil or incus, and the stirrup or stapes.[55,82,89,99] The three bones form an uninterrupted chain from the tympanic membrane, to which the malleus is attached, to the oval window where the base of the stapes (footplate) matches like a lid and is attached by means of a ligament. The three auditory ossicles are interconnected by small synovial joints and connected to the walls of the tympanic cavity by means of fine ligaments.[55,82] In the dog, the malleus consists of a head, a neck, and a handle, the rostral or long process of which is largely embedded in the tympanic membrane (Fig. 2.8). The head of the malleus articulates with the body of the

incus in the epitympanic recess.[55,99] The incus measures about 4 x 3 mm in dogs and has two crura, located on each side of a transverse ridge that forms the caudal limit of the epitympanic recess.[55] The shorter crus points caudally into the incudal fossa which is dorsal to this ridge. The longer crus is also directed caudally, but presents a small bone, the lenticular bone, that extends rostrally and somewhat medially from its distal end.[55] The stapes is the smallest bone in the body and approximately 2 mm in length in the dog. It consists of a head, a neck, two crura, a base (footplate), and a muscular process.[55] The footplate articulates with the fibrocartilaginous ring covering the edge of the oval window. Histologically, the three ossicles consist of compact bone (but with a central marrow cavity in the head of the malleus and in the body of the incus), and moreover, the footplate partly consists of hyaline cartilage.[68,82] The surface of the bones and ligaments is covered with mucosa which is firmly attached to the periosteum of the bones.

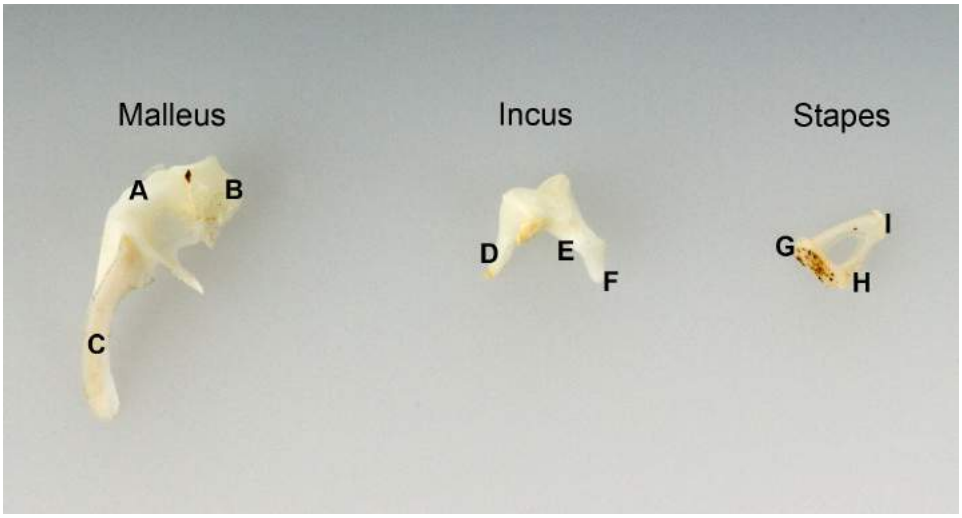


Fig. 2.8. The auditory ossicles in the middle ear of the dog: malleus, incus, and stapes. A = Neck. B = Head. C = Handle. D = Short crus. E = Long crus. F = Lenticular bone. G = Footplate. H = Crus. I = Head.

Associated with the auditory ossicles are two small striated muscles, the tensor tympani muscle and the stapedius muscle. In dogs, the tensor tympani muscle is spherical, has its base in the fossa tensor tympani, and attaches to the hook on the apex of the muscular process of the malleus via a short tendon of insertion.[55] The muscle is innervated by the trigeminal nerve and its contraction pulls the handle of the malleus medially. The tympanic membrane thereby

becomes more tense and the footplate is pressed against the oval window. The stapedius muscle, which is the smallest striated muscle in humans and dogs, springs from the posterior wall of the tympanic cavity and attaches to the head of the stapes.[55,68,82] It is innervated by the facial nerve and its contraction pulls back the stapes so that its footplate swings slightly away from the oval window. The two muscles thus exert opposite actions on the stapes, introducing another mode of vibration, which reduces sound transmission.

Auditory tube

The auditory tube or Eustachian tube is almost 4 cm long. It connects the nasopharynx with the anterior surface of the tympanic cavity in humans[82] and with the rostral portion of the tympanic cavity proper in dogs.[55] It consists of an osseous part and a cartilaginous part. The mucosa of the auditory tube is ciliated pseudostratified columnar epithelium continuing from the nasopharynx. Goblet cells are present in large numbers in the cartilaginous part, especially adjacent to the opening of the pharynx. The lateral wall of the auditory tube in dogs is supported by the tensor veli palatini muscle, which arises in the groove of the petrous temporal bone ventrolateral to the tensor tympani muscle.[55] The branch of the trigeminal nerve that supplies the tensor tympani muscle enters the tympanic cavity in association with its tendon of origin.[55]

Inner ear

The inner ear is also called the labyrinth due to its complex anatomy and consists of a bony part and a membranous part. The inner ear can be divided into three portions: the vestibule, the semicircular canals, and the cochlea. The vestibule and the semicircular canals contain the vestibular end organs, which are involved in the sense of equilibrium, whereas the cochlea contains the sensory organ for hearing. The following detailed description of the inner ear is based on findings in human cochleas, specific features of canine cochleas being mentioned when they differ from those in humans.

Cochlea

The cochlea in dogs resembles the shell of a common snail and points ventrorostrally and slightly laterally within the promontory of the petrous temporal bone.[55] It is a hollow, fluid-filled bony tube that spirals around a central bony axis, the modiolus, containing the auditory or cochlear nerve and the vascular supply to the cochlea. In humans the cochlea makes 2½ turns and in dogs it makes 3¼ turns (Fig. 2.9).[82,99] The base of the modiolus opens into the internal acoustic meatus.

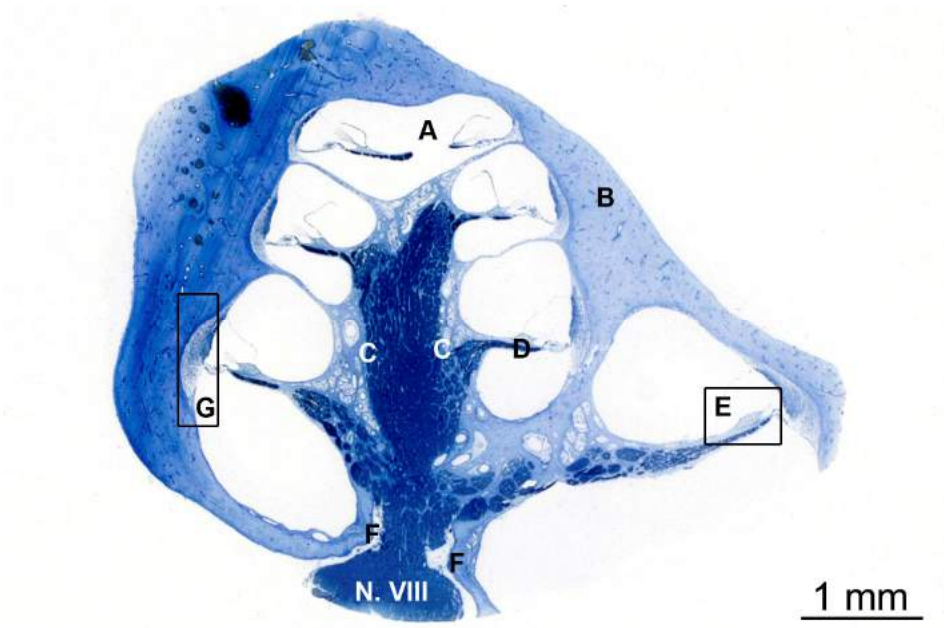


Fig. 2.9. Midmodiolar section through the cochlea of a normal dog. N.VIII = Vestibulocochlear nerve. A = Helicotrema. B = Bony capsule. C = Modiolus. D = Osseous spiral lamina. E = Organ of Corti. F = Internal acoustic meatus. G = Lateral wall.

From the modiolus, the osseous spiral lamina protrudes into the lumen of the cochlea, and winds around the entire modiolus from the base to the apex (Fig. 2.9).[82,144,288] This osseous spiral lamina contains the afferent and efferent nerve fibers that branch off from the intramodiolar portion of the cochlear nerve and end in the organ of Corti. These fibers fan out in a spiral fashion from the modiolus to pass into the channel near the base of the osseous spiral lamina, called Rosenthal's canal.[165] The cell bodies or perikarya of the afferent neurons constitute the spiral ganglion (Fig. 2.10). There are two populations of afferent neurons. Type-I neurons are bipolar cells with a large cell body surrounded by a relatively thick myelin sheath and comprise 95% of the total population. Type-II neurons are pseudomonopolar cells which are much smaller and are surrounded by only a few myelin layers.[165]

The osseous spiral lamina and its nonosseous extension, the basilar membrane, divide the lumen of the cochlea into two fluid-filled compartments, the scala vestibuli and the scala tympani. At the level of the basilar membrane, the two scalae are separated from one another by a third compartment, the scala media (Fig. 2.11). However, the scala vestibuli and scala tympani communicate at the apex of the cochlea through an opening called the helicotrema (Fig. 2.9). The fluid in the scala vestibuli and scala tympani is low in K^+ and high in Na^+

and is called perilymph. The fluid in the scala media is of a different biochemical composition, high in K^+ and low in Na^+, and is called endolymph.[82,247]

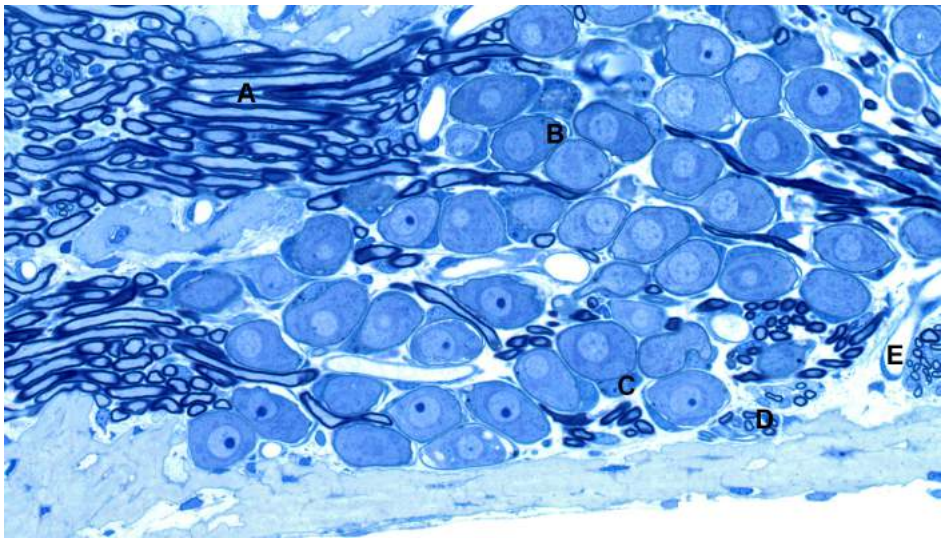


Fig. 2.10. Cross-section through the spiral ganglion of a normal dog. A = Nerve fiber. B = Spiral ganglion cell. C = Schwann cell. D = Nerve fiber. E = Capillary.

The scala vestibuli begins in the basal turn of the cochlea near the oval window, whereas the scala tympani begins at the round window. In a cross-section, the scala media resembles a right triangle, the hypotenuse formed by Reissner's membrane, which consists of two cell layers separated by a basement membrane. Reissner's membrane separates the scala media from the scala vestibuli and extends from the spiral limbus to the uppermost part of the stria vascularis.[82,165,217] One leg of the triangle is formed by the stria vascularis and the spiral ligament. The latter is the thickened periosteum on the internal surface of the outer wall of the bony capsule of the cochlea.[217] Together with the stria vascularis, the spiral ligament forms the lateral wall (Fig. 2.9). The second leg of the triangle is formed by the floor of the scala media separating the scala media from the scala tympani. It consists of a nonosseous extension of the osseous spiral lamina, the basilar membrane, on the surface of which lies the organ of Corti. The basilar membrane is attached to the basilar crest of the spiral ligament (Fig. 2.11).

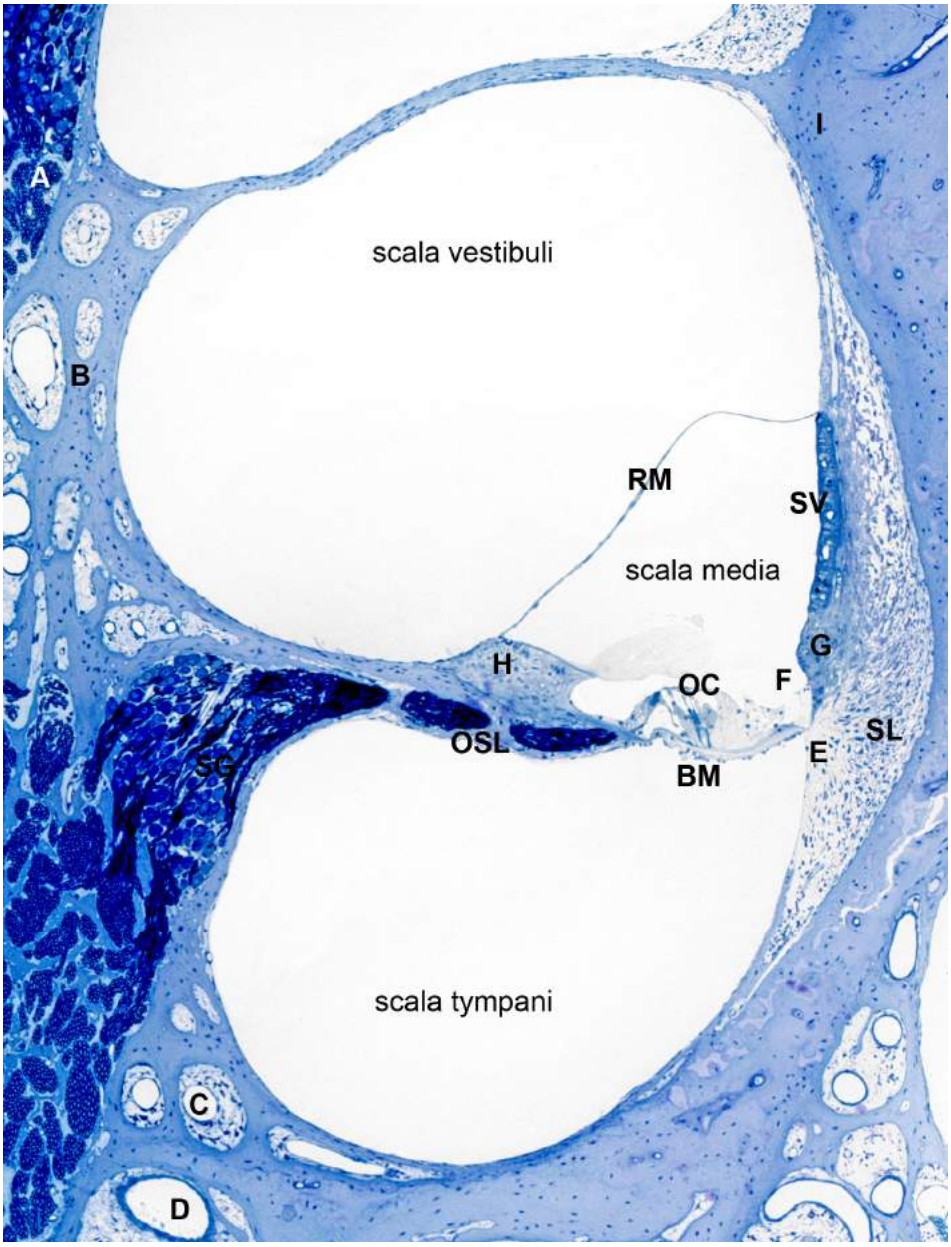


Fig. 2.11. Midmodiolar section through the cochlea of a normal dog. BM = basilar membrane. OC = organ of Corti. OSL = osseous spiral lamina. RM = Reissner's membrane. SG = spiral ganglion. SL = spiral ligament. SV = stria vascularis. A =Modiolus N. VIII. B = Bony part of modiolus. C = Modiolus venules. D = Modiolus arterioles. E = Basilar crest. F = Outer sulcus. G = Spiral prominence. H = Spiral limbus. I = Bony capsule.

Stria vascularis

The stria vascularis is highly specialized and vascularized epithelium on the medial surface of the spiral ligament and separated from it by a basement membrane.[68,82,202,247] The stria vascularis consists of three main types of epithelial cells, arranged in three layers (Fig 2.12). Facing the scala media is a single superficial layer of so-called marginal cells. These contain large numbers of mitochondria and endocytotic vesicles and possess numerous plasmalemmal infoldings on their basal surface.[217,228] They are involved in the production of endolymph.

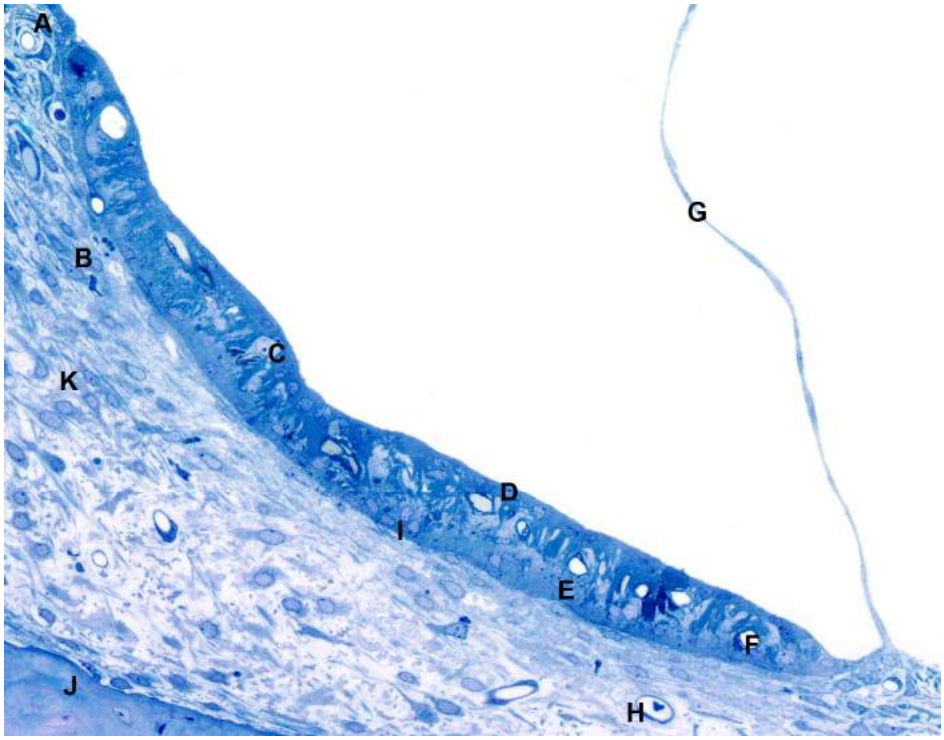


Fig. 2.12. Lateral wall in the lower middle turn of the cochlea of a normal dog. The lateral wall consists of the spiral ligament, the spiral prominence, and the stria vascularis. A = Spiral prominence. B = Fibrocyte. C = Intermediate cell. D = Marginal cell. E = Basal cell. F = Strial capillary. G = Reissner's membrane. H = Capillary. I = Stria vascularis. J = Bony capsule. K = Spiral ligament.

Beneath the marginal cells a discontinuous layer is formed by the intermediate cells, which form extensions at their apical end that intermingle with the basolateral foldings of the marginal cells. These cells are melanocytes derived from the neuroectoderm and are involved, together with the marginal cells, in

the generation of the endocochlear potential.[165] Facing the spiral ligament are several layers of flat overlapping basal cells.

Spiral prominence

The spiral prominence is formed by a protuberance of the spiral ligament into the scala media (Fig. 2.12) and contains several blood vessels. The luminal surface of the spiral prominence is covered by a layer of cuboidal epithelial cells that are continuous with the marginal cells of the stria vascularis.[217] The transition between the spiral prominence and the basilar membrane forms a concavity called the outer sulcus, which is lined by the outer sulcus cells (or root cells), the Claudius' cells, and the Böttcher's cells.[144,228]

Spiral limbus

The peripheral edge of the osseous spiral lamina is pierced by fibers of the cochlear nerve; this region is called the habenula perforata.[144] On top of the osseous spiral lamina rests a fibroepithelial mound, the spiral limbus, which acts as an attachment point for Reissner's membrane (Fig. 2.11). The spiral limbus has an upper and a lower lip separated by an intervening groove, the inner sulcus.[228] The upper or vestibular lip overhangs the inner sulcus and carries the tectorial membrane, whereas the lower or tympanic lip continues into the basilar membrane and is lined by the inner sulcus cells. On its apical side, the spiral limbus has teeth-like extracellular projections called Huschke's teeth, which are separated by epithelial cells, the interdental cells or Huschke's cells, which cover Huschke's teeth completely.[217,228] The secretory product of the interdental cells forms the tectorial membrane.[247]

Tectorial membrane

The tectorial membrane consists of fibrils embedded in a homogenous gelatinous mass of extracellular material which extends over the inner sulcus and the organ of Corti.[82] It is attached to the vestibular lip of the spiral limbus, where it extends over the entire luminal surface of the spiral limbus and covers the interdental cells (Fig. 2.13). The free part extends laterally and has a thicker part that terminates at the level of the Hensen's cells. The superior surface facing the scala media is convex, whereas the undersurface is fairly smooth and faces the organ of Corti. Only the tips of the longest stereocilia of the outer hair cells are in direct contact with the tectorial membrane.[202,217]

Basilar membrane

The basilar membrane is divided into a pars tecta, lying beneath the tunnel of Corti, and a pars pectinata, which extends to the basilar crest.[217] The sensory and nonsensory cells of the organ of Corti are separated by a basement membrane from the connective matrix of the basilar membrane. The inferior

surface of the basilar membrane is covered by mesothelial cells which are spindle-shaped and although they are connected, there are large intercellular areas. The basilar membrane is mainly composed of extracellular matrix material with collagen fibers embedded in a homogeneous ground substance.[247] The pars tecta contains single fibers arranged in a regular order, parallel to each other. In the pars pectinata the fibers are organized in bundles. The diameter of the fibers increases from the apex to the base, which increases the stiffness of each fiber about 100 fold.[82, 217]

Organ of Corti

The structure of the cochlear sensory epithelium or organ of Corti is very complex. Basically, it consists of two populations of cells: hair cells and supporting cells. The hair cells are the true sensory receptors. There are two subpopulations of hair cells: inner hair cells, arranged in a single row, and outer hair cells, arranged in 3-5 rows (Fig 2.13 and 2.14).

The supporting cells can be divided into six different types, from medial to lateral: inner border cells, inner phalangeal cells, inner pillar cells, outer pillar cells, outer phalangeal cells (or Deiters' cells), and Hensen's cells. At the apical side of the organ of Corti, both inner and outer hair cells, pillar cells, and phalangeal cells are attached to each other and their apical cytoplasm contains large numbers of microtubules and microfilaments, forming the reticular lamina of the organ of Corti.[217,228,247]

Unlike the supporting cells, the hair cells do not reach the basilar membrane. The inner hair cells are surrounded by the inner border, inner pillar, and inner phalangeal cells, whereas only the basal regions of the outer hair cells are enclosed by the Deiters' cells. The inner and outer pillar cells form a triangular structure called the tunnel of Corti.[82] This fluid-filled space communicates through clefts between the outer pillar cells with Nuel's space, which surrounds the outer hair cells and extends from the outer pillar cells to the innermost of the Hensen's cells. The fluid in these spaces is called the cortilymph and in biochemical composition it is similar to perilymph, since fluid exchange is possible between the tunnel of Corti and the scala tympani.[217,228,247] The inner hair cells are entirely surrounded by inner phalangeal cells.

Hair cells

The inner hair cells, about 3,500 in number, are pear-shaped cells with a large, round central nucleus and a narrow neck just below the apical (luminal) membrane.[82,165] The apical membrane contains numerous stereocilia in a horizontal array without kinocilia.[144] The cytoplasm contains numerous mitochondria and profiles of rough endoplasmic reticulum. An array of smooth endoplasmic reticulum with a limited number of profiles is present on the inner

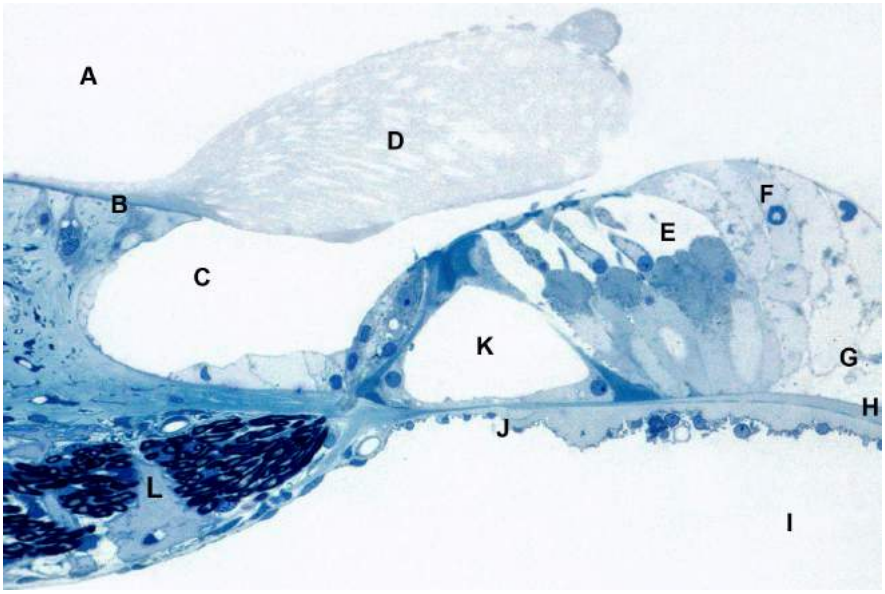


Fig. 2.13. Organ of Corti in the lower middle turn of the cochlea of a normal dog. A = Scala media. B = Spiral limbus. C = Inner sulcus. D = Tectorial membrane. E = Space of Nuel. F = Hensen's cell. G = Claudius'cell. H = Boettcher's cell. I = Scala tympani. J = Basilar membrane. K = Tunnel of Corti. L = Osseous spiral lamina.

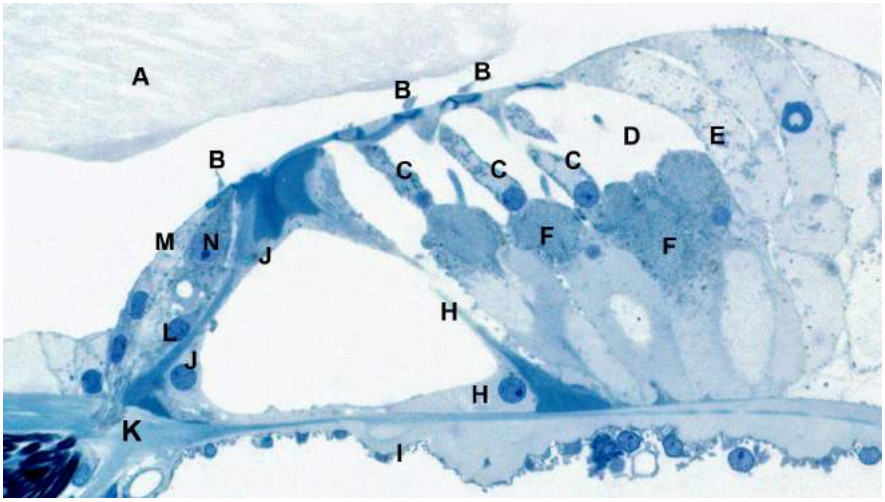


Fig. 2.14. Higher magnification view of the organ of Corti in the lower middle turn of the cochlea of a normal dog. A = Tectorial membrane. B = Stereociliae. C = Outer hair cells. D = Phalangeal process of Deiters' cell. E = Hensen's cell. F = Deiters'cell. H = Outer pillar cell. I = Basilar membrane. J = Inner pillar cell. K = Habenula perforate. L = Inner phalangeal cell. M = Border cell. N = Inner hair cell.

surface of the lateral membrane.[217] The apical part of the cell is tightly connected to the adjoining inner border cells and inner pillar cells by junctional complexes. There are up to approximately 50 stereocilia per cell in three to five parallel rows, with length increasing from row to row in a lateral direction.[82,247] None of the stereocilia of the inner hair cells appears to be in direct contact with the overlying tectorial membrane. Each inner hair cell is innervated by many type-I afferent nerve fibers, each cell receiving approximately 20 nerve fibers.[165,261] Connections of efferent fibers of the olivocochlear bundle (or Ramussen's bundle) to inner hair cells are more sparse. They originate from the lateral part of the superior olivary complex, are small and unmyelinated, and terminate on type-I afferent synapses at the base of the inner hair cells.[261]

The outer hair cells**,** about 20,000 in number, are arranged in three to five rows. They have a more elongated columnar shape than the inner hair cells and the nucleus is located in the basal part.[82,165] At its base each outer hair cell makes contact with a number of synapses of the type-II afferent nerve fiber branches from the cochlear nerve in a niche between the outer hair cell and its Deiters' cell. The stereocilia occur in numbers of up to 100 per cell and are arranged in the form of a "W" with its base pointing peripherally in a lateral direction.[82,217,247] Unlike those of the inner hair cells, the longest stereocilia are clearly embedded in the overlying tectorial membrane.[217] Innervation of the outer hair cells differs from that of the inner hair cells. A single type-II afferent nerve fiber connects to several outer hair cells, and outer hair cells receive the largest number of large efferent synapses.[165,261] Many large efferent myelinated fibers originating from the medial part of the superior olivary complex terminate directly on outer hair cells.[165]

Supporting cells

The inner border cells form numerous rows of slender low cuboidal cells at the transition with the inner sulcus and gradually increase in height in a lateral direction (Fig. 2.14).[82,217] The inner phalangeal cells are tall columnar supporting cells interposed between the inner border cells and the inner pillar cells.[82,217] The nucleus is located in the basal part of the cell. The main part of the cell body is generally located on the lateral part of the inner hair cell. The nerve fibers and their synapses at the base of the inner hair cells are entirely enclosed by the cell bodies of the inner phalangeal cells. The inner pillar cells rest on the basilar membrane and their broad, almost triangular bases contact the inner phalangeal cells medially, whereas laterally they are in contact with the bases of the outer pillar cells.[82,217,228] The nucleus of the cell is located in the lateral part of the base. From the medial part of the base, a bundle of tonofibrils extends up through the entire cell body to end in a density just below the apical membrane.

The outer pillar cells basically have the same structure as the inner pillar cells. The pillar-shaped part of the cell body ends apically in an expansion with a shape different from that of the inner pillar cells. Its convex apical end accommodates the concavity on the apical part of the inner pillar cell, but also continues laterally into a plate-like luminal phalangeal process.[82,217,247] The latter contains a condensed cuticular plate and forms occluding junctions with the phalangeal processes from adjoining outer pillar cells and outer phalangeal cells. The slender cell bodies of the outer pillar cells are separated from each other by large intercellular clefts.

The outer phalangeal cells or Deiters' cells support the outer hair cells and are located at their base in a corresponding number of rows.[82,217] The lower part of the outer phalangeal cell is columnar and its base rests on the basilar membrane. Here, the cells closely contact each other as well as the bases of the outer pillar cells and Hensen's cells. This columnar part of the cell contains the nucleus and has a deep concavity, which encloses the basal part of the outer hair cell, together with the afferent and efferent synapses.[165,247] The columnar part of the outer phalangeal cell does not reach the apical surface of the organ of Corti, but a long, slender phalangeal process arises from its lateral surface and ends in a flattened expansion facing the scala media.[82,217] The outer border cells or Hensen's cells are columnar cells that terminate the organ of Corti laterally. They rest on the basilar membrane and closely contact each other without intercellular clefts.

Outside the organ of Corti, two additional types of supporting cells are present. Claudius' cells and Böttcher's cells are cuboidal to columnar cells that cover the most lateral part of the basilar membrane. Böttcher's cells are only present in the basal turn. Their function is as yet unknown.[82,217]

Computed Tomographic Anatomy

The canine ear is a very complex structure and its morphology is difficult to evaluate with diagnostic imaging techniques. Radiography was widely used in the past to evaluate the middle ear cavities but it has proved to be too insensitive to identify lesions of the middle ear or to image such normal structures as the middle ear ossicles, much less the even smaller structures of the inner ear.[220,225] The best imaging techniques available in veterinary medicine today are computed tomography (CT) and magnetic resonance imaging (MRI). Recent reports have emphasized the use of MRI in patients with peripheral vestibular ataxia to diagnose otitis media and otitis interna.[77] However, there have been no reports on the use of MRI in the diagnosis of hearing loss in dogs. CT may be preferable to MRI when osseous involvement is suspected and it is essential for pre- and postoperative imaging in patients receiving middle or inner ear implants, because the nature of the implants prevents the use of MRI. The

computed tomographic anatomy of the inner and middle ear in normal dogs was described recently.[225]

CT scans were used for diagnostic imaging in this thesis. The structures of the outer ear, middle ear, and inner ear that can be identified on CT scans are shown in Figures 2.15 and 2.16.

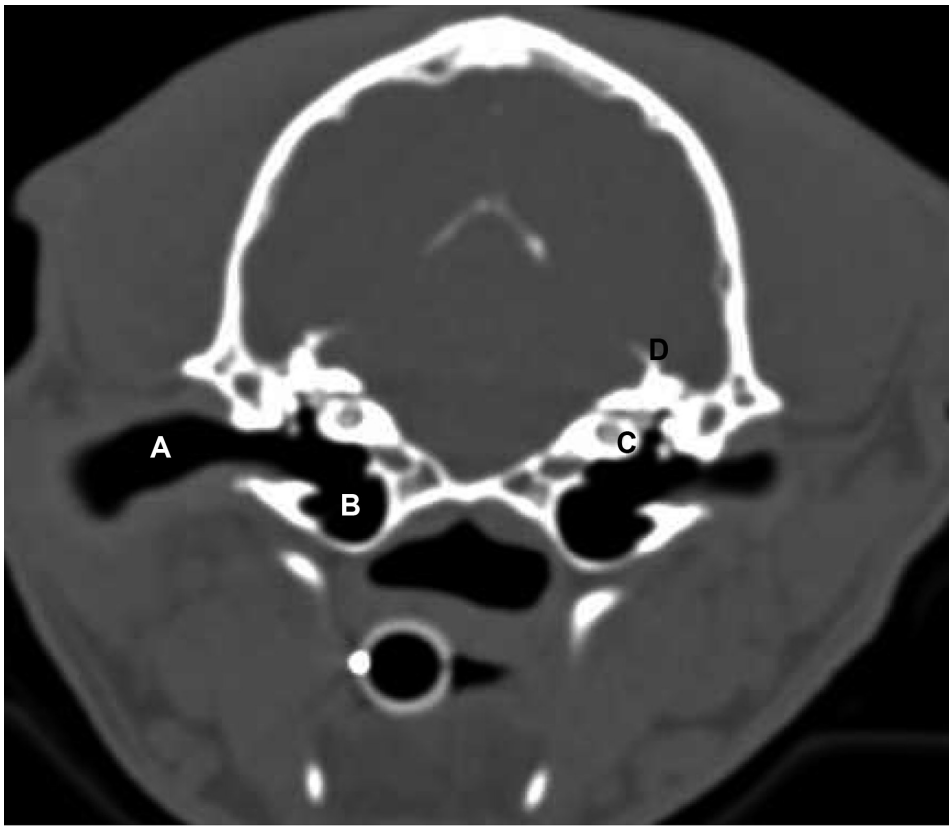


Fig. 2.15. Transverse CT image at the level of the middle ear of a dog. A = Outer ear canal. B = Middle ear cavity. C = Cochlea. D = Petrosal Bone.

Fig. 2.16. This figure is located on the following page and shows three consecutive slices of transverse CT-images of the middle ear of a dog, with 2 mm spacing. *Inserts on the left figures are shown enlarged on the right. A) A = Malleus. B = Epitympanic recess. C = Cochlea. D = Septum bulla. E = Tympanic bulla. B) A = Incus. B = Oval window. C = Aqueductus vestibule. D = Cochlea. E = Tympanic bulla. F = Outer ear canal. C) A = level of round window. B = Tympanic bulla.*

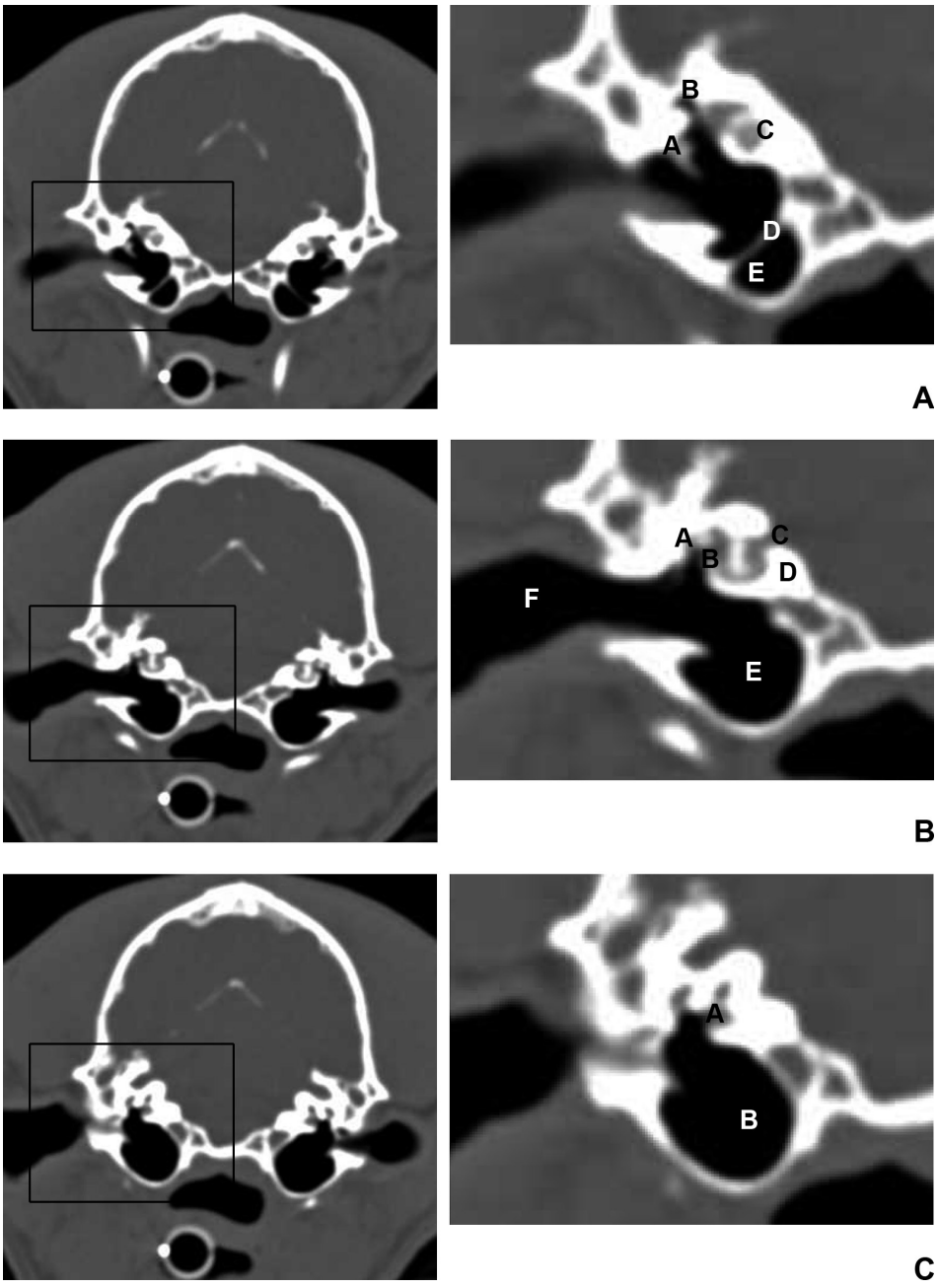


Fig. 2.16. The legend of this figure can be found on the previous page.

Physiology of the Ear

Introduction

Sound normally reaches the cochlea via the outer and middle ear, but it may also reach the cochlea by conduction through bone. The auricle and ear canal have two roles in transmitting sound to the tympanic membrane. They aid in sound localization and they increase the sound pressure at the tympanic membrane by resonance.[166,201,213] The middle ear acts as an acoustic impedance transformer, transmitting energy from low-impedance air over the middle ear ossicles to the higher-impedance cochlear fluids.[166,201,213] Sensory transduction occurs in the cochlea, where the mechanosensory cells in the organ of Corti transform sound into a train of nerve impulses in the auditory nerve, thus conveying information to the brain. The cochlea separates sounds according to their frequency components so that different populations of hair cells become activated by sounds within different parts of the audible frequency range.[166,201,213] This frequency specificity is determined by the location of maximal amplitude of the traveling wave over the basilar membrane, which depends on the membrane's characteristics.[166,201,213] Furthermore, it depends on the outer hair cells, which play an important and active role in increasing the frequency selectivity of the basilar membrane, especially at low sound intensities.[166,201,213] Humans are generally capable of hearing sounds in the frequency range of 20 Hz to 20 kHz[167], whereas in dogs the high-frequency cut-off is much higher at 43 kHz.[98] In addition, dogs are capable of hearing sounds at lower intensities than are humans.

Hearing involves the perception of sounds, which are air pressure waves generated by vibrating air molecules. Sound waves propagate in three dimensions, creating spherical shells of alternating compression and rarefaction.[213] Like all wave phenomena, sound waves have four major features: waveform, phase, amplitude, and frequency. These determine the perception of sound, especially the frequency and the amplitude of the waves. Sounds composed of a single sine wave are, however, extremely rare in nature; most sounds consist of acoustically complex waveforms.[213] The frequency of a sound, expressed in cycles per second or Hertz (Hz) roughly corresponds to its pitch, whereas the amplitude, usually expressed in decibels (dB), determines its loudness. A change in the frequency composition and/or amplitude of a sound causes a change in stimulation of the ear and it is this that results in perception.[213]

Outer ear

As mentioned above, the auricle, the ear canal, and the head influence the sound that reaches the tympanic membrane. In a free sound field, the head causes the sound pressure at the entrance of the ear canal to be higher than it would be at

the same location in the field in the absence of the body.[166] This effect depends on the frequency of the sound and on the direction of the head relative to the sound source. Lower frequency sounds pass around the head, whereas middle and higher frequencies are captured by the auricle. The difference in time of arrival of a sound at the two ears is the physical basis for directional hearing in the horizontal plane, together with the difference in intensity of the sound at the two ears.[166] Intra-aural time differences in the onsets of sounds are most important for sounds below 1,500 Hz, while it is the difference in the intensity that is most important for high-frequency sounds.[166] The raised ridges of the pinna and conchae aid in reflecting sound waves into the ear canal and are believed to provide clues on the direction of the sound source as coming from the front or behind or from above or below.[201]

The outer ear collects sound waves over the large area of the pinna and concha and funnels them into the narrower ear canal. The sound pressure at the tympanic membrane not only depends on the acoustic properties of the auricle and the head, but also on those of the outer ear canal. The concha of the auricle and the outer ear canal act as resonators, increasing the sound pressure at the tympanic membrane.[166] The gain is greatest near 2.5-3 kHz (the resonance frequency) in humans, being approximately 10-20 dB.[166,201] In the cat, the pinna can produce a gain of up to 21 dB in sound pressure at high frequencies.[176] A gain as a result of resonance in the auricle and outer ear canal has not been studied in dogs, but is likely to be similar to that in cats. Even though the auricle in dogs varies greatly in size and shape, more so than in cats, studies have shown that the type and position of the pinnae (erect versus pendulous ears) at least do not appear to have a large effect on the free-field audiogram.[98] Little is known about the effects of the frequent auricle movements in dogs and cats on sound localization. Research in cats has shown that for sound sources in front of the cat, the midfrequency components provide the clue for source localization.[218]

Middle ear

The middle ear acts as an impedance transformer that matches the high impedance of the cochlear fluids to the low impedance of the air in the outer ear canal. The middle ear transformer uses two principles. First, the pressure is increased because the area of the oval window is smaller than that of the tympanic membrane. [166,201,213] Second, the lever action of the ossicles increases the force acting on the oval window.[201] It is the difference between the force that acts on the two windows of the cochlea that sets the cochlear fluid in motion. Normally, the force on the oval window is much larger than that acting on the round window because of the gain of the middle ear, via the ossicular chain.[166] A broken chain results in hearing loss. The gain of the middle ear is frequency dependent and the increase in sound transmission to the cochlear

fluid, due to improvement in impedance matching, is close to 30 dB in the midfrequency range in people.[166] In dogs the gain of the middle ear is unknown, but in cats the greatest transmission is produced in the range around 1-2 kHz and is also around 30 dB.[181,222,302] Transmission through the middle ear is affected by the middle ear muscles, which reduce the transmission of low-frequency sounds. This may serve to protect the ear to some extent from noise damage.[201]

Inner ear

The movement of the stapes footplate in the oval window sets up a fluid wave inside the scala vestibuli in the cochlea, displacing the fluid toward the round window. As the basilar membrane is displaced by this traveling fluid wave, the hair cells within the organ of Corti are also displaced. This displacement causes deflection of the stereocilia, which results in opening of the ion-selective channels by the shearing forces on the stereocilia. This allows K^+ ions to enter the cell, causing depolarization of the hair cell.[40,167,202,213] This generates a sensory action potential which, when summed with numerous hair cell sensory action potentials, generates the all-or-none nerve compound action potential. The inner hair cell clearly forms the center of the cochlear apparatus, as it is solely responsible for transducing the mechanical energy brought by sound and initiating the receptor potential and the auditory nerve action potential.[40] Deflection of the stereocilia by the traveling wave opens and closes ion channels in the stereocilia, thereby modulating the current being driven into the hair cells, its magnitude determined by the combined effects of the positive endocochlear potential and the negative intracellular potential.[167,202] Changes in the electrical potentials can be measured in the hair cells with fine microelectrodes, and grossly in the cochlea with larger electrodes. The potentials that can be recorded with an electrode at or near the cochlea became known as the cochlear microphonics (CM), the source of which is mainly the outer hair cells. Synchronized activity of auditory nerve fibers and of neurons in the passageway through the brainstem toward the auditory cortex can be recorded as far-field potentials with large electrodes on the skull (brainstem-evoked response audiometry, see Chapter 3).

The range of sound frequencies and intensities that the ear is capable of handling is impressive. The frequency selectivity of the cochlea has been the topic of many studies and in the past ten years the knowledge concerning this subject has increased dramatically. It has been known for a long time that frequency selectivity of the cochlea is based on the basilar membrane characteristics. Only recently, it became known that it is the active function of the outer hair cells that provides the fine-tuning of the frequency selectivity and that it is responsible for the amplitude compression of sounds and the creation of otoacoustic emissions.

In the 19th century, Von Helmholtz proved that the ear performs a spectral analysis of sounds. He suggested that the basilar membrane functions as a series of resonators tuned to different frequencies covering the audible range.[100] This became known as the resonance theory.[167] Georg von Békésy showed that the tone of a certain frequency caused the highest vibration amplitude at a certain point along the basilar membrane and that a frequency scale could be laid out along the cochlea with high frequencies located at the base and low frequencies at the apex.[301] Von Békésy showed that sounds set up a traveling wave motion along the basilar membrane. Traveling waves produced by sounds of high frequency reach a peak near the base of the cochlea, whereas waves produced by low-frequency sounds reach a peak closer to the apex.[40,167,202,213] The physical make-up alone, however, cannot explain the fine-tuning properties as implied by psychoacoustic data on the frequency discrimination in the auditory system.[167] It was not until much later that the actual source of the fine tuning was discovered to be the outer hair cells.

The properties of the outer hair cells were further appreciated by the studies of Brownell and co-workers, who analyzed in vitro solitary outer hair cells removed from the cochlea. As the outer hair cells were stimulated, they were found to actually expand, stretch, and contract.[25] This property is known as electromotility or as the cochlear amplifier.[167,202,213] The sharply tuned peak of the traveling wave arises because the outer hair cells, when stimulated by the movement, make an active mechanical response that amplifies the vibration of the basilar membrane as the traveling wave passes through.[202] The traveling wave therefore increases in amplitude as it passes along the cochlear duct, until it dies away abruptly when it reaches a point where the cochlear partition can no longer sustain vibrations of that particular frequency.[202]

It is now also known that the ability of the basilar membrane to separate the frequency components of sounds is intensity dependent because of the active role of the outer hair cells.[167] For low sound intensities the outer hair cells actively compensate for the energy losses in the basilar membrane. The active amplification by the outer hair cells has its largest effect at low stimulus intensities and makes a smaller contribution at higher intensities.[167,202] Therefore, the frequency selectivity of the cochlea decreases with increasing stimulus intensity and the location of the maximal response of the basilar membrane shifts towards the base of the cochlea.[167] This way the cochlea compresses the amplitude of sounds before initiating nerve impulses in the auditory nerve. This has an important functional consequence, because it allows the auditory system to discriminate stimuli over a very wide range of stimulus intensities.[167]

The finding of the active outer hair cells brought the understanding of the functioning of the cochlea a large step forward and answered many, but not all, unresolved questions that had been troubling researchers for many years.

The active role of the outer hair cells explains why metabolic energy is necessary to maintain the normal sensitivity and frequency selectivity of the ear. Furthermore, it explains why oxygen deprivation causes the threshold of auditory nerve fibers to increase and the tuning to become wider.[167] The fact that efferent neural activity controls the function of the outer hair cells implies that the mechanical properties of the basilar membrane can be modulated by the brain. This will undoubtedly be the subject of many future studies.

REVIEW: Age-Related and Noise-Induced Hearing Loss in Dogs: Current Status and Future Directions in Diagnosis and Treatment

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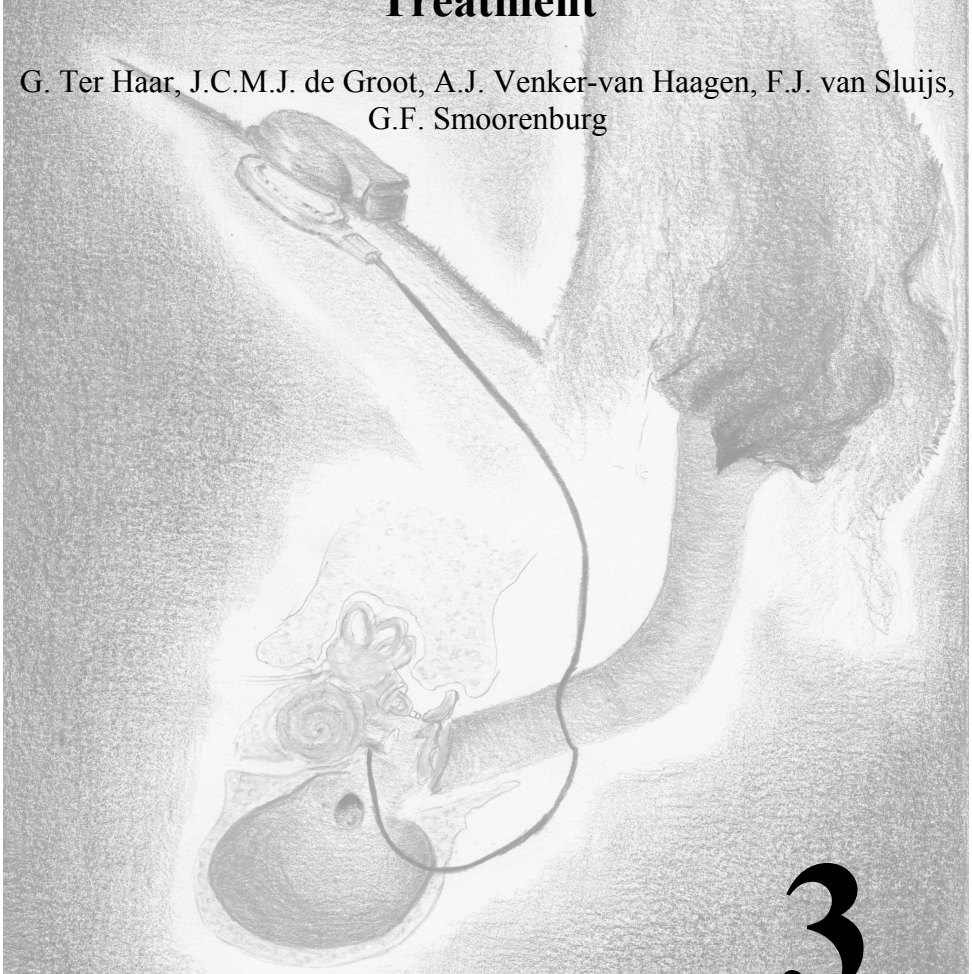

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Manuscript in preparation for submission

Abstract

The forms of acquired sensorineural hearing loss (SNHL) in dogs for which owners most often seek veterinary advice are noise-induced hearing loss (NIHL) and age-related hearing loss (ARHL). This article reviews current knowledge about the etiopathogenesis, diagnosis, and treatment of ARHL and NIHL in dogs and discusses current and future directions in hearing research on prevention and treatment. ARHL is increasingly recognized in dogs and has many similarities to presbycusis in humans. Objective evaluation of hearing is essential for diagnosis and in dogs it is usually obtained with Brainstem Evoked Response Audiometry (BERA). The use of BERA with click and tone burst stimulation for diagnosis of NIHL and ARHL is discussed. In humans, prevention and treatment of SNHL focuses on otoprotective agents, hearing aids, implants, hair cell recovery, and stem cell therapy. Treatment of ARHL in dogs with implantable hearing aids is being investigated and has shown promising preliminary results.

Introduction

Hearing loss is a common disorder in many breeds of dogs and the auditory dysfunction and its clinical consequences can vary from mild to severe.[106,215,277,314] The most frequently observed forms of hearing loss in dogs are: (1) congenital sensorineural (inherited deafness), (2) acquired sensorineural (age-related hearing loss [ARHL] or presbycusis, noise-induced hearing loss [NIHL], and ototoxicity), and (3) acquired conductive hearing loss due to chronic otitis externa and media.[265,277] A thorough history and physical, neurological, and otoscopic examinations usually make it possible to differentiate between conductive and sensorineural hearing loss.[283] Advanced CT- and MR-imaging is necessary to examine the middle ear and inner ear in order to rule out conductive deafness and to plan treatment.[17,77,220,225] Although essential for the diagnostic work-up of all patients with hearing disorders, CT- and MR-imaging can only identify anatomical abnormalities of the petrous bone and the inner ear structures. Diagnosis of functional abnormalities requires electrophysiological methods such as impedance audiometry, evoked response audiometry, and electrocochleography.[33,245,282] Of these, brainstem-evoked response audiometry (BERA) is used most frequently in veterinary medicine because it yields objective and reproducible information. Furthermore, it is relatively easy to perform, noninvasive, safe, and cost-effective.[282,314]

Sensorineural hearing loss (SNHL) cannot be cured as yet, but modern hearing aids, assistive listening devices, middle ear implants, cochlear implants, and brainstem implants provide valuable aids for communication in hearing-

impaired people.[7,80,112,241,313] The use of hearing aids for dogs with SNHL has been reported occasionally, but there have been no comprehensive studies of their clinical use and benefits.[155,277] The clinical use of middle ear implants in dogs with mild to severe SNHL is currently under investigation.[285,286] This article reviews our present knowledge about the etiopathogenesis, diagnosis, and treatment of ARHL and NIHL in dogs and discusses current and future directions in hearing research on treatment to restore hearing in dogs with these forms of SNHL.

Diagnosis of SNHL in dogs

Hearing in dogs has been evaluated by several methods, ranging from behavioral studies to electrophysiological measurements of neural responses after auditory stimulation. A psychoacoustic audiogram can be constructed by measuring behavioral responses to a sound. However, it requires extensive training of both the dog and the observer before reliable results are obtained, which renders the technique unsuitable for young puppies and clinical cases.[98,221,314] Two important conclusions about hearing in dogs can be drawn especially from the behavioral studies by Heffner.[98] First, the audible range of frequencies is much broader in dogs than in humans, particularly in the high-frequency range. Upper cut-off frequencies range from 41 to 47 kHz in dogs, compared with the cut-off frequency around 20 kHz in humans.[165] Second, neither the size of the dog (from Chihuahua to Saint Bernard) nor the type of auricle (erect versus pendulous) affects hearing thresholds or high-frequency cut-offs. Several more objective methods have been employed to test hearing ability in dogs, ranging from impedance audiometry and BERA to electrocochleography.[107,119,152,245] Whereas these techniques have mainly been used and evaluated in research settings, BERA is also commonly used in clinical veterinary practice.

Auditory stimulation evokes electrical responses in the auditory pathway in a consistent manner.[165,202] When a nerve impulse is generated in the cochlea, the signal travels along the auditory nerve to the cochlear nuclei in the brainstem. From the cochlear nuclei, many projections lead to other nuclei in the brainstem and ultimately to the primary auditory cortex.[165,202] These electrical changes can be recorded from scalp electrodes (Fig. 3.1).

When amplified and averaged, they typically consist of a complex wave having several distinct peaks, usually 5-7, occurring during the first 10 ms after the presentation of a transient sound.[114,115] In dogs, these wave peaks are numbered with Roman numerals, as they are in humans. In dogs, wave peak VII is usually absent and wave peaks III and IV often merge, which has led to confusion about the correct labeling of peaks.[12,19,124,125,242,245,295,298,314]

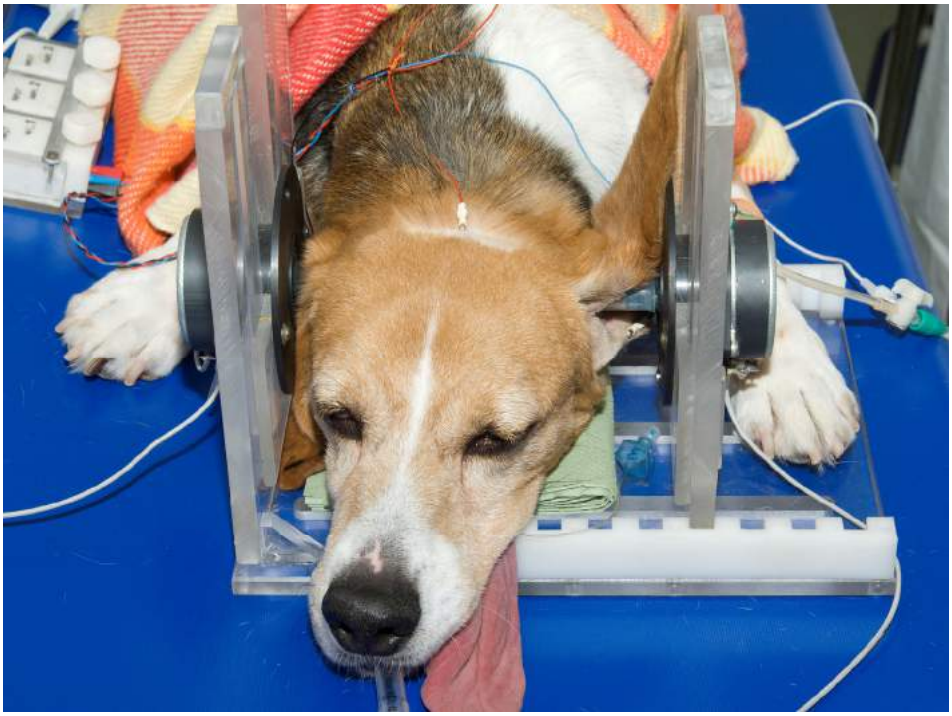

Fig. 3.1. *Recording of BERA with the dog in sternal recumbency under a light plane of anesthesia. Three recording electrodes are inserted subcutaneously: 1) recording electrode at the base of the pinna of the ipsilateral stimulated ear, 2) common (ground) electrode at the base of the pinna of the contralateral ear, and 3) reference electrode over the occipital protuberance on the midline. Stimulations are delivered to the ear via a flexible transmission tube, 4.5 cm long with an internal diameter of 8 mm, inserted 1 cm into the vertical ear canal.*

Most authors therefore use the following criteria for labeling the wave peaks in dogs (Fig. 3.2): wave peak I is the first recognizable wave with a positive deflection and wave peak V is the positive peak occurring immediately before the deep negative trough in the second half of the recording.[245,282,295,298,314]

The time between stimulation and the electrophysiological response is referred to as latency. Generators of the early-latency components—responses recorded 0–10 milliseconds after the stimulus—are thought to be located almost completely within the brainstem[245,314] and therefore this series of waves is referred to as the brainstem auditory evoked response. Neuroanatomical structures were eventually identified for the different waves, which have proved to be very similar in humans, dogs, and other mammalian species.[26,67,107,115,267,293]

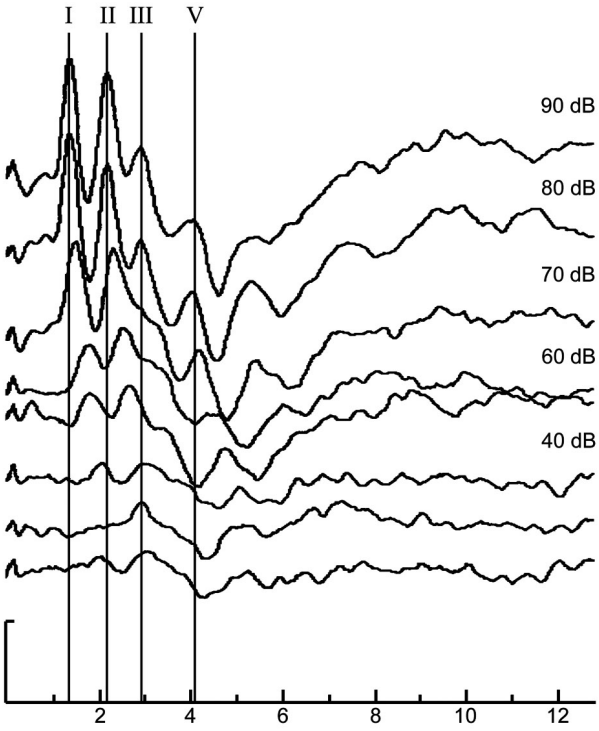

Fig. 3.2. *Representative sample of BERA with an 8 kHz (90-20 dB SPL) tone burst stimulation in the left ear of a healthy 5-year-old dog. The positive peaks are labeled with Roman numerals. Wave peak IV is missing in this tracing and peak V is identified by the waveform and latency. The vertical bar represents 1 μV.*

Brainstem auditory evoked responses do not reflect a simple sequential transfer of information, but rather the complexity of different auditory brainstem structures and the variable timing of activity arising from them.[230] In early studies it was assumed that each peak could be attributed to a single neural generator, but it is now generally accepted that each wave peak represents information from more than one anatomical structure, with the exception of peaks I and II.[26,164,166,230] Wave peak I is generated by the distal part of the vestibulocochlear nerve and peak II by the intracranial portion of this nerve.[166,293] The other peaks represent electrical activity in the cochlear nucleus, the superior olivary complex, the lateral lemniscus, the inferior colliculus, the medial geniculate nucleus, and thalamocortical radiations.

In dogs, factors known to influence the latencies and amplitudes of the elicited waves are related to the gender, age, head size, and body temperature of the animal being examined and to the stimulation and recording protocols, whereas the effect of anesthesia on the evoked responses is generally considered to be negligible.[33,34,153,163,175,177,210,244,245,270-272] There have been various reports of the use of BERA in dogs, including descriptions of its basic principles, the equipment used, and the stimulus and recording factors. Most authors have used click stimulation to assess auditory function.[12,124,131,153,178,244,274] Click stimuli are very brief (0.1 ms) and most of their energy is in the range of 500 to 4000 Hz.[314] Click stimulation is valuable for differentiating sensorineural from conductive deafness, detecting brainstem lesions, and for intraoperative monitoring.[67,148,178,267] In dogs, BERA with click stimulation has been used most extensively and successfully in the diagnosis of congenital forms of SNHL. No frequency-specific thresholds are required, since hearing loss is complete in these cases.[278]

The main disadvantage of BERA with click stimulation is its lack of frequency specificity, which makes it less useful for determining hearing thresholds across the full range of hearing frequencies. Frequency-specific information is needed to determine the extent of loss of auditory function in ARHL, NIHL, and ototoxicity.[61,249,263,277,295] Frequency-specific areas in the cochlea can be tested by using click stimulation with high-pass noise or notch-noise masking, and by direct stimulation with tone pips (tone bursts). In addition, auditory steady-state evoked potentials can be used for frequency-specific threshold measurements and their use in dogs has also been reported.[152] The latter method appears to be valid for obtaining frequency-specific thresholds in dogs, but frequencies higher than 8 kHz were not tested. Tone burst stimulation has been used more widely, is easy to perform, and has been shown to provide reliable information about pure-tone thresholds.[44,45,61-63,133,188,263] Tone bursts are tones of short duration that attempt to combine the rapid onset of a click with the frequency specificity of a pure tone. However, frequency specificity is technically difficult to achieve with tone bursts. Spread of energy to frequencies other than the nominal center frequency, a phenomenon known as spectral splatter, is of concern when using tone bursts.[208,243,282,284,314] Thus, the BERA threshold obtained using tone bursts is not completely determined by the response of the neurons at the nominal frequency but also by the response at the side lobe frequencies. In patients with hearing loss, these side lobes could lead to an underestimation of the hearing loss at the nominal frequency of the tone burst.[243] In dogs, threshold measurements using tone burst stimulation have been performed in a few studies during the past decade.[208,295] Again, frequencies higher than 8 kHz were not tested in these studies. Stimulations that cover the audible frequency range of dogs more completely should be used for the evaluation of ARHL, NIHL, and ototoxicity.

In our laboratory, a method was developed to deliver tone bursts ranging from 1-32 kHz for frequency-specific assessment of cochlear function in dogs.[282]

Thresholds of brainstem auditory evoked responses to click stimulation (CS) and to different tone burst stimulations (TS) were determined in a group of healthy middle-aged dogs. Individual wave peak latencies at 80 dB sound pressure level stimulus intensity were significantly longer after TS than after CS. With TS wave peak I latencies were significantly shorter at 12 and 16 kHz than at other frequencies. Amplitudes of peaks I and V were usually lower for TS. There were marked differences in the thresholds for the different stimulations, the lowest being for CS and for TS at 12 and 16 kHz. The thresholds after the other TS were significantly higher than after CS. A threshold audiogram encompassing the entire audible frequency range in normal-hearing dogs was constructed from these data, demonstrating that the highest sensitivity of the dog's ear is between 12 and 16 kHz (Fig. 3.3).

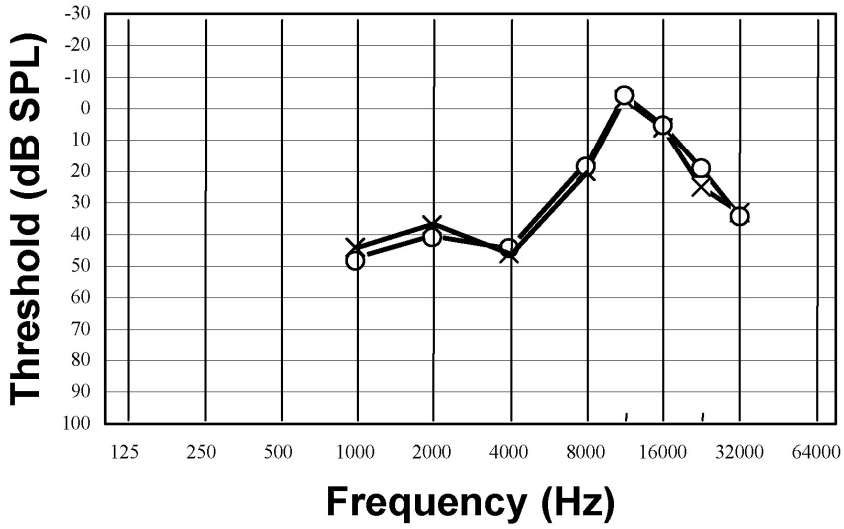

Fig 3.3. *Threshold audiogram showing mean threshold values of 10 healthy dogs with a mean age of 6 years. X = left ear, O = right ear. The standard deviation of mean threshold values ranges between 6.7 and 24.6 dB.*

Noise-induced hearing loss (NIHL)

Exposure to loud noise causes hearing loss in humans and laboratory animals.[101,167,191,192,249] There have been no reports on acoustic trauma in dogs, but NIHL may occur in dogs used for hunting with firearms.[277] Hearing thresholds in humans may return to their normal values after minutes, hours, or days, depending on the intensity and duration of the noise exposure and the individual's susceptibility.[167] Genetic variation, age, health status, smoking, drugs, and hypertension are known to influence the susceptibility to NIHL.[23,65,67,308] In addition, conductive hearing loss has been shown to act as an ear protector, decreasing SNHL from exposure to noise.[185] Reversible NIHL is characterized by temporary threshold shifts as measured by BERA. In contrast, NIHL that remains after a recovery period is due to irreversible damage to the sensory epithelium and/or auditory nerve fibers and is characterized by a permanent threshold shift.[101,167,202]

Noise can induce damage to most of the cochlear tissues, but primarily the outer hair cells are affected, especially in the basal turn of the cochlea. Outer hair cell stereocilia can be broken, fused, or have broken tip links, leading to loss of functional integrity.[142,200,291] After prolonged exposure to noise, inner hair cells, auditory nerve fibers, and even the stria vascularis are affected[101] and eventually the cellular damage progresses to more apical locations. With impulse noise exposure, in addition to the changes described above, the organ of Corti can detach from the basilar membrane.[92] Loud noise can damage the cochlea instantaneously, but the damage can also progress gradually over a period of 2 to 30 days following exposure.[1,92] Recently, free radicals, and in particular reactive oxygen species (ROS), have been implicated in causing hair cell damage from noise exposure, as well as from ageing and exposure to ototoxic drugs.[135,137,191] Free radical scavengers were shown to be able to reduce the effect of acoustic trauma, especially when administered before the noise exposure.[137]

NIHL affects higher frequencies more than lower frequencies in humans and there is usually a dip at or near 4 kHz.[138,167,214] Hearing thresholds above the affected frequency are usually better, which distinguishes NIHL from ARHL, in which hearing loss increases at every frequency above a certain frequency.[167] The amount of hearing loss depends on the intensity of the noise, the duration of exposure, and the frequency spectrum and time pattern of the noise. NIHL in humans is usually associated with continuous loud recreational or occupational noise exposure. The cumulative effects of repetitive noise exposure exceeding a maximum permissible daily dose are well known.[41,122,156,192,269] Unlike continuous environmental and occupational noise, firearms and firecrackers are sources of impulse noise exposure. Most rifles, shotguns, handguns, and firecrackers produce very high peak sound pressure

levels capable of producing permanent NIHL instantaneously if ear protection is not used.[122,156,249,269] It is very likely that NIHL after either continuous exposure to environmental noise or impulse noise is also common in dogs. At present, no data are available on the prevalence, audiometric characteristics, and histopathological consequences of NIHL in dogs.

The effects of noise-induced damage in the ear are difficult to separate from those of ageing and it is plausible that the two interact. Not only can hearing preservation be demonstrated in individuals raised in a relatively noise-free environment, but animal models have shown the existence of two windows of increased susceptibility to noise exposure, early in life (adolescence to early adulthood) and late in life, when hearing thresholds increase as a result of degeneration of the sensory epithelium accompanying ageing.[191] Studies in humans have reached similar conclusions.[288] In our studies on ARHL in dogs we found increased thresholds at 4 kHz in a group of middle-aged dogs, which could have been the result of noise-induced damage.[284] These dogs had been housed lifelong in kennels with sound-reflecting walls and although ambient noise produced by barking was significant, it was not high enough to produce NIHL in humans.[249] However, the effect of repetitively high levels of ambient noises on hearing thresholds in dogs is unknown and requires further research with determination of frequency-specific auditory thresholds.

Age-related hearing loss (ARHL)

Age-related hearing loss (presbyacusis or presbycusis in the U.S.A.) is the most common form of hearing loss in industrialized countries, affecting approximately 40% of the population by age 65.[80,112,138,183,191,226,313] The disorder is more prevalent and severe among males than among females.[39,103,197] ARHL is also the most common form of acquired hearing loss in dogs[48,277,284,285], but little is known about its prevalence, etiology, and audiometric characteristics. In both dogs and humans presbycusis reflects the cumulative effects of heredity, disease, noise, and ototoxic agents superimposed upon those of the ageing process itself.[80]

Although it is difficult to differentiate between the effects of noise-induced damage and ageing per se, chronic noise exposure leads to significant increases in hearing thresholds in the elderly. Furthermore, noise exposure at an early age might even trigger progressive hearing loss later in life.[191,288] A causal relationship between NIHL and ARHL is likely to exist in dogs, but remains to be demonstrated. Many animal models have been used to study SNHL, but mice have become the predominant model. Research in mice has examined NIHL as well as the genetic aspects of ARHL. At least 10 loci that promote ARHL have thus far been identified.[112,191] Evidence for genetic effects on the inheritance of presbycusis in humans is supported by data from Gates et al..[78] He found a clear

familial aggregation for age-related hearing levels. While congenital hearing loss in dogs obviously has a genetic basis and has been the topic of many studies, genetic effects on ARHL in dogs remain to be identified.

In addition to noise and heredity, systemic degenerative changes also appear to contribute to the development of ARHL. The process of ageing is associated with many biochemical and physiological changes, including increases in mitochondrial DNA damage and reduction in mitochondrial function. Ageing is the progressive accumulation of changes associated with or responsible for the ever-increasing susceptibility to disease and death with advancing age.[93] Although there are several hypotheses to explain the degenerative changes of ageing, the "membrane hypothesis of ageing (MHA)" or "mitochondrial clock theory of ageing" has received the most attention.[135,226,237,313] This theory is based on the progressive accumulation of oxidative damage due to the activity of ROS. There is an increase in ROS as the body ages, accompanied by depletion of endogenous free radical scavengers (e.g., glutathione) and reduced levels of protective enzymes, such as glutathione peroxidase, catalase, and superoxide dismutase.[226,237] ROS are generated in various cellular compartments by multiple intracellular enzymes. They are produced within the plasma membrane by enzymes such as NADPH oxidases and by various cytosolic enzymes such as cyclooxygenases, but approximately 90% of intracellular ROS can be traced to mitochondrial oxidative phosphorylation.[8] ROS-dependent degeneration of sensory epithelia plays an important role in presbycusis. The recent discovery that the enzyme NADPH oxidase 3 is highly expressed within the inner ear makes it a prime candidate as a source of ROS during ageing of various cochlear tissues.[135,237]

Many studies have documented the audiometric characteristics of ARHL in humans.[24,53,79,171,198,219] Cross-sectional studies of differences between age groups have shown that pure-tone hearing thresholds increase with age, particularly at higher frequencies.[18,80,81,138,183] Longitudinal studies provide a better description of the course of changes with age within individuals, with each subject acting as its own control, and single out intrinsic interindividual differences. There are only a few reports of longitudinal studies in humans but they show that a significant reduction in hearing capacity occurs from the age of 60 years onward.[24,53,79,138,198] This hearing loss begins at higher frequencies (6-16 kHz), but gradually progresses to encompass the entire frequency range. Between the ages of 70 and 80 years this reduction in hearing amounts to 1-2 dB per year, depending on the frequency tested. Longitudinal and cross-sectional studies of ARHL have also been reported for various animal species, including dogs.[21,38,49,96,191,284] Most of these studies have revealed elevated hearing thresholds in aged animals. The difference between behavioral and BERA thresholds is larger in older persons than in the young.[22] This potentially leads to an overestimation of the extent of hearing loss in older individuals,

which may also apply to older animals, when BERA is used to diagnose and characterize ARHL. Yet, since behavioral tests cannot be used in clinical studies, this is the only way to gain objective information in animals. Ageing itself does not appear to influence BERA wave peak latencies if no threshold elevation occurs.[22,194] Even when threshold elevation is accounted for, most studies suggest a reduction in peak amplitudes in older individuals and, typically, the amplitude of wave peak I is more affected by age than is peak V.[22,38,227]

In a cross-sectional and longitudinal study on ARHL in dogs, marked differences in auditory BERA thresholds at different frequencies were found between the three age groups studied (Fig. 3.4) and within the group in which BERA assessment was performed once yearly for 7 years (Fig. 3.5).[284] The cross-sectional study revealed significantly higher thresholds at all frequencies tested (1-32 kHz) in geriatric dogs as compared to young and middle-aged dogs.

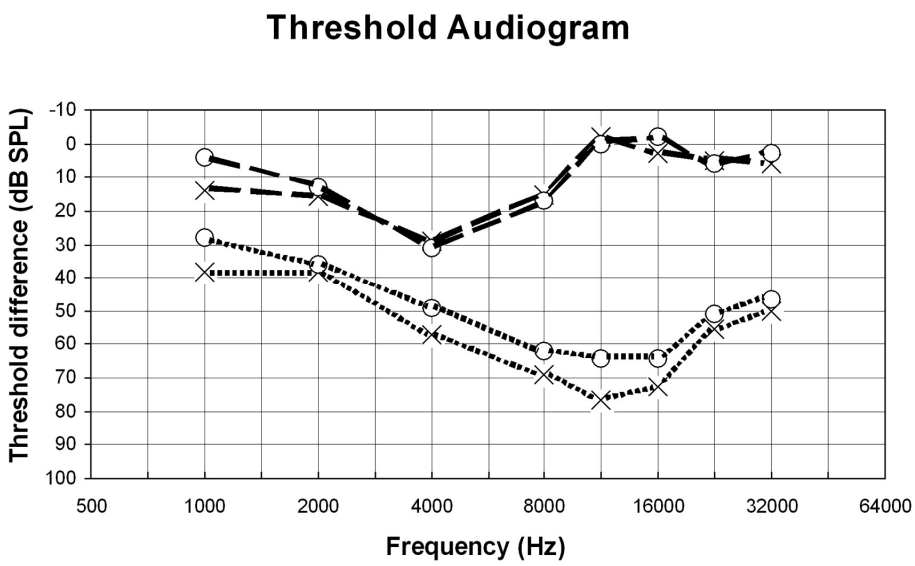

Fig 3.4. *Cross-sectional pure-tone threshold audiogram showing the threshold differences (X = left ear, O = right ear) between the young dogs with a mean age of 1.9 years and the middle-aged dogs with a mean age of 5.7 years (dashed lines) and between the young dogs and the geriatric dogs with a mean age of 12.7 years (dotted lines). For all points, SEM is between 2 and 6 dB.*

The highest absolute thresholds were found in the middle- to high-frequency region (8-32 kHz) and the increase in thresholds (Fig. 3.4) was significantly greater in this region than in the low-frequency region (1-4 kHz). The audiograms of the dogs in the longitudinal study reveal a progressive

increase in hearing thresholds with ageing, starting at 8–10 years. The effect is again most pronounced at the middle to high frequencies (8-32 kHz). While there were considerable differences in the severity of hearing loss among these dogs, statistical analysis of the average increase in thresholds revealed that the thresholds at 8, 12, 16, 24, and 32 kHz were significantly higher at a mean age of 12 years than at a mean age of 6 years.[284]

Less information is available on the histological changes in the cochlea associated with ageing and on the relationship between these changes and the shape of the audiogram. The most widely referenced framework for describing these histological changes in humans is that proposed by Schuknecht.[80,108,189,232,233,235,259] He divided ARHL into four types: sensory (predominantly loss of outer hair cells [OHCs]), neural (loss of afferent neurons and spiral ganglion cells [SGCs]), metabolic (atrophy of stria vascularis), and cochlear conductive. Later, he added two more categories: mixed (sensory, neural, and metabolic) and indeterminate (no morphological findings at cochlear level). Although >25% of cases can be classified as indeterminate, in most cochleas from aged humans there is a mixture of histopathological changes.[22,80,183,189] Age-related loss of hair cells and SGCs has been reported in several animal species, including dogs.[80,109,132,183,211,240,259] Schuknecht et al. described histological changes in the cochlea of a 20-year old dog.[234] Johnsson and Hawkins found histological changes in canine inner ears that were similar to those in elderly humans, including strial atrophy, degeneration of the organ of Corti, and vascular abnormalities.[118] Knowles et al. found a loss of SGCs in a group of deaf dogs in which auditory thresholds were completely lacking.[132] Shimada et al. reported varying degrees of SGC loss and atrophy of the organ of Corti and the stria vascularis in dogs over 12 years of age, predominantly affecting the basal turn of the cochlea.[240]

Based upon the assumption that the shape of the audiogram reflects the type of ARHL[233], the above findings imply a high-frequency hearing loss of the mixed type in dogs, comparable to that found in most cases of human ARHL. However, frequency-specific hearing thresholds were not determined in these studies. In a study of the effects of ageing on inner ear morphology in dogs and their brainstem responses to tone burst auditory stimuli, elevated hearing thresholds and cochlear lesions were found in all geriatric dogs, aged 11–14 years.[285] Together with loss of OHCs and IHCs there was a significant loss of SGCs and a reduction in the cross-sectional area of the stria vascularis (SVCA). The histological changes were primarily in the basal turn, which is consistent with the occurrence of the largest threshold shifts and lowest absolute thresholds in the middle- to high-frequency regions. It was concluded that the concomitant degeneration of OHCs and SGCs in the basal turn was primarily responsible for the elevated hearing thresholds, similar to findings in humans. It is noteworthy that SVCA was smaller in all turns of the cochlea in the geriatric

dogs. In gerbils raised in quiet environments, degeneration of the stria vascularis is an early event in ARHL, usually beginning at both the base and apex and extending to midcochlear regions with advancing age.[80,183,236,259] While the reduction in SVCA in all turns may explain the loss of hearing sensitivity over the entire frequency range in the geriatric dogs, it cannot explain the difference between low- and middle-to-high-frequency loss. The combination of OHC loss, SGC loss, and the reduction in SVCA thus seems to be the best explanation for the audiometric results. Despite Schuknecht's statements, several studies have failed to show a close correspondence between the audiometric data and histological changes, and our study is no exception.[35,182,285] The auditory thresholds found in these dogs do not indicate whether histological changes were primarily sensory, neural, or strial. This is most likely because there were mixed lesions in all cases and the severity of OHC loss, IHC loss, SGC loss, and reduction in SVCA also varied in all cases. Individual audiograms do, however, reflect the severity and location of the histological changes. In general, histological changes are more extensive in dogs with more advanced hearing loss. It is concluded that tone audiograms can, therefore, be used to diagnose and characterize ARHL in dogs, because they not only indicate the severity of hearing loss but also the extent and location of the histological changes. This information is helpful in planning treatment of ARHL with hearing aids or middle ear or cochlear implants.[285]

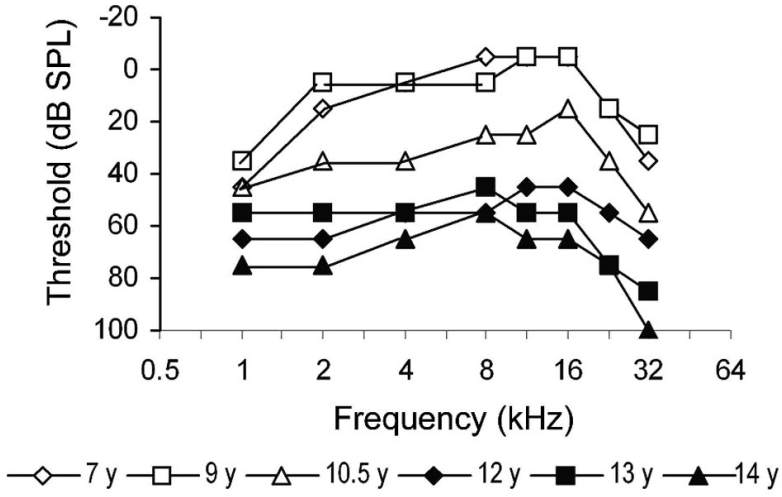

Fig 3.5. *Longitudinal tone audiograms of the left ear of 1 dog from the longitudinal group at octave frequencies from 1 to 32 kHz. This dog entered the study at 7 years of age.*

Providing a remedy for presbycusis makes an important contribution to improving the quality of life of geriatric patients. For hearing-impaired individuals, modern hearing aids, assistive listening devices, middle ear implants, cochlear implants, and brainstem implants are valuable aids for communication.[7,80,112,226,241,313] Generally, in humans with mixed SNHL and average hearing thresholds of $\geq$ 40 dB on the audiogram, amplification is indicated. Amplification is primarily accomplished with conventional hearing aids and most hearing impaired human patients are helped by these devices. Despite great technological progress and the evident advantages that digital hearing aids have over conventional (analogue) hearing devices, there remains some controversy regarding their benefits in some people. In addition, hearing aids have drawbacks, being subject to acoustic feedback, gain limitations due to anatomical factors, sound and voice distortion, ear canal occlusion, and the need for frequent servicing and maintenance, besides being unsightly, uncomfortable, and costly.[66,72,149,286] Conventional hearing aids have been used in dogs[155], but not with great clinical success and there have been no clinical reports on their efficacy.

Implantable hearing devices (middle ear and cochlear implants) have been developed for elderly people with moderate to severe SNHL who do not benefit from conventional external amplification.[66,72,149,251,252,268] Such patients with moderate to severe SNHL are typically not considered good candidates for cochlear implantation because their relatively good residual hearing may be damaged by the procedure.[149] Hence these implants are only indicated in patients with bilateral severe-to-profound hearing loss that is not improved by other means.[80] A bone-anchored hearing aid (BAHA) is a surgically implantable system for treatment of hearing loss that functions by direct bone conduction and it has mainly been used to aid persons with certain types of conductive hearing loss.[254,256] Middle ear implants provide acoustic amplification of residual hearing and transmission of sound energy by coupling a vibratory element (implanted transducer) directly to the middle ear ossicular chain or to the round window membrane (Fig. 3.6).[72,128,241,260]

The Vibrant Soundbridge (VSB) middle ear implant is the only middle-ear-implantable hearing device with US Food and Drug Administration approval that is currently available.[7] There is significant clinical experience with this implantable hearing device in Europe and several studies have been reported on the successful short-, medium- and long-term use of it in patients with moderate to severe SNHL[66,72,149,172,231,253,268,290,300] or both conductive and sensorineural hearing loss.[14,36]

No reports on the clinical use of this device in dogs with ARHL have been published, but a feasibility study demonstrated that the VSB can be implanted successfully in dogs using a lateral approach to the tympanic bulla.[286] Implant surgery is usually uneventful in humans, but postoperative infection,

healing difficulties, vertigo, facial nerve paralysis, damage to chorda tympani, tinnitus, and pain over the implant site have been reported occasionally.[14,66,72,128,149] The surgery itself does not negatively influence the residual hearing of human patients. Air-conducted hearing thresholds with nonactivated devices implanted in the ear reportedly remain unchanged. Further research is needed to determine whether postoperative problems arise in dogs as a result of this surgery, whether there is a postoperative improvement in hearing, and what are the effects on the quality of life of client-owned dogs. Ongoing clinical trials are promising, however, and implantation with the VSB middle ear implant may soon be a viable option in the treatment of dogs with moderate to severe SNHL associated with ageing. However, a clear benefit in audibility leading to a substantial improvement in patient-owner communication will have to be demonstrated in future studies to justify the high costs of the implant.

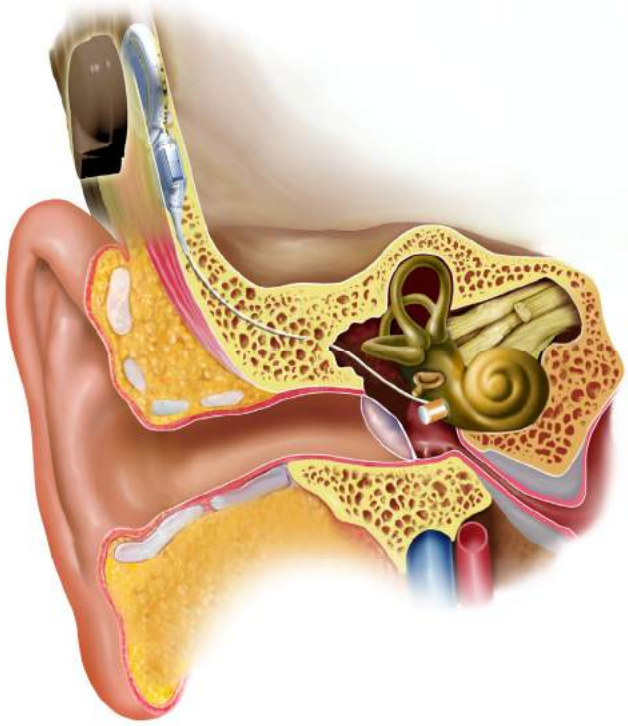

Fig 3.6. *Vibrant Soundbridge Middle Ear Implant depicted in a model of the human ear with implantation and coupling of the vibratory element (floating mass transducer) on the round window membrane (Reprinted with permission of MED-EL).*

Potential future treatment options for SNHL

Although some degree of hearing loss associated with ageing is inevitable, the deterioration can be reduced or partly prevented by avoiding hazardous noise or by suitable hearing protection.[80,159] Cardiovascular disease, hyperlipidemia, diabetes mellitus, and smoking are risk factors affecting hearing in humans and to some extent they may be reduced by maintenance of good general health and fitness. Apart from the use of hearing devices and their obvious benefits, auditory rehabilitation and patient counseling also improve general health in the elderly.[80,174] Dogs can also be expected to benefit from avoidance of the environmental health risks of smoke and noise and from prompt and correct treatment of otologic disease and avoidance of exposure to ototoxic agents.

In recent decades progress has been made in the description of the causes, symptoms, and epidemiology of presbycusis and encouraging steps have been taken to slow its course, ameliorate its severity, and reverse its effects.[313] Although current research is now focused on replacing hair cells after degeneration of the sensory epithelium in the cochlea, protecting the hair cells from damage causing the degeneration may be a more achievable goal. Several possibilities under investigation include pharmacological protection of the hair cells from free radicals, identifying and enhancing the hair cell's defensive mechanisms, and inhibiting cell death once the hair cell has been damaged.[202]

Recent studies in both rodents and humans have shown that aspirin can be used to protect hair cells from aminoglycoside-induced oxidative damage.[202,238] Many oxidants and other molecules can act as otoprotective agents and help to reduce the effects of aminoglycosides, acoustic trauma, and possibly ageing.[150,202] Recent examples are N-acetylcysteine[16], acetyl-L-carnitine[134], vitamin C[162], ebselen[130], and T-817MA[319]. Animal experiments have revealed that cellular levels of the enzyme superoxide dismutase 2 decrease markedly with ageing and another useful approach may be to induce overexpression or modulation of this enzyme within the cochlea.[159] The cochlea becomes more resistant to acoustic damage (sound conditioning) following repeated bouts of acoustic stimulation.[2,186] This may in part be explained by enhanced antioxidant defenses, since antioxidant enzymes increase in the inner ear following sound conditioning.[2,95,186,202] Furthermore, the intracellular stress pathways evoked by excessive noise can be suppressed, and hearing loss in the chinchilla has been prevented by inhibition of the Src protein tyrosine kinase signaling cascade.[94] In addition, several reports have indicated that other molecules might rescue hearing from noise-induced damage. These are basic fibroblast growth factor[323], corticosteroids[281], the glutamatergic neurotransmission blocker riluzole[305], and the glutamate receptor antagonist caroverine[31]. In addition to acoustic damage, ototoxic drugs[317] and possibly ageing lead to generation of ROS, and intracellular stress pathways within the

organ of Corti are subsequently activated. Molecules that prevent acoustic trauma are also effective as otoprotective agents in ototoxicity and may be used to prevent ARHL. Finally, neurotrophic factors such as neurotrophin-3 and brain-derived neurotrophic factor play a highly important role in the development of the auditory system[207] and are normally expressed by hair cells.[280] Both of these factors as well as glial-derived neurotrophic factor have been found to be effective against kanamycin-induced hair cell loss in rodents.[224] However, more research is needed on the clinical applicability of these otoprotective agents before they can be used in clinical veterinary practice.

Cell survival pathways are active and cell death-promoting pathways are inactive in healthy animals under physiological conditions. Cell stress disrupts this balance and can lead to the activation of apoptosis-promoting pathways and subsequent cell death. In cultures of mammalian or avian vestibular epithelia, inhibition of programmed cell death can lead to survival of hair cells after an otherwise lethal aminoglycoside insult.[32,69] NF-κB is an ubiquitous transcription factor that plays a major role in the regulation of apoptosis and was detected in an active form in the organ of Corti in young rats.[179] It has been shown that NF-κB in rats keeps the cells alive and that inhibition of NF-κB results in rapid hair cell loss.[179] These results suggest that survival of functional hair cells can be enhanced by reducing or inhibiting apoptosis in the sensory epithelium of the cochlea. Reducing apoptosis might help ameliorate ARHL, but further research is certainly needed.

Cochlear hair cells that are destroyed in humans and other mammals are not replaced. In contrast, in birds and reptiles new hair cells are generated after the inner ear has been exposed to noise or ototoxic drugs, leading to functional restoration of hearing. In the avian cochlea, the new hair cells develop after mitotic proliferation of the supporting cells or by transdifferentiating, i.e., direct conversion of supporting cells to hair cells.[85,273,304,316] It is possible to induce mitotic proliferation of supporting cells in explant cultures from the organ of Corti of the neonatal mouse.[307] Proliferation is more limited in the mature cochlea, but supporting cells could provide a source for new hair cells.[320] The proliferation of supporting cells in the mammalian cochlea is controlled by the cell cycle inhibitor known as $p27^{(Kip1)}$. Knockout of this gene in mice allows proliferation of excess supporting cells with the possible production of hair cells.[30,147,202] Transdifferentiation of mammalian supporting cells into hair cells is possible only in the presence of the key gene *Math-1*.[15,139] If this gene is introduced into the cochlea by inoculating the ear with a virus engineered to contain *Math-1*, some supporting cells transdifferentiate into hair cells, generating both inner and outer hair cells. BERA measurements demonstrate lowered thresholds in these inoculated cochleas, but although the new, transdifferentiated cells have many of the cellular characteristics of outer hair

cells, they are not completely differentiated.[111,123] Hair cell recovery strategies obviously require extensive research, especially in mammals, before their use in treatment of ARHL can be examined.

Also in an experimental, yet promising, phase is the use of stem cells in the treatment of SNHL. Stem cells have the ability to develop into specific cell types depending on their origin and local environmental factors. Some types of adult tissue harbor their own endogenous stem cells, but the regenerative capacity of such cells is sometimes limited.[20] Exogenous stem cells come from sources other than the target tissue and they often possess a higher regenerative capacity than endogenous stem cells.[20] The inner ear of mice contains endogenous stem cells and these can differentiate in vitro into hair-cell-like cells[140] and in other types of cells from the inner ear.[84,193] Exogenous stem cells have been used in a variety of studies in different animal models to replace lost cochlear hair cells and neurons. Different types of stem cells have been used, ranging from embryonic stem cells[141], bone marrow-derived stem cells[113,157] and neural progenitor cells.[180] These stem cells have the potential to repair various sites of damage in the cochlea and exciting discoveries in this field are to be expected in the near future.

Conclusions

NIHL has not been documented in dogs, but ARHL is increasingly recognized. Brainstem-evoked response audiometry using tone burst stimulation allows determination of auditory thresholds over the entire audible frequency range and is necessary for an early and complete diagnosis of acquired SNHL in dogs. Management of ARHL in humans focuses on the augmentation of hearing by hearing aids and middle ear and cochlear implants, as well as by the use of otoprotective agents and hair cell replacement. Implantation of a middle ear implant in dogs is feasible and may become a viable option for treatment of ARHL in clinical veterinary practice in the future. This would raise the standard of treatment of ARHL in this species to a level comparable to that employed in human patients. In addition, considering the similarities between dogs and humans, for future research, the dog should be considered as a model for human ARHL.

Acknowledgements

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Click and low-, middle-, and high-frequency toneburst stimulation of the canine cochlea

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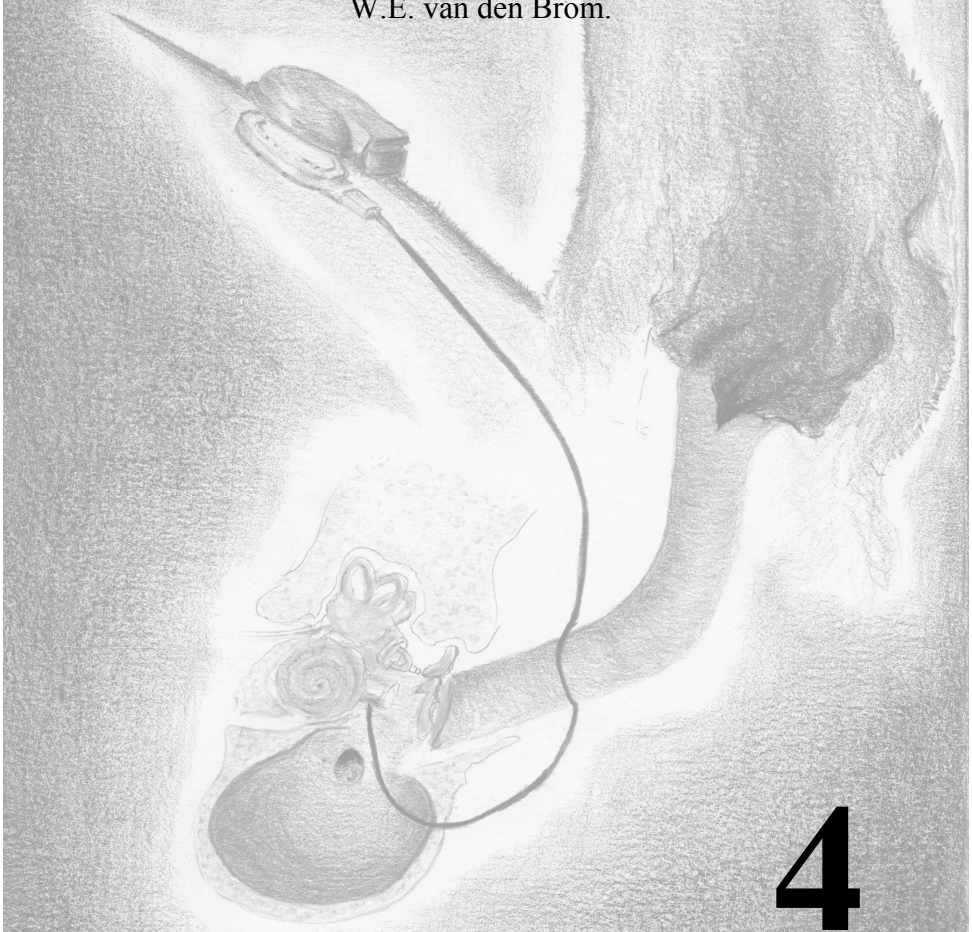
W.E. van den Brom.



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Journal of Veterinary Internal Medicine 2002;16:274-280

Abstract

A method was developed to deliver tonebursts ranging in frequency from 1 - 32 kHz for frequency-specific assessment of the canine cochlea. Brainstem auditory evoked responses (early latency responses, 0 - 10 ms) to a click (CS) and to 1-, 2-, 4-, 8-, 12-, 16-, 24-, and 32-kHz toneburst stimulations (TS) were compared at 80-dB sound pressure level (SPL) stimulus intensity in 10 adult dogs.

All stimulations yielded a 5-7 positive wave pattern, with the exception of the 1-kHz TS, which evoked a frequency-following response (FFR). Thresholds were lowest for the CS and the 12- and 16-kHz TS. All individual peak latencies for TS were significantly ($P \leq 0.05$) longer than for CS. Peak I latencies were significantly ($P \leq 0.05$) shorter for 12- and 16-kHz TS than for other TS. Interpeak latencies I-V were significantly ($P \leq 0.05$) longer for the 4- to 32-kHz TS than for CS. Differences in interpeak latencies I-III were not significant. Amplitudes of waves I and V were significantly ($P \leq 0.05$) lower for TS than for CS, except for higher wave V amplitude ($P \leq 0.05$) at 2- and 32-kHz TS. Peak I – Peak V amplitude ratios were significantly ($P \leq 0.05$) higher for the 2-, 4-, 16-, 24-, and 32-kHz TS and lower for the 8- and 12-kHz TS, compared to CS.

We conclude that reproducible information on frequency specificity of the canine cochlea can be obtained using TS. This report provides a normative database for parameters needed to evaluate frequency-specific hearing loss in dogs.

Introduction

Several methods have been employed to test hearing ability in dogs, ranging from behavioral studies to measurement of electrical responses after auditory stimulation, by impedance audiometry (tympanometry, acoustic reflex testing), evoked response audiometry (brainstem [BAER] and middle latency auditory-evoked responses), and cochlear microphony.[33,98,115,199,245,271,315]

During the past 2 decades, BAERs have been used for this purpose with increasing frequency in veterinary medicine. The acoustic signal usually has consisted of a click, which stimulates a large part of the cochlea.[19,124,125,131,245,263,298] Brainstem evoked response audiometry by a click (CS) is useful for differentiating neurologic from conduction deafness [154,266] and is useful in assessing some brainstem pathologic changes.[178,267] Frequency-specific information is needed to assess the extent of neurologic deafness, such as noise-induced deafness and that caused by ototoxicity and presbycusis, each of which can be partial and frequency specific.[11,43,178,249,276,294]

Considerable research has been conducted on factors that influence wave latency, amplitude, and thresholds in dogs, and on the clinical applicability of these factors.[13,143,163,170,177,210,264,295,298] Behavioral studies indicate that dogs can hear frequencies up to 45 kHz[98], considerably higher frequencies than heard by humans[263], but the tonebursts used in BAER assessment of hearing in dogs have been at frequencies only as high as 8 kHz.[13,264,295]

The aim of this study was to collect reference values for BAER wave peak latencies, interpeak latencies, amplitudes, amplitude ratios, and thresholds in response to CS and toneburst stimulations (TS) from 1 to 32 kHz for a more complete assessment of thresholds of hearing in dogs.

Materials and Methods

The protocol of this study was approved by the Animal Experimentation Committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

This study was carried out in 10 clinically healthy dogs, 3.5-7.0 years of age (mean, 5.7 years) weighing 12.5-21.3 kg (mean, 17.8 kg). Seven were mixed-breed littermates, and 3 were Beagles. Five were intact males, 1 was a castrated male, and 4 were intact females. None of the dogs had any clinical signs of neurologic or otic disease or showed abnormal behavior suggestive of hearing impairment. Otoscopic examination under anesthesia verified normal external ear canals and normal tympanic membranes in all 20 ears. A light plane of anesthesia was induced with medetomidine[a] (100 µg/kg IV), followed by propofol[b] (1 mg/kg IV) within 5 minutes after medetomidine sedation. Anesthesia was maintained with medetomidine (25 µg/kg IV, every 30 minutes) and propofol (1 mg/kg IV every 15 minutes during the 1st hour and every 20 minutes during the remainder of the session). At the end of the procedure, anesthesia was antagonized with atipamezole[c] (250 µg/kg IV). Body temperature as indicated by rectal temperature was maintained at 36.5-37.5 °C by means of circulating water heating pads placed under the dog.

With the dog in sternal recumbency, 3 recording needle electrodes[d] were inserted subcutaneously: a recording electrode at the base of the pinna of the ear to be stimulated, a common (ground) electrode at the base of the pinna of the other ear, and a reference electrode over the occipital protuberance on the midline. The electrodes were cleaned mechanically and electrolytically before each session. The nonstimulated ear was plugged with an adjustable wax ear plug[e]. The electrodes were attached to an amplifier[f] with a total gain of 20,000-

50,000 and a bandpass filter of 3-300 Hz. Positive electric activity at the recording electrode produced an upward deflection on the recording.

CS were generated[g] as rectangular waves of 0.2-ms duration at a nominal repetition rate of 10 Hz; phase-synchronized triggering was used to reduce power line-derived noise. The generation of CS was triggered by the data acquisition software, and the stimuli were amplified by a variable headphone attenuator.[h] They were delivered to the ear via a high-frequency bandpass speaker (tweeter[i]) with a frequency response up to 38-40 kHz. The tweeter was connected to the ear via a flexible transmission tube, 4.5 cm long with an internal diameter of 8 mm, extending 1 cm into the vertical ear canal. Because of the flexibility of the tube, a tight fit in the ear canal could be accomplished visually without markedly altering or obstructing the internal dimensions of the tube or the ear canal .

TS (1, 2, 4, 8, 12, 16, 24, and 32 kHz) were generated by software, transmitted to a digital-to-analog converter (DAC),[j] and delivered to the ear in the same way as CS. Two or more sine waves were used for rise and decay times, and 1 was used for plateau time, with a total duration of at least 1 ms. The 1-, 2- and 4-kHz TS therefore had a 2-1-2 configuration, the 8-kHz TS had a 4-1-4 configuration, the 12-kHz TS had a 6-1-6 configuration, and so on (Fig. 4.1). Stimuli alternated between rarefaction and condensation with a nominal repetition frequency of 10 Hz.

The intensity output of the tweeter was measured at the end of the flexible tube by a sound pressure meter[k] to determine the stimulus sound pressure levels in decibels (dB SPL). The measurements were made with a linear filter setting of 1 Hz-70 kHz. The output of the tweeter also was checked with a microphone[l], from which the electrical output was displayed on an oscilloscope to visually confirm the shape and duration of the stimulus and the number of peaks.

All amplified response signals were input to an analog-to-digital converter (ADC)[m] interfaced to a personal computer. Signal acquisition, averaging, and analysis were controlled by dedicated software developed in-house. Signals were recorded for 12.8 ms, with a sampling time per data point of 0.05 ms. The responses to 256 stimuli were averaged. During the recordings, the running average was displayed in real time. Results were stored for subsequent analysis by the dedicated software developed in-house.

For each ear, hearing was assessed by delivering CS, beginning at 80 dB SPL and decreasing in intensity in steps of 10 dB until the threshold was reached. Threshold was defined as the intensity 5 dB above that at which no visually recognizable brainstem wave V was elicited. Two recordings were made for each set of stimulus variables to confirm reproducibility. The entire procedure then was repeated, frequency by frequency, by TS of 1, 2, 4, 8, 12,

16, 24, and 32 kHz to determine the threshold at each frequency. For all of the 80 dB SPL recordings (CS and TS), peak latencies and interpeak latencies were measured. The latency of each wave was considered to be the time from stimulus onset to the maximal amplitude of that wave. The time between 2 peaks was the interpeak latency. Interpeak latencies were calculated for pairs I-III and I-V. Amplitudes of waves I and V were measured from apex to nadir. The wave amplitude ratio (A-ratio) was calculated by dividing wave V amplitude by wave I amplitude. In each dog, a single ear was assessed during a session lasting about 2 hours. The second ear was tested 3 –12 weeks later.

Differences in peak latency, interpeak latency, amplitude, amplitude ratio, and threshold between CS and TS and within the different stimulations were analyzed by analysis of variance for repeated measures.[322] If significant differences were found, multiple comparison tests were applied: Tukey's test and Dunnett's test for comparison with a control. $P \leq 0.05$ was considered significant.

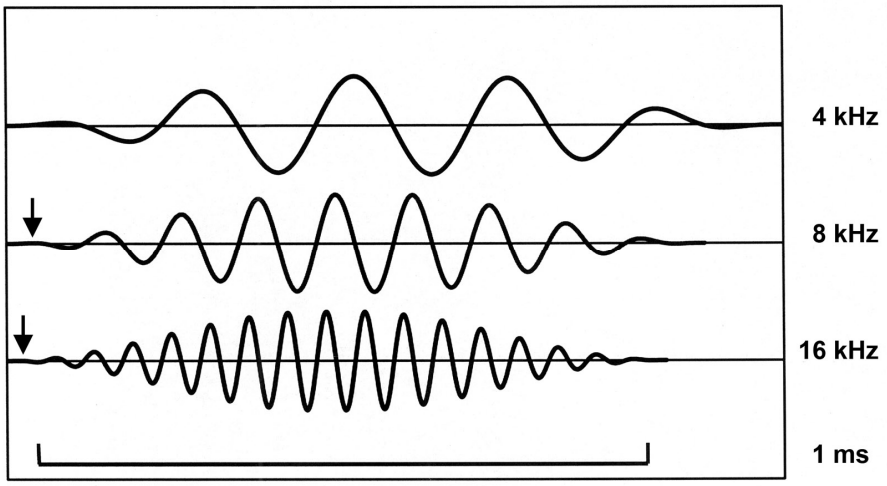


Fig 4.1. *For 4, 8, and 16 kHz TS, stimulus waveforms are shown with a 2-1-2, 4-1-4, and 8-1-8 configuration (number of sine waves for rise, plateau, and decay time respectively). Arrows indicate the first sinewave after start of TS.*

Results

The brainstem evoked response after CS at 80 dB SPL resulted in the characteristic 5-7 positive wave pattern. The waves were labeled in the conventional way with Roman numerals, the 1st identifiable wave more than 1.2 ms after stimulation (to bypass stimulus artifacts) being labeled I and the wave preceding the deepest negative trough more than 3.5 ms after stimulation being V (Fig 4.2).[124,131,153,244,245,298] The most readily identifiable peaks usually were I, II, III, V, and VI. Wave IV was not readily identifiable in all recordings, and wave VII was identified infrequently and therefore was excluded from further analysis. All TS at 80 dB SPL yielded the same characteristic pattern of at least 5 identifiable waves. The only exception was the 1-kHz TS, which elicited a frequency-following response (FFR) overlapped by biphasic potentials (Fig. 4.2), but even in these tracings, waves I and V could be identified. As the intensity of the stimulus decreased, the evoked response decreased in amplitude, whereas peak latency increased until threshold was reached.

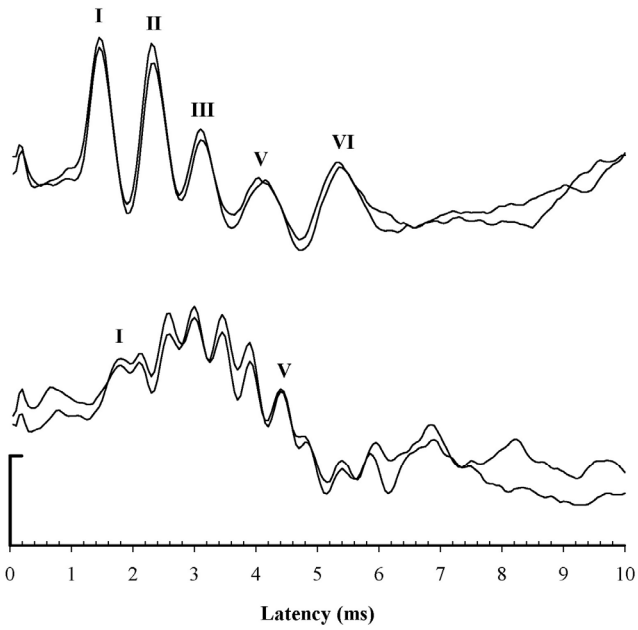


Fig 4.2. *Representative sample (duplicate recording) of BAER after an 80 dB SPL CS (upper tracing) and a 1 kHz TS (lower tracing), in the left ear of one dog. The positive peaks are labeled with Roman numerals (see text). The vertical bar represents 1 μV.*

At 80 dB SPL, peak latencies were markedly greater after TS than after CS (Fig. 4.3). Because the increase in peak I latency after TS accounts for most of the increase in latencies of all subsequent peaks, the peak I latencies of all TS were analyzed further. Peak I latency after the 12- and 16-kHz TS was markedly shorter than that after the 1-, 2-, 4-, 8-, 24-, and 32-kHz TS. Peak I latency after the 32-kHz TS was markedly longer than that at all other frequencies. The increase in individual peak latencies after TS was not caused by an increase in peak I latency alone, because there also were marked differences in interpeak latencies (Table 1).

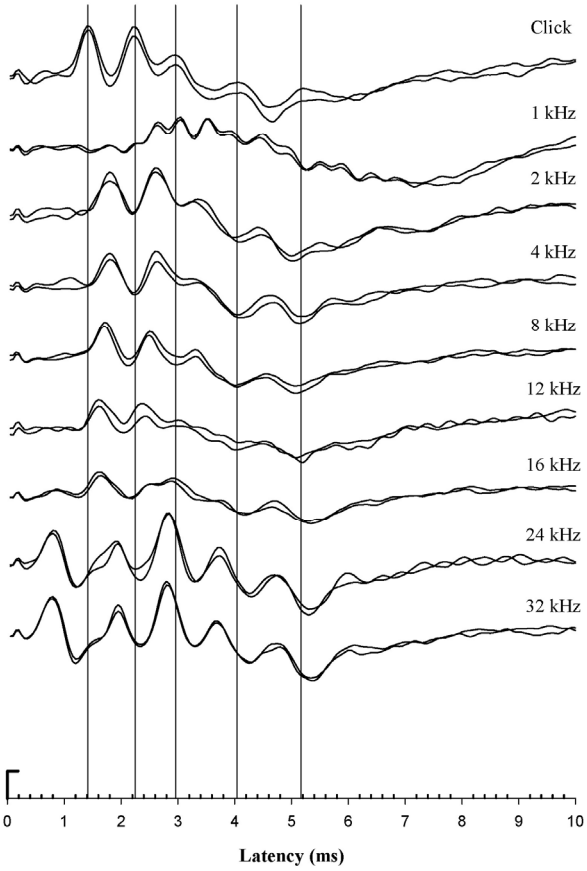


Fig 4.3. *Comparison of BAER after 80 dB SPL CS and TS ranging from 1 to 32 kHz. Duplicate recordings after stimulation of the left ear of one dog. The positive peaks are labeled with Roman numerals (see text). The vertical bar represents 1 μV.*

The interpeak latencies for peaks I-V were markedly longer after the 4-, 8-, 12-, 16-, 24-, and 32-kHz TS than after CS, but after the 2-kHz TS, they were markedly shorter, and after the 1-kHz TS, they did not differ markedly from those after CS. Marked differences in interpeak latencies also were found after all TS. The IPL I-V after the 16-kHz TS was markedly longer than that at all other TS frequencies. From 2 to 8 kHz, IPL I-V lengthened progressively, and the differences between successive frequencies were marked.

	Auditory Stimulus								
		Toneburst (kHz)							
	Click	**1**	**2**	**4**	**8**	**12**	**16**	**24**	**32**
Absolute latency (ms) (mean ± SD)									
Lat I	1.43 (0.08)	1.77 (0.09)	1.78 (0.08)	1.75 (0.10)	1.74 (0.11)	1.63 (0.09)	1.67 (0.11)	1.77 (0.15)	1.83 (0.13)
Lat II	2.28 (0.09)		2.63 (0.13)	2.58 (0.12)	2.54 (0.14)	2.44 (0.13)	2.61 (0.21)	2.77 (0.17)	2.71 (0.21)
Lat III	3.01 (0.10)		3.32 (0.16)	3.33 (0.14)	3.30 (0.19)	3.23 (0.24)	3.28 (0.41)	3.55 (0.23)	3.55 (0.21)
Lat IV	3.38 (0.10)		3.63 (0.23)	3.60 (0.14)	3.68 (0.06)	3.68 (0.14)	3.72 (0.20)	3.89 (0.32)	3.94 (0.24)
Lat V	4.09 (0.10)	4.45 (0.11)	4.35 (0.13)	4.50 (0.17)	4.59 (0.21)	4.48 (0.19)	4.63 (0.20)	4.53 (0.16)	4.61 (0.17)
Lat VI	5.29 (0.14)		5.51 (0.22)	5.67 (0.27)	5.84 (0.18)	5.75 (0.26)	5.88 (0.32)	5.72 (0,23)	5.69 (0.26)
Interpeak intervals (ms) (mean ± SD)									
IPI: I-III	1.60 (0.10)		1.56 (0.13)	1.58 (0.09)	1.58 (0.15)	1.61 (0.19)	1.63 (0.34)	1.76 (0.16)	1.72 (0.17)
IPI: I-V	2.67 (0.11)	2.68 (0.16)	2.56 (0.10)	2.75 (0.10)	2.85 (0.14)	2.85 (0.16)	2.97 (0.15)	2.78 (0.09)	2.78 (0.14)
Amplitude (μV) (mean ± SD)									
Ampli-I	1.41 (0.69)		1.12 (0.58)	1.06 (0.48)	0.81 (0.39)	0.63 (0.32)	0.54 (0.33)	1.03 (0.97)	1.17 (1.12)
Ampli-V	1.37 (0.72)		1.42 (0.70)	1.14 (0.65)	0.51 (0.29)	0.52 (0.36)	0.68 (0.38)	1.33 (0.71)	1.47 (0.78)
Ampli-ratio	1.25 (0.88)		1.79 (1.57)	1.36 (1.01)	0.90 (1.15)	1.03 (1.16)	1.86 (1.83)	2.37 (1.80)	3.32 (4.73)
Threshold (dB SPL) (mean ± SD)									
Thresh.	0.53 (14.5)	46.5 (9.8)	39.25 (6.7)	43.5 (16.3)	17.0 (24.6)	-3.5 (16.3)	5.5 (20.6)	23.0 (13.2)	33.5 (8.1)

Table 1. *Mean peak latency, interpeak interval, amplitude, and amplitude-ratio for click and different toneburst stimulations in 10 dogs (20 ears) using a 80 dB SPL stimulus and mean threshold are given as mean ± standard deviation. Lat = latency, IPI = interpeak interval, Ampli = amplitude, Ampli-ratio = peak I to peak V amplitude ratio, and Thresh. = threshold. Roman numerals refer to the individual waves.*

The interpeak latencies for peaks I-III after TS were not markedly different from those after CS, except for IPL I-III after the 24-kHz TS, which was markedly longer than IPL I-III for CS. Interpeak latency I-III could not be determined after the 1-kHz TS, because peak III could not be identified. The IPL I-III after TS was markedly longer at 24 and 32 kHz than at 2, 4, and 8 kHz.

The mean amplitude of wave I was markedly lower after all TS than after CS. The amplitude decreased progressively with increasing frequency for TS from 2 kHz to 16 kHz but increased again for TS of 24 and 32 kHz. Wave I amplitude for 8-, 12-, and 16-kHz TS was markedly lower than that for other

frequencies. The differences in mean wave I amplitude among these 3 frequencies also were marked.

The mean amplitude of wave V was markedly lower after the 4-, 8-, 12-, and 16-kHz TS than after CS, whereas that after the 32-kHz TS was markedly higher, and that after the 2- and 24-kHz TS did not differ markedly from that after CS. The mean amplitude of wave V after the 4-, 8-, 12-, and 16-kHz TS was markedly lower than after the other TS, being lowest after the 8-kHz TS. Compared with the amplitude ratio after CS, the ratios after TS were markedly higher at 2, 16, 24, and 32 kHz, markedly lower at 8 and 12 kHz, and not markedly different at 4 kHz. A trend could be recognized in amplitude ratios for the TS: The ratio decreased progressively from 2 to 8 kHz and then increased progressively from 8 to 32 kHz, the difference at each succeeding step being marked. The responses to the 1-kHz TS were excluded because the potentials were biphasic.

Marked differences occurred in the threshold for different stimulations (Table 1), the lowest being for CS and for the 12- and 16-kHz TS; the differences among these 3 were not marked. The thresholds for all other TS were markedly higher than for CS. The 2-kHz threshold was markedly lower than the 1-kHz threshold but was not markedly different from the 4-kHz threshold. The 8-kHz threshold was markedly lower than the 4-kHz threshold and higher than the 12-kHz threshold. The 24-kHz threshold was markedly lower than the 32-kHz threshold. A threshold audiogram for TS was constructed from these values (Fig. 4.4).

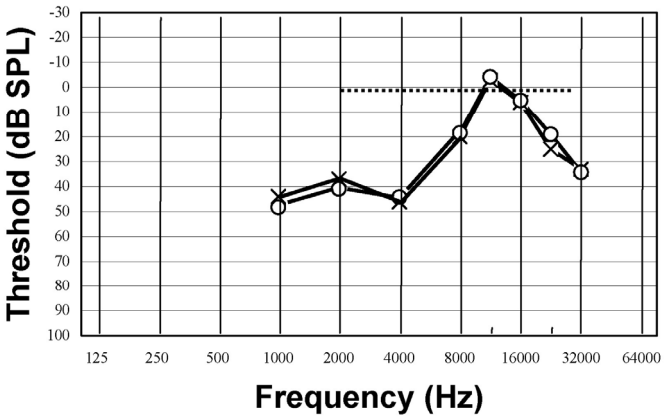


Fig 4.4. *Threshold audiogram showing mean threshold values of 10 normal dogs with a mean age of 6 years. X = left ear, O = right ear. The dotted line represents the mean click threshold (-0.5 dB SPL) for both ears. The standard deviation of mean threshold values ranges between 6.7 and 24.6 dB (Table 1).*

Discussion

There have been several reports on brainstem auditory-evoked potentials by CS to assess auditory function in dogs.[12,124,131,153,178,244,274] Whereas this method is valuable for differentiating neurologic from conduction deafness[153,274], for detecting brainstem lesions[67,178,267] and for intraoperative monitoring[148], frequency-specific information is needed to determine the extent of neurologic deafness.[249,263,277,295]

Factors known to influence the latencies and amplitudes of the elicited waves are related to the dog being examined (eg gender, age, and head size), body temperature, the anesthetic, and the stimulation and recording protocols.[33,34,153,163,177,244,245,272]

To minimize biologic variability, we used dogs of similar age and weight maintained in our facilities for at least 4 years before examination. None had clinical signs of neurologic or otic disease, and none had been exposed to potentially ototoxic drugs or excessive noise capable of inducing hearing loss. Gender influences on BAER peak latency are subordinate to body and especially head size in dogs. Meij et al. showed that an increase of 1 kg in body weight was accompanied by an increase of 0.001 ms in the latency of peak I.[163] The range of body weights in the patient groups of our study was 12.5-21.3 kg, and an increase of 0.009 ms in peak I latency for the largest dog compared with the smallest dog could be expected. This difference was considered insignificant, as confirmed by the small standard deviation in peak I latencies. No age-related differences in thresholds were observed in this group. Presbycusis therefore can be ruled out as a relevant factor influencing peak latencies.

The effect of anesthesia on the BAER generally is considered negligible. Thiamylal and methoxyflurane, however, have been found to affect peak latencies and interpeak latencies.[177,245] Medetomidine and propofol (used in this study) have not been reported to affect BAER.

Testing of frequency-specific areas of the cochlea can be accomplished by use of CS with high-pass masking, pure tone masking (derived response), or notch noise or white noise masking or by use of direct stimulation with tone pips (tonebursts). The last procedure has been shown to be easy to perform and to yield reliable information about pure tone thresholds.[37,44,45,60-63,133,188,195,263,321] To overcome the high-frequency limitation of headphones for human testing, we generated specific TS digitally and delivered them from the tweeter to the ear canal via a flexible tube. The tweeter had a frequency response up to 38-40 kHz; hence, the upper limit of TS used was 32 kHz, this being the highest applicable frequency with octave-based intervals, starting at 1 kHz, as commonly used in testing.

Many problems also occur in low-frequency testing.[44,86,136] Controversy exists about the frequency specificity of low-frequency TS, and the evoked responses are different from those of CS-evoked responses. Low-frequency testing has been performed in dogs and cats by TS of 1 kHz or less.[76,136,295] The responses were reported to be FFRs, which are short-latency scalp-recorded evoked potentials elicited by the presentation of low-frequency acoustic stimuli. They are ascribed to the electrical activity in brainstem auditory nuclei synchronous with each wave in the acoustic signal.[74,76,86] Although the origin of these responses is not very clear[74,76,86], scalp-recorded FFR can be used to ascertain low-frequency hearing sensitivity.[76] For this study, we therefore decided to use the 1-kHz TS as the lower limit of specific stimulation.

The responses to 256 stimuli were averaged, this number proving to give a reliable, stable response in all instances. Increasing the number of stimuli to 512 did not yield more stable responses. A standard number of stimuli was given, despite the fact that in most instances, a stable response was obtained well before all 256 stimuli were given. The BAER after CS or TS always consisted of the characteristic 5-7 positive wave pattern, except after the 1-kHz TS, which resulted in a FFR as reported by others and discussed above.

The markedly greater latency after all TS compared to CS might be due to the differences in extent of stimulation of the cochlea and to differences in stimulus duration. The stimulus duration of $\geq$ 1 ms for TS compared to 0.2 ms for CS may account for some of the observed differences, and this factor is known to influence peak latency dramatically.[60,133,245,272] It could be proposed, however, that with CS, the cochlea perceives a functionally louder stimulus than with relatively pure TS that only activate that portion of the cochlea most sensitive to that frequency.

Differences in latency between different TS most likely reflect the shape of the audiogram, with the progressive decrease in wave I latency from 1 to 12 kHz and the progressive increase in wave I latency from 16 to 32 kHz reflecting the normal reduction in hearing sensitivity for dogs at very low and high frequencies, whereas sensitivity was greatest at 12 and 16 kHz. However, the high amplitudes after the 24- and 32-kHz TS are not consistent with this explanation.

Two other factors may influence specific TS latency.[46,133] First, a slight decrease in latency could be expected with increasing frequency because the base of the cochlea is more sensitive to high-frequency sounds, and the apex is more sensitive to low-frequency sounds.[213] This explanation could account for the shorter peak latencies after the 12- and 16-kHz TS than after the 8-kHz TS, but not for the increased latencies after the 24- and 32-kHz TS. Second, Debruyne and Forrez concluded that the BAER is a pure onset response with a very short integration time and that the 1st oscillation of the TS is responsible

for the brainstem response.[46] The mechanism for increased latency, then, is a decrease in amplitude of the 1st oscillation. However, only 1 TS frequency (3 kHz) was used by these authors, and only wave V was analyzed, so the effect on wave I latency and interpeak latencies is unknown. If only the 1st oscillation (amplitude) of a TS is responsible for the BAER, one would expect increasing latencies with increasing frequency, because the latter results in a progressively shorter 1st oscillation of decreasing amplitude. This hypothesis, however, was not in agreement with our findings. Moreover, the results of Kodera et al. are contrary to those of Debruyne and showed that each cycle of 500, 1,000, and 2,000 Hz TS contributed to the increase in response amplitude.[133]

The dramatically greater wave I latency after TS than after CS accounts for most of the increases in latency of all subsequent waves (II - VI). However, differences also were observed in interpeak latencies, which contribute to the differences in individual wave latency. Differences in interpeak latencies I-V between CS and TS and between individual TS were marked, but those for interpeak latencies I-III were not. Interpeak latencies I-III represent the approximate time required for the action potential created in the auditory nerve to arrive at the level of the pons, whereas interpeak latencies I-V represents the approximate time required to reach the mesencephalon.[245] The latter is referred to as the central conduction time.[245] Alterations in interpeak intervals usually are indicative of retrocochlear lesions affecting the auditory nerve or the auditory pathways within the brainstem, provided that all nonpathologic factors affecting interpeak latencies have been ruled out. Increasing the stimulus intensity in the dog markedly increases the interpeak latency.[245] Decreasing the stimulus intensity results in a greater increase in latency for wave I than for wave V, effectively decreasing the interpeak interval at very low stimulus levels.[245,272] The explanation may lie in the recruitment of more rapidly conducting nerve fibers at higher sound levels or in a shift in predominance between 2 neural populations in the cochlea.[245,272] Thus, the differences in interpeak interval again seem to reflect the shape of the audiogram. At increasing frequencies up to 12-16 kHz, the sensation level (intensity) increases with the subsequent increase in interpeak intervals I-V. This does not, however, explain why there was no marked difference in the interpeak intervals I-III.

A similar shift in neuronal populations could theoretically occur with different stimulus frequencies. If any shift occurs, however, it is most likely to occur in the rostral pons, which is the probable generator site of waves IV and V, whereas differences in interpeak latencies for waves I-III among all stimulations were not significant.[213,245,272] Whether or not these differences also are biologically significant is not yet clear.

The amplitudes of waves I-VI have been reported to range from <1 μV to approximately 6 μV.[245,272] For most stimulus intensities and rates, waves I, II, and V in dogs have large amplitudes, and waves III, IV, and VI have small

amplitudes. Because amplitude is highly variable among individuals and among recording sessions in an individual, absolute amplitude measurements are of little practical use for diagnostic purposes. However, amplitude ratios are useful, provided the stimulus intensity is taken into account. The amplitude ratio (A-ratio) of waves I-V can be used to detect probable brainstem lesions. In our study, the amplitudes of waves I and V could be measured for all stimulations to calculate the ratio. The mean amplitudes of waves I and V for all TS were markedly lower than for CS, except for wave V amplitude after the 2- and 32-kHz TS. Decreased amplitude after TS has been reported before.[60,295] The click A-ratio of 1.25 is comparable to that reported by others[245,272,295], and the toneburst A-ratios all differed markedly from the click A-ratio, except for that of the 4-kHz TS. The A-ratios for the 24- and 32-kHz TS were much larger than for the CS. This observation is attributable to a larger wave V and supports our hypothesis that a neuronal shift could be responsible for these findings.

There have been only a few reports on frequency range thresholds for dogs, most of them obtained with behavioral tests.[98,199] Some authors have reported thresholds for frequency-specific stimulation of the canine cochlea. Uzuka et al. found thresholds for the 1- to 8-kHz TS comparable to those found in behavioral studies, the lowest thresholds being for the CS and the 4-kHz TS.[295] We found the lowest thresholds to be for the CS and the 12- and 16-kHz TS, in agreement with the results of the behavioral studies.

It is concluded that a specific TS of the canine cochlea with high frequencies yields reproducible results and that results are in agreement with results of behavioral studies on frequency thresholds and hearing sensitivity in dogs. For testing hearing in terms of sound intensity (dB) in dogs, CS is sufficient. In addition, to determine the extent of neurological damage due to ototoxic drugs, presbycusis, or noise, frequency-specific assessment should be done. This report provides a normative database for parameters necessary to evaluate frequency-specific hearing losses in dogs.

Acknowledgements

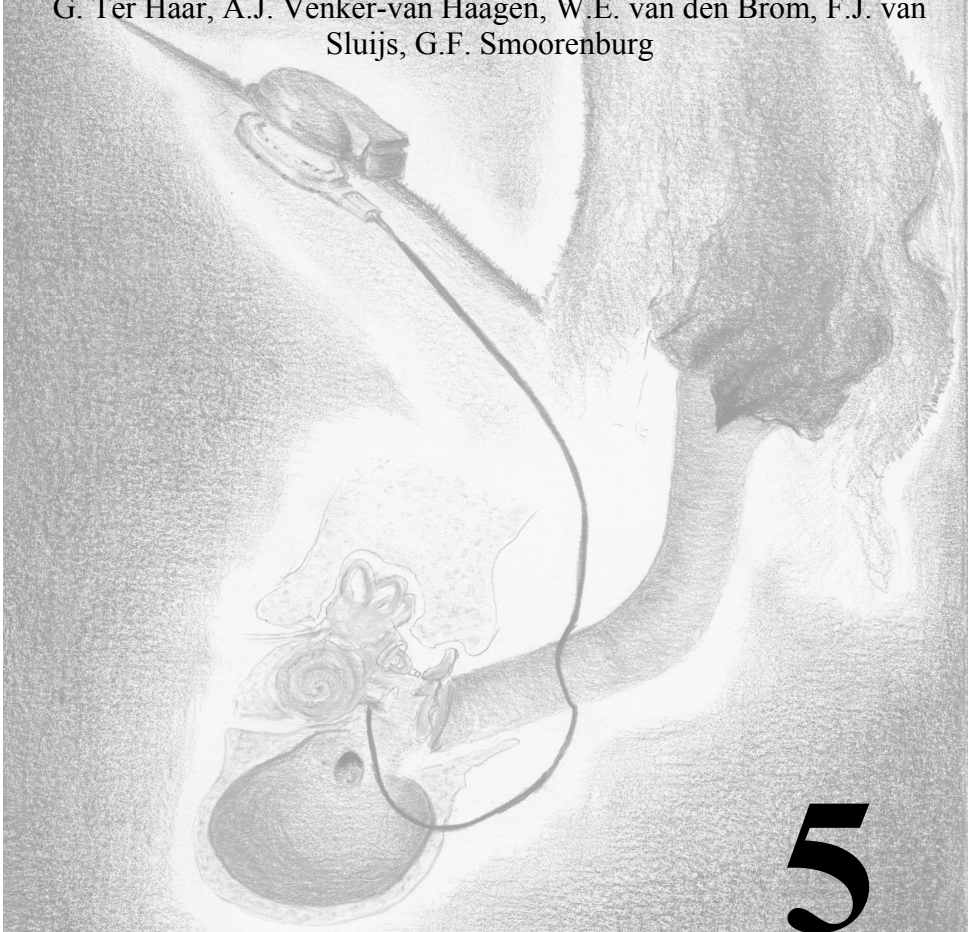
The authors are grateful for assistance of the Department of Anesthesiology and the supporting technicians. The critical reading of the manuscript by Dr. B.E. Belshaw is greatly appreciated.

Footnotes

[a] Domitor, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands
[b] Rapinovet, Mallinckrodt Veterinary, Houten, The Netherlands
[c] Antisedan, Pfizer Animal Health, Capelle a/d Ijssel, The Netherlands
[d] 13L61, 35 mm, 0.7 mm diameter, Dantec Medical, Scovlunde, Denmark
[e] Ohropax, OHROPAX GmbH, Wehrheim, Germany
[f] AB601G, Nihon Kohden Co, Tokyo, Japan
[g] Grass stimulator S88, Astro-Med. Inc., Grass Instrument Division, West Warwick, RI
[h] Variable headphone attenuator, Biophysical Laboratory, Department of Clinical Sciences of Companion Animals, Utrecht
University, The Netherlands
[i] DHT9 tweeter, 8µV, frequency range: 1-40 kHz, Visaton, Haan, Germany
[j] DAC converter, Physical Laboratory, Utrecht University, The Netherlands
[k] Sound pressure meter, type 2231, Brüel & Kjaer, Naerum, Denmark
[l] Modell EM 30, 600 µV, Vivanco Gruppe AG, Ahrensburg, Germany
[m] 14-bit, PCL 816 ADC, America Adventech Corp., Sunnyvale, CA

Effects of Aging on Brainstem Responses to Toneburst Auditory Stimuli: A Cross-sectional and Longitudinal Study in Dogs

G. Ter Haar, A.J. Venker-van Haagen, W.E. van den Brom, F.J. van Sluijs, G.F. Smoorenburg



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Journal of Veterinary Internal Medicine 2008;22:937-945

Abstract

It is assumed that the hearing of dogs becomes impaired with advancing age, but little is known about the prevalence and electrophysiological characteristics of presbycusis in this species. We hypothesized that as in humans, hearing in dogs becomes impaired with aging across the entire frequency range, but primarily in the high-frequency area. This can be assessed quantitatively by brainstem-evoked response audiometry (BERA).

Three groups of 10 mixed-breed dogs with similar body weights but different mean ages were used. At the start of the study the mean age was 1.9 years (range 0.9-3.4) in group I, 5.7 years (3.5-7) in group II, and 12.7 years (11-14) in group III. In a cross-sectional study, the BERA audiograms obtained with toneburst stimuli were compared among the 3 groups. In a longitudinal study, changes in auditory thresholds of group II dogs were followed for 7 years.

Thresholds were significantly higher in group III than in groups I and II at all frequencies tested, and higher in group II than in group I at 4 kHz. The audiograms in group II indicated a progressive increase in thresholds associated with aging starting around 8-10 years of age and most pronounced in the middle- to high-frequency region (8-32 kHz).

We concluded that age-related hearing loss in these dogs started around 8-10 years of age and encompassed the entire frequency range, but started and progressed most rapidly in the middle- to high-frequency area. Its progression can be followed by BERA with frequency-specific stimulation.

Introduction

In humans, age-related hearing loss (ARHL), or presbycusis, is one of the most prevalent chronic health conditions among the elderly and the most common form of sensorineural hearing loss encountered in industrialized nations.[80,112,138,183,226,313]

Although it is difficult to separate the effects of noise injury on hearing ability from those of aging, chronic noise exposure leads to substantially increased hearing thresholds in the elderly, and noise exposure at an early age may trigger progressive hearing loss later in life.[191,288] In addition, ARHL in mice has been found to have a genetic basis, and 10 loci that promote ARHL thus far have been identified.[191,288] Evidence for a genetic effect on the inheritance of presbycusis in women and a mixed, genetically acquired cause in men is supported by data from Gates et al..[78] In addition to noise and heredity, systemic degenerative changes also appear to contribute to the development of ARHL. The process of aging is associated with many molecular, biochemical, and physiological changes, including increases in mitochondrial DNA damage

and reduction in mitochondrial function.[8,237] Reactive oxygen species-dependent destruction of sensory epithelia has been shown to play an important role in presbycusis.[135,237] In the broadest sense, the term presbycusis therefore refers to the cumulative effects of heredity, disease, noise, ototoxic agents, and probably other environmental and dietary factors superimposed upon those of the aging process itself, although it is sometimes used in relation to the effect of aging only.[80,112,226]

Many studies have documented age-related hearing impairment in humans.[24,53,79,171,198,219] These fall into 2 categories, cross-sectional studies and longitudinal studies. Cross-sectional studies, describing differences among age groups, have shown that pure tone hearing thresholds increase with age, particularly at high frequencies.[80,138,183,226] Longitudinal studies measure changes with age within individuals, where each subject can act as his or her own control. This type of study provides a better description of the course of changes with age and identifies intrinsic interindividual differences. There have been few reports with longitudinal design in humans.[24,53,79,138,198] Substantial reduction in hearing capacity appears to occur from the age of 60 years onward and begins at the high frequencies (6-16 kHz), but gradually encompasses the entire frequency range.[24,53,79,80,138,171,198,219] Between the ages of 70 and 80 years, the reduction of hearing amounts to 1-2 dB per year, depending on the specific frequency tested.[24,53,138,171,198]

Previous reported studies of hearing loss in dogs have concerned the hearing capacity of puppies, have been cross-sectional in nature, or have employed click stimulation in brainstem-evoked response audiometry (BERA).[131,132,152,208,209,240] Because hearing loss can be partial and starts at the higher frequencies in humans, its accurate description in dogs requires determination of audiograms over the entire frequency range. The technique of BERA using frequency-specific stimulation of the canine cochlea over a wide audiometric range, from 1 to 32 kHz, was described previously and was used for this study.[282] Recently, other techniques for determination of high-frequency hearing loss in dogs have been reported, of which the use of auditory steady-state evoked potentials (ASSEP) seems to have the most potential.[152,209]

Hearing loss in humans affects the individual's psychosocial situation and, if left untreated, contributes to social isolation, depression, and loss of self-esteem.[80,138] Although it is known that dogs also develop hearing loss with advancing age, little is known about its prevalence, electrophysiological characteristics, or psychosocial effects in this species.[80,131,132,240]

The aim of this study was to test the hypothesis that dogs, similar to humans, develop hearing impairment across the entire frequency range with increasing age, first and most noticeably in the high-frequency area, and that this impairment can be quantitatively assessed by BERA by toneburst stimulation. Results of both a cross-sectional and a longitudinal study in dogs

by BERA with frequency-specific stimulation of the cochlea over a wide audiometric range (1-32 kHz) are presented.

Materials and Methods

The protocol of this study was approved by the Animal Experimentation Committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

Animals

This investigation consisted of a cross-sectional study and a longitudinal study. The cross-sectional study compared the frequency-specific BERA thresholds (audiograms) of 3 groups of dogs with substantially different mean ages, designated groups I (young), II (middle-aged), and III (geriatric). At the start of the study, the mean age in group I was 1.9 years (median, 1.8, range 0.9-3.4, n = 10), in group II it was 5.7 years (median 5.5, range 3.5-7.0, n = 10), and in group III it was 12.7 years (median 13.3, range 11.0-14.0, n = 10). Smaller breeds of dogs live longer than do larger breeds and are considered to become geriatric at a later age.[87] To enable comparisons to be made between middle-aged and geriatric dogs, only dogs from the same weight category using the Goldston and colleagues classification were used in groups II and III.[87] They categorized dogs, according to their weight, as being small (0-9 kg), medium (10-23 kg), large (24-40 kg), or very large (>40 kg) and considered these categories to become geriatric at 11.5, 10.9, 8.9, and 7.5 years, respectively (Goldston, 1995). Using this classification, all dogs in our groups II and III were medium-sized and all in group III were geriatric (>10.9 years).

Group I consisted of 10 privately owned female dogs of different breeds, having a mean weight of 20.8 kg (median, 21.5 kg; range, 9.0-30.6), referred to the Faculty of Veterinary Medicine, Utrecht University for neutering. Their hearing was assessed during recovery from the surgery. Group II consisted of 10 clinically healthy dogs housed in the kennels of the Department. Their mean body weight was 17.8 kg (median, 18.6; range, 12.5-21.3 kg) at the start of the study. Seven were mixed-breed littermates and 3 were Beagles. Five were intact males, 1 was a castrated male, and 4 were intact females. The initial audiograms of these dogs were reported previously.[282] For the longitudinal study, frequency-specific hearing thresholds were determined in this group once yearly or once every other year for 7 years, from a mean age of 5.7 years to a mean age of 12.3 years. Group III consisted of 10 clinically healthy elderly dogs maintained in the kennels of the Department from an early age onward. They included 6 mixed-breed intact females and 4 Beagles, of which 3 were intact

females and 1 was an intact male. Their mean body weight was 15.8 kg (median, 15.3; range, 13.2-21.5).

None of the dogs had any clinical signs of neurologic or otic disease, and otoscopic examination under anesthesia verified normal external ear canals and normal tympanic membranes in all ears. The privately owned dogs had no known history of ototoxic drug administration or hearing impairment and had not been exposed to excessive noise. The 20 dogs from the department kennel had no history of ototoxic drug administration but were exposed to the noise of barking dogs in the kennel. The sound pressure level of this noise measured with a sound level meter[a] with A-weighted filter settings was 82 dB (dB_A SPL; A-weighting according to the frequency dependent response of the [human] ear [IEC 179[b] and ISO 1999[c]]) with short peaks of 104 dB SPL during feeding. Since these dogs were not housed separately or trained to respond individually to commands from the technicians, there was no simple means of determining their hearing ability other than that they all exhibited what was considered to be normal behavior.

Auditory testing

In groups II and III, a light plane of anesthesia was induced with medetomidine[d] (100 µg/kg IV), followed by propofol[e] (1 mg/kg IV) within 5 minutes and was maintained with these drugs during auditory testing, as described previously.[282] At the end of the procedure, anesthesia was antagonized with atipamezole[f] (250 µg/kg IV). Rectal temperature was maintained at 36.5 to 37.5°C by means of a circulating water heating pad placed under the dog. In group I, anesthesia was induced in the same way with medetomidine and propofol, after which the dogs were intubated and surgical anesthesia was maintained by inhalation of isoflurane[g] (delivered in a 1:1 mixture of oxygen and air). After completion of ovariectomy and closure of the abdominal wound, isoflurane administration was stopped and dogs were allowed to recover to a light plane of anesthesia, similar to that in groups II and III, for auditory testing, after which anesthesia was antagonized. Although different anesthesia protocols were used, the effect of anesthesia on the BAER is generally considered to be negligible and the protocols used in this study have not been reported to affect the BAER.[282]

Auditory testing with determination of hearing thresholds by means of BERA was performed as described previously.[282] Briefly, with the dog in sternal recumbency, 3 recording needle electrodes[h] were inserted SC, 1 at the base of the pinna of each ear and the third as the reference electrode over the occipital protuberance on the midline. The nonstimulated ear was plugged with an adjustable wax plug[i]. The electrodes were attached to an amplifier[j] with a total gain of 20,000–50,000 and a bandpass filter of 3–300 Hz. Click stimuli (CS) were generated[k] as rectangular waves of 0.2 ms duration. Toneburst stimuli (TS) of 1, 2, 4, 8, 12, 16, 24, and 32 kHz were generated by software,

transmitted to a digital-to-analog converter-DAC,[l] and delivered to the ear via a high-frequency bandpass speaker (tweeter[m]) with a frequency response up to 38–40 kHz. The tweeter was connected to the ear via a flexible, well-fitting transmission tube inserted deep in the ear canal. Stimuli alternated between rarefaction and condensation with a nominal repetition frequency of 10 Hz. The intensity output of the tweeter was measured at the end of the flexible tube with a sound pressure meter[n] to determine the stimulus sound pressure levels in decibels (dB SPL). The measurements were made with a linear filter setting of 1-70 kHz. The output of the tweeter was checked with both a waveform analyzer[o] and a microphone[p], from which the electrical output was displayed on an oscilloscope to visually confirm the shape and duration of the stimulus and the number of peaks. All tonebursts were confirmed to have a center lobe at the aimed frequency. Side lobes of the nominal frequency were present, but their power was at most a few percent of that of the central lobe. All amplified response signals were fed to an analog-to-digital converter[q] interfaced to a personal computer. Further details on signal acquisition, averaging, and analysis controlled by dedicated software were reported previously.[282]

For each ear, hearing was assessed by delivering CS, beginning at 80 dB SPL and decreasing in intensity in steps of 10 dB until the threshold was reached. Two recordings were made for each set of stimulus variables to check reproducibility. The entire procedure then was repeated, frequency-by-frequency, using TS of 1, 2, 4, 8, 12, 16, 24, and 32 kHz to determine the threshold at each frequency. Threshold was defined as the intensity 5 dB above the 1st decreasing level at which no visually recognizable brainstem wave V was elicited. Wave V was identified as the wave preceding the deepest negative trough in the tracing more than 3.5 ms after stimulation.[282] Wave V was chosen to determine threshold because its amplitude is usually the least affected by aging and the last to disappear with decreasing stimulus levels.[22,80,209] When no response to stimulation was observed at 80 dB SPL, the intensity of the stimulus was increased in steps of 10 dB to a maximum of 100 dB. If still no response was seen at 100 dB, then 100 dB was arbitrarily assigned as the threshold value. In group I, both ears were tested once in a single session. In group III, both ears were tested in 2 separate sessions 3–12 weeks apart. In group II, both ears were tested in 2 separate sessions 3-12 weeks apart and then this procedure was repeated at intervals of 12–24 months until (a) there was a marked increase in the pure tone threshold, or (b) the dog reached the median age of group III, or (c) the dog was removed from the study for unrelated reasons (eg, malignancy).

Statistical Analysis

For the cross-sectional study, a linear mixed effects model[r,s] was applied with a normal distribution for threshold. Two random effects associated with each dog were included: a random intercept and a random frequency level effect. The

threshold was the dependent variable and the independent grouping variables were the ear, frequency level, age group, and the 2-way interactions among the 3 independent variables. A variance model was used, which allows a different variance per frequency level. The final model included the effects of frequency level, ear, the interaction between ear and age group, and the interaction between age group and frequency level.

A paired Student's *t*-test was used to determine whether significant differences existed in group III between threshold increases (compared with groups I and II) in the low-frequency area (1-4 kHz) and the high-frequency area (8-32 kHz).

For the longitudinal study, the initial model was the same as for the cross sectional study. Three random effects associated with each dog were included: a random intercept, a random effect for moment of observation (age), and a random frequency level effect. A variance model was used, which allows a different variance per moment of observation (age). The final model included frequency level, ear, and the interaction between moment of observation (age) and frequency level.

In both models the restricted maximum likelihood method was used to estimate the covariance parameters. The maximum likelihood method was used for estimating the fixed effects. The Akaike's Information Criterion was used to select the best model.[196] The models were fitted using the statistical program R, version 2.5.1.[r] $P \leq 0.05$ was considered significant.

Results

The brainstem-evoked responses after click and toneburst stimulation resulted in the characteristic 5-7 positive-peak pattern at 80, 90, or 100 dB SPL in all but one of the dogs tested.[124,131,153,244,245,282,298] In the exception, a dog aged 13.9 years in group III, there was no response to 32 kHz toneburst stimulation at 100 dB SPL but there were measurable thresholds at all other frequencies tested. In all dogs, as the intensity of the stimulus decreased the amplitude of the evoked response decreased, while peak latency increased until the threshold was reached (see Fig. 5.1). There were marked differences in thresholds at different frequencies not only in the same animal, as reported previously[282], but also among the three age groups and within group II during the longitudinal study.

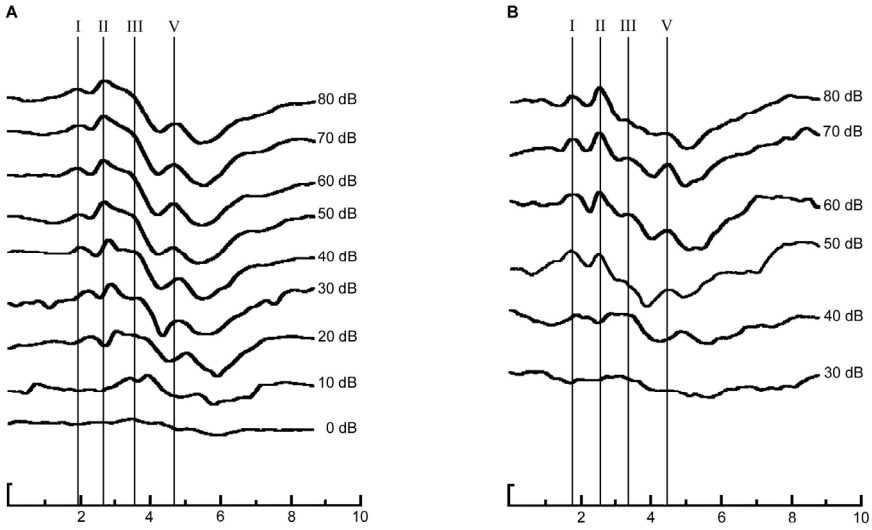

Fig 5.1.(A) *Representative sample of BAER with 4 kHz toneburst stimuli(TS) decreasing from 80 to 0 dB SPL in the left ear of 1 dog in group I (threshold was determined to be 5 dB) and* ***(B)*** *with 8 kHz TS decreasing from 80 to 30 dB SPL in the right ear of 1 dog in group II (threshold was determined to be 35 dB). The positive peaks are labeled with Roman numerals. Peak IV is missing in both tracings and peak V is identified by waveform and latency[282]. The vertical bar represents 1 μV. The time scale marker is 1 ms.*

The composite audiogram for the 3 groups in the cross-sectional study with the mean threshold values is shown in Figure 5.2. The difference in thresholds between groups I and II was not significant except at 4 kHz, where the threshold was significantly higher in group II ($P < 0.05$). The cross-sectional study identified markedly higher thresholds in group III than in groups I and II, the difference being significant at all frequencies tested ($P < 0.05$). The highest absolute thresholds were in the middle- to high-frequency region (8-32 kHz), and the increase in thresholds was significantly greater ($P < 0.01$) for this region than for the low-frequency region (1-4 kHz) in group III, compared with both groups I and II. None of the differences in threshold between the left and right ear was significant.

Individual audiograms were constructed for all 10 dogs from group II in the longitudinal study, 6 of which are shown in Fig 5.3. Dogs II-H, II-J, and II-K were still alive at the end of this study, the other 7 having been euthanized during the study, most often because of malignancy.

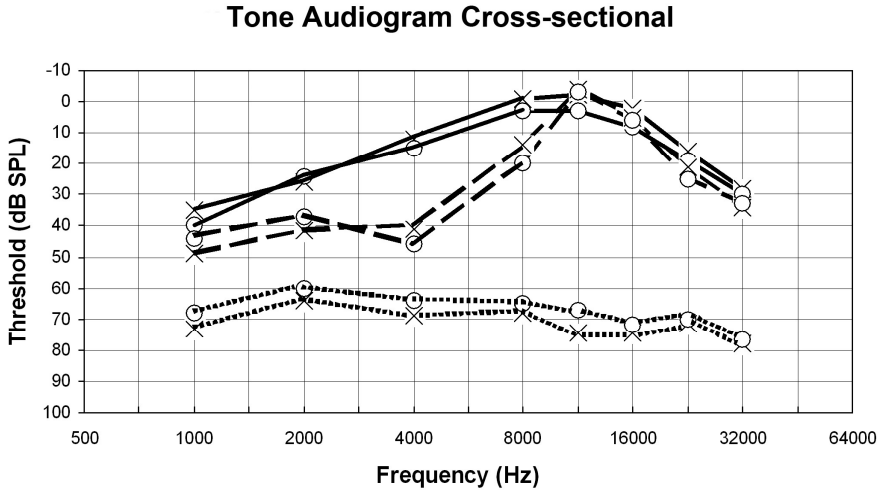

Fig 5.2. *Tone audiogram indicating mean threshold values in 3 groups of 10 dogs: group I (solid lines) mean age 1.9 years, group II (dashed lines) mean age 5.7 years, group III (dotted lines) mean age 12.7 years. The standard deviation of the data sets (mean thresholds for 1–32 kHz) ranged between 5.2–12.2 for group I, between 6.7–24.6 for group II, and between 11.0–15.6 for group III (X = left ear, O = right ear).*

Dogs II-A, II-B, and II-C were tested three times during the period between the mean ages of 6 and 9 years (see Fig. 5.3A for the audiogram of dog II-A). No increase in threshold was significant in these 3 dogs, nor were any of the differences in threshold between the left and right ear significant. The audiograms of the other 7 dogs disclosed a progressive increase in hearing thresholds with aging, starting at 8–10 years of age. The effect was most pronounced at the middle to high frequencies (8-32 kHz). However, there were considerable differences in severity and rate of hearing loss among these dogs (see Fig. 5.3A+B for the audiograms of dogs II-D, II-E, II-G, II-J, and II-K).

The average decline in hearing at all tested frequencies in group II between the mean ages of approximately 6 and 12 years is shown in Figure 5.4. All dogs of group II were still alive at a mean age of 10 years (n = 20, both ears tested), but only 7 dogs (n = 14) were still alive at a mean age of 12 years. Three dogs were still alive at the end of the study, with a mean age of 14 years, but this number was too small to be included in these calculations. Individual thresholds increased gradually over the years starting around 8-10 years of age (Fig. 5.4). Statistical analysis of the average increase in thresholds identified a significantly higher threshold at a mean age of 12 years than at a mean age of 6 years for 8, 12, 16, 24, and 32 kHz ($P < 0.05$).

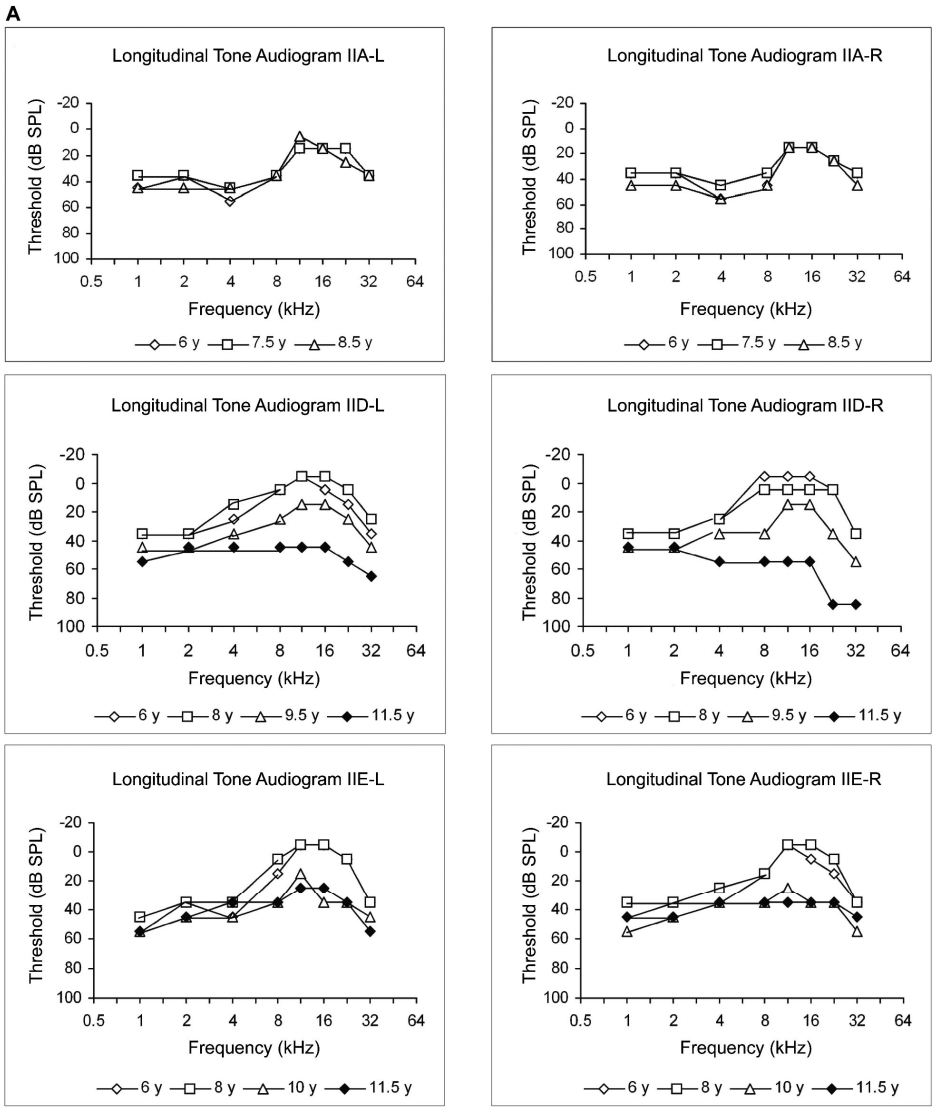

Fig 5.3. (A, B) *Longitudinal tone audiograms of selected dogs of group II at octave frequencies from 1 to 32 kHz. Left panel = left ear, right panel = right ear.*

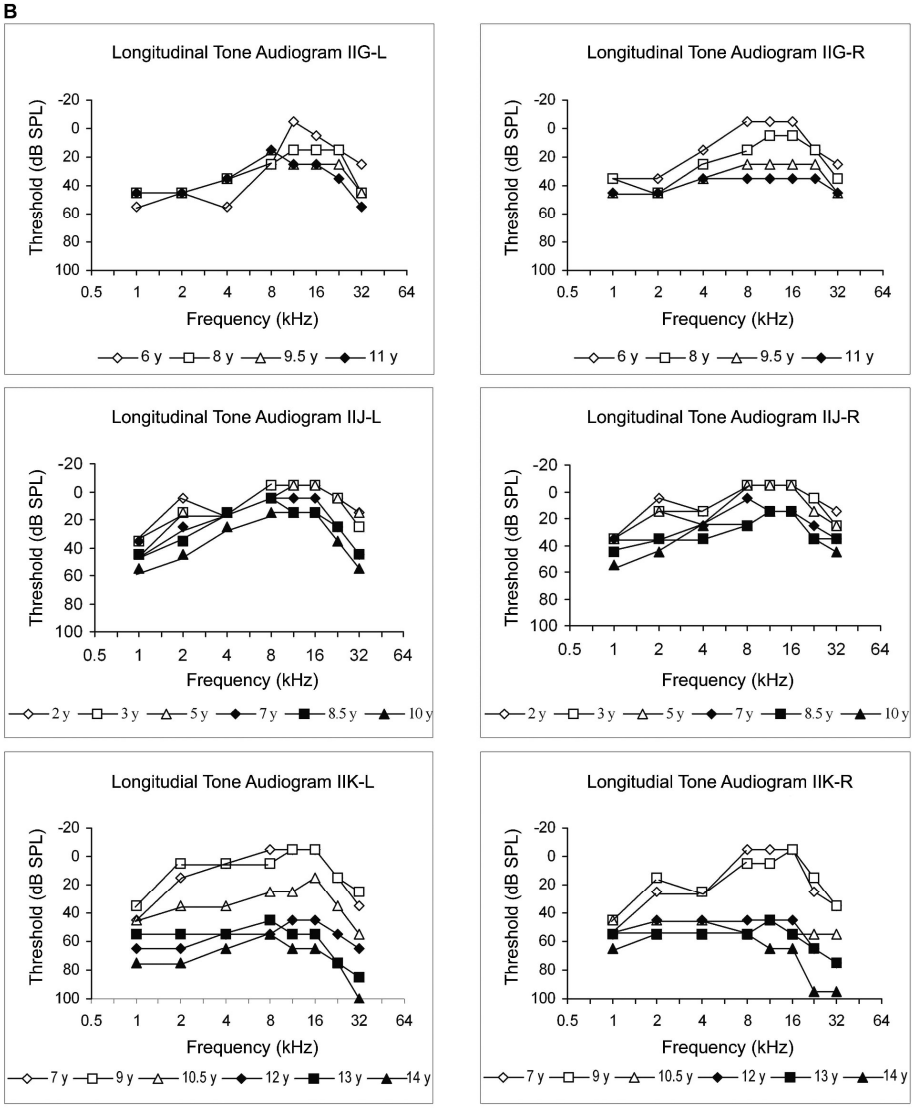

Fig 5.3. (A, B) *Continued.*

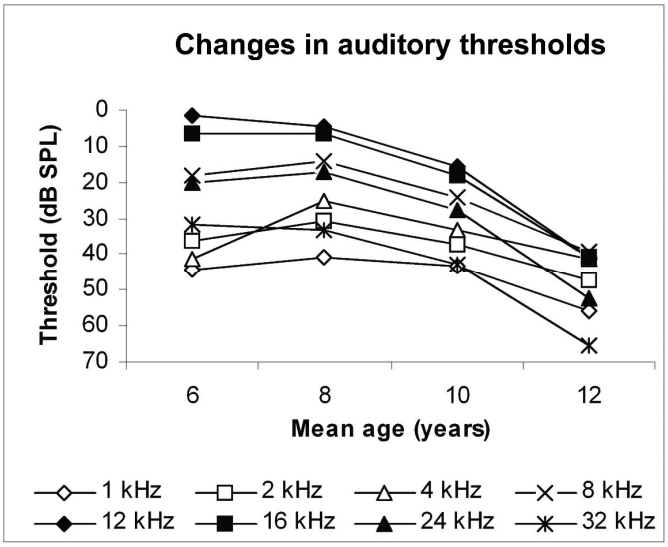

Fig 5.4. *Changes in auditory thresholds at octave frequencies from 1 to 32 kHz in group II at mean ages of 6-12 years. At 6, 8, and 10 years, n = 20 (both ears tested), and at 12 years, n = 14 (both ears tested). The standard deviation of the data sets (mean thresholds for 1-32 kHz) ranges between 5.8 and 13.8.*

Discussion

Presbycusis, or ARHL, is the major type of hearing loss and the predominant neurodegenerative disease of aging in humans.[189] There have been many reports about this increasingly common form of sensorineural hearing loss in humans and its incidence, prevalence, auditory brainstem response characteristics, and pure-tone thresholds have been described in detail in experimental, cross-sectional, and longitudinal studies and in review papers.[22,24,53,78-80,93,112,138,171,183,190,191,198,219,226,288,313] Presbycusis most likely reflects the cumulative effects of heredity, disease, noise, and ototoxic agents superimposed upon those of the aging process itself.[80,112,226] Especially, the effects of noise injury are difficult to separate from those of aging, and there are obvious interactions between them. Not only is considerable hearing preservation demonstrated in individuals raised in a relatively noise-free environment, but also animal models have shown the existence of 2 windows of increased susceptibility to noise exposure, early in life (adolescence to early adulthood) and late in life, with subsequent increases in hearing thresholds as a result of degeneration of sensory epithelium later in life.[191] Studies in humans have

reached similar conclusions.[288] In a recent study, it was found that early noise exposure in mice can trigger a progressive neuronal loss later in life.[191]

The decrease in function of the cochlea as a result of aging leads to hearing loss, which can be assessed with both behavioral studies and electrophysiological techniques. Hearing thresholds usually are measured behaviorally in humans, especially in adults.[53,80] Even though this is also possible in dogs after appropriate training, as has been shown by Heffner et al.[98] among others, it is very impractical in the clinical setting and thus objective assessment of hearing requires electrophysiological measurements. Thresholds measured behaviorally are highly correlated with those measured electrophysiologically in humans, although the latter tend to be somewhat higher, especially in the elderly, which can lead to a slight underestimation of hearing ability.[22] The difference depends on the stimulus frequency and ranges from several dB at high frequencies to as much as 15-20 dB at lower frequencies.[212,263] In considering this difference in thresholds, it should be noted that frequencies of 8 kHz and above are high for human hearing, whereas dogs can hear much higher frequencies. Both the electrophysiologically defined thresholds reported in our previous study and those found in this study in the group I dogs are in close agreement with behaviorally measured thresholds in the original report by Heffner on hearing in large and small dogs.[98,282] The lowest thresholds in the latter study were found to be between 2 and 16 kHz. For 3 of the 4 dogs reported in Heffner's study, the thresholds at 16 kHz were equal to or lower than those at 2 kHz. Whereas the lowest absolute thresholds were found in the 8 kHz area compared with the 12 kHz area in our study, threshold differences between 8 and 16 kHz in the Heffner study are only between 0 and 5 dB in three of the four dogs. Unfortunately, 12 kHz was not examined separately, but we can conclude that thresholds measured behaviorally in dogs are highly correlated with those measured electrophysiologically.

BERA has been the most commonly used electrophysiological method for assessing hearing in dogs, and the most common stimulus has been a click with a broad spectral energy distribution. Although it is known that the threshold for click-evoked potentials can give a rough idea of the overall threshold for hearing, it cannot provide accurate information about hearing capacity over the entire audible frequency range.[262,318] Because hearing loss in presbycusis primarily begins in and mostly affects the higher frequencies in humans, frequency-specific thresholds should be determined in dogs as well. This can be achieved with either toneburst stimulation or by masking clicks with high-pass noise or noise with a spectral gap.[44,60,133,208,209,263,321] The applicability of techniques with masking to increase frequency selectivity was considered, but these techniques were found to be unreliable by Gorga and Worthington[88], because of the tuning curve of the high-frequency neurons.

Threshold measurements in dogs have been reported using toneburst stimulation, but only in puppies or in dogs without hearing impairment.[208,282,295] A major concern in using tonebursts is the spread of energy to frequencies other than the nominal center frequency, which is also known as spectral splatter.[202,243] Thus, the BAER threshold obtained with tonebursts is not completely determined by the response of the neurons at the center frequency but also that of the side lobe frequencies. Recruitment of lower and higher frequencies by the side lobes could have altered the responses at threshold in our normal hearing dogs and hence could have made the results less frequency-specific. However, as mentioned before, the results are in close agreement with those obtained with behavioral methods. In patients with hearing loss, these side lobes could lead to an underestimation of the hearing loss at the nominal frequency of the toneburst.[243] However, this seems to be particularly true for hearing loss limited to specific frequencies, such as noise-induced hearing loss, where surrounding frequencies usually are unaffected. In presbycusis, where pathology is not limited to a small area or one specific frequency, this problem is probably less relevant. Theoretically, hearing loss at certain frequencies could have been greater than reported in this study, and this phenomenon could have contributed to the flatness of the slope of the audiogram of the aged dogs (group III) in this study.

A promising new technique for frequency-specific threshold measurement in dogs was reported recently by ASSEP.[152] Although the technique appears to be a valid method for obtaining frequency-specific thresholds in dogs, the authors state that work remains to be done on the low variability of interindividual thresholds, and frequencies of 16 kHz or higher were not tested. Although still inferior to behavioral testing, because of problems with spectral splatter in the tonal stimuli and the aforementioned discrepancy between behaviorally and electrophysiologically obtained thresholds, the most direct approach to obtaining frequency-specific thresholds in the dogs at the beginning of our study was frequency-specific TS.[262,282]

To the best of our knowledge, ours is the 1st report on presbycusis in dogs documented with audiograms by BERA. There have been reports of decreased hearing in aged dogs documented by BAER but with click stimulation or only 1 stimulus level. No thresholds have been reported in aged dogs with impaired hearing by means of either click or toneburst stimulation. Knowles reported increased latency and decreased amplitude of responses in 4 dogs with reduced hearing and the absence of recognizable peaks at 84 dB SPL stimulus intensity in 5 dogs.[131,132] In a study of age-related changes in the cochlea and cochlear nuclei, Shimada et al.[240] reported that in 13 dogs older than 10 years the responses to click stimulation at 90 dB SPL were diminished or absent.

Cross-sectional studies in humans have shown that pure tone hearing thresholds increase with age, particularly in the high frequencies.[53,80,138,183,226] Although cross-sectional studies can describe differences among age groups, the observed differences are confounded by cohort differences, making it difficult to match the demographic and clinical features of young and older subjects.[53,138] In addition, the potential for bias must be considered in these studies. In our study, information bias was minimized, and all materials and methods as well as data interpreters were identical from the beginning to the end of the study. Also, selection bias appears to have been small in this study, because all dogs of groups II and III were chosen at random from the same pool and were used throughout the entire study, and their housing, diet, and other environmental factors were unchanged.

The most important conclusion of our cross-sectional study is that auditory thresholds at all frequencies tested were significantly higher in elderly than in young and middle-aged dogs. Although differences were significant at all frequencies, the most dramatic increase in thresholds was seen at middle to high frequencies (8-32 kHz), which is similar to hearing loss in human presbycusis.[24,53,79,80,138,171,198,219] Considering the audiogram in dogs, which encompasses a much wider range of audible frequencies than in humans, especially high frequencies, the greatest loss of hearing capacity in elderly dogs occurred in the area of the audiogram with the lowest initial thresholds in dB SPL. The cochleas of all dogs of group III have been examined histologically, and the findings are compatible with sensorineural hearing loss. These findings will be reported separately.

The number of dogs studied was relatively small for a cross-sectional study, but the individual differences within each group were small, which lends credence to the findings. Relative to the lifespan of dogs, those in group III were of advanced age and were all considered to be geriatric. Most otogerontologic studies in humans available for comparison have reported hearing loss occurring between the ages of 65 and 80 years.[24,53,79,171,198,219] Presbycusis is reported to be slight in normal individuals at the age of 60 years, but thereafter a significant reduction in hearing capacity occurs, beginning at the high frequencies (6-16 kHz).[24,53,79,138,171,189,198,219] Between the ages of 70 and 80 years, the reduction of hearing is obvious and generally amounts to 1-2 dB per year, depending on the specific frequency tested.[24,53,138,171,198] In addition, the amount and rate of decrease in hearing capacity also depend on gender and noise exposure as well as the level of hearing capacity at the start of the investigation.[22,24,138] Presbycusis is more prevalent and more severe in men than in women and the rate of hearing deterioration in men is more than twice that experienced by women.[197,226] These authors state that decreases in hearing sensitivity may be detected at all frequencies in men by age 30, whereas the onset is later in women. Once the impairment is clinically detectable, women tend to have better

hearing than men at frequencies above 1000 Hz, whereas men have better hearing at lower frequencies.[197,226] In view of the small number of male dogs in this study, sex differences could not be examined.

The contribution of kennel noise exposure to the observed increase in auditory thresholds is unknown, but probably played a role in both groups II and III. Noise-induced hearing loss in humans typically occurs in the 2-6 kHz range, with initial changes at 6 kHz and the greatest increase in threshold at 4 kHz.[138,198,249] This suggests that the increased threshold at 4 kHz in group II could have been the result of noise damage. Ambient noise measured in the kennel of groups II and III, mainly produced by the barking dogs, was not high enough to produce such alterations in humans.[249] However, the influence of frequently occurring high levels of ambient noises on hearing thresholds in dogs is unknown. Evidence from mice and other species (including cats, hamsters, and guinea pigs) has indicated 2 windows of increased susceptibility to noise exposure with implications for thresholds and assumed ARHL later in life: early in life (up to about 4 months) and late in life.[191] The dogs of groups II and III had been housed lifelong in kennels with sound-reflecting walls, and noise-induced hearing loss could also account for some of the differences between groups I and II. In light of the other thresholds in group II, especially when compared with group I, the 4 kHz threshold stands out and could conceivably have been because of unexpected focal interference or technical errors that were not recognized during the measurements. The increase in the 4 kHz threshold in the cross-sectional study in group II over that in group I is unlikely to be the result of presbycusis.

Although thresholds could still be determined in all of the dogs in group III, most were dramatically increased in comparison with those in the other 2 groups, implying that presbycusis was present in a very advanced stage. To study the progression of presbycusis before the end-stage is reached, 1 or 2 additional age groups would have been desirable in the cross-sectional study and would have enabled more accurate comparisons with human thresholds. Nonetheless, the present study clearly demonstrates the hearing loss associated with aging in dogs.

A longitudinal design overcomes many of the problems of a cross-sectional study design by measuring changes with age within individuals, where each subject can act as its own control, thus providing a better description of the course of changes with age and identifying intrinsic interindividual differences. Although environmental effects also differ among subjects in longitudinal studies, because it takes many years to obtain results and it is difficult to follow subjects over long periods of time, longitudinal studies are the preferred method for studying presbycusis.[24,53,79,138,198]

This study indicated that thresholds in group II gradually increased with advancing age from the age of 8 years onward in all dogs in which there was

sufficient follow-up. There were differences in audiograms among dogs but not between ears of the same dog. The audiograms of group II are difficult to compare because the number of audiograms and the intervals between them were not similar for all dogs. Also, there was a 4-year difference in age between the youngest and oldest dogs in the group, and not all dogs could be followed to old age.

There were enough measurements, however, to enable comparison of thresholds in all dogs at the same age, from a mean age of 6 years to a mean age of 12 years. Interesting conclusions can be drawn from the changes in auditory thresholds tested at octave frequencies from 1 to 32 kHz during these years. Thresholds at a mean age of 12 years were significantly higher than those at a mean age of 6 years for 8, 12, 16, 24, and 32 kHz ($P < 0.05$), demonstrating that presbycusis starts in the middle- to high-frequency area in dogs.

The average increase between 10 and 12 years was lowest (around 10 dB) at 1, 2, and 4 kHz. Increases in thresholds were significantly greater at higher frequencies, ranging from 15 dB at 8 kHz to more than 22 dB at 12, 16, 24, and 32 kHz. This difference between low and high frequencies also is observed in longitudinal studies in presbycusis in humans. In 2005, Lee et al. reported the average rate of change in thresholds in humans to be 0.7 dB per year at 0.25 kHz, 1.2 dB at 8 kHz, and 1.23 dB per year at 12 kHz.[138] Enrietto et al. also reported an average yearly increase of 0.8 dB in the 0.5 kHz threshold and 1.7 dB in the 8 kHz threshold.[53] The threshold increases that we observed in dogs are 10 times greater than those reported in humans. This may in part be explained by our use of 10 dB steps in lowering stimulus intensity, but using steps of 5 dB would double the anesthesia time and would be very impractical for clinical use in dogs. Another hypothesis for the larger increase in thresholds in aging in dogs could be the dog's much shorter lifespan, so that any age-related deterioration might occur at a more rapid rate than in humans.

Although our observations and conclusions are derived from a small group of medium-sized dogs and therefore not necessarily applicable to all dogs, we conclude that presbycusis does exist in dogs, as in humans. The impairment can be demonstrated and its progression can be followed by means of BERA with frequency-specific stimulation of the cochlea. Our findings indicate that increases in auditory thresholds occur from a mean age of 8-10 years onward, eventually encompass the entire frequency range, and occur initially and are greatest at middle- to high- frequencies (8-32 kHz).

Acknowledgements

The authors are grateful for the assistance of Harry de Groot, the Department of Anesthesiology and the supporting technicians, Dr. J.C.M. Vernooij for his help with the statistical analysis of the data, and Dr. B.E. Belshaw for editing.

Footnotes

[a] Brüel and Kjaer microphone (type 4135) and measuring amplifier (type 2610), Naerum, Denmark
[b] IEC 179: Precision sound level meters, 1973
[c] ISO 1999: Determination of occupational noise exposure and estimation of noise-induced hearing impairment, 1990
[d] Domitor, Pfizer Animal Health, Capelle a/d IJssel, The Netherlands
[e] Rapinovet, Mallinckrodt Veterinary, Houten, The Netherlands
[f] Antisedan, Pfizer Animal Health, Capelle a/d Ijssel, The Netherlands
[g] Isoflurane, Abbott Laboratories Ltd., Maidenhead, Berkshire, England
[h] 13L61, 35 mm, 0.7 mm diameter, Dantec Medical, Scovlunde, Denmark
[i] Ohropax, OHROPAX GmbH, Wehrheim, Germany
j AB601G, Nihon Kohden Co, Tokyo, Japan
[k] Grass stimulator S88, Astro-Med. Inc., Grass Instrument Division, West Warwick, RI, USA
[l] Biophysical Laboratory, Department of Clinical Sciences of Companion Animals, Utrecht
University, The Netherlands
[m] DHT9, 8Ω, frequency range: 1-40 kHz, Visaton, Haan, Germany
[n] Brüel & Kjaer, type 2231, Naerum, Denmark
[o] Stanford Research Systems, SR760 spectrum analyzer, Sunnyvale, California, USA
[p] Brüel & Kjaer, ¼ inch, type 4136, bandpass (-6dB; 20 Hz – 100 kHz), Naerum, Denmark
[q] 14 bit, PCL 816, America Adventech Corp., Sunnyvale, California, USA
[r] R Development Core Team (2007). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.
[s] Jose Pinheiro, Douglas Bates, Saikat DebRoy and Deepayan Sarkar, the R Core team (2007). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-86.

Effects of Aging on Inner Ear Morphology in Dogs in Relation to Brainstem Responses to Toneburst Auditory Stimuli

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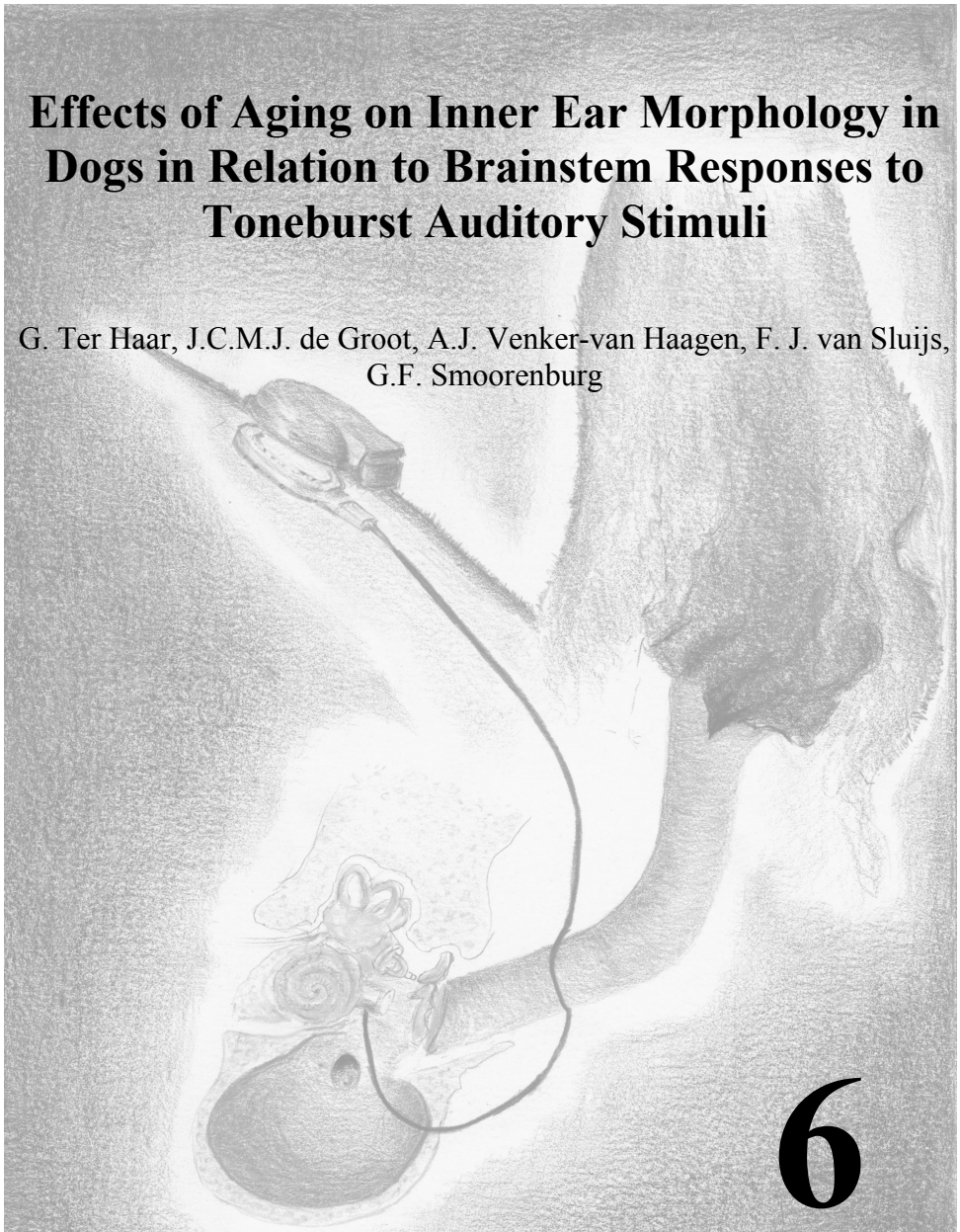

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Abstract

Age-related hearing loss (ARHL) is the most common form of hearing loss in humans and is increasingly recognized in dogs. We hypothesized that cochlear lesions in dogs with ARHL are similar to those in humans and that the severity of the histological changes is reflected in tone audiograms. For this observational study, 10 geriatric dogs (mean age: 12.7 years) were used and three 9-month-old dogs served as controls for histological analysis. Auditory thresholds were determined by recording brainstem responses (BERA) to toneburst auditory stimuli (1, 2, 4, 8, 12, 16, 24, and 32 kHz). After euthanasia and perfusion fixation, the temporal bones were harvested and processed for histological examination of the cochleas. The numbers of outer hair cells (OHCs) and inner hair cells (IHCs) were counted and the spiral ganglion cell (SGC) packing density and stria vascularis cross-sectional area (SVCA) were determined. A combination of cochlear lesions was found in all geriatric dogs. There were significant reductions ($P < 0.001$) in the OHC (42%, 95% confidence interval [CI]; 24-64%) and IHC counts (21%, 95% CI; 62-90%) and SGC packing densities (323, 95% CI; 216-290) in the basal turn, SVCA was smaller in all turns. The greatest reduction in auditory sensitivity was at 8-32 kHz. We concluded that ARHL in this specific population of geriatric dogs was comparable histologically to the mixed type of ARHL in humans. The predominance of histological changes in the basal cochlear turn was consistent with the large threshold shifts observed in the middle- to high-frequency region.

Introduction

Age-related hearing loss (ARHL) is the most common form of hearing loss in humans.[189] The audiometric characteristics of ARHL in humans and the longitudinal changes in hearing thresholds are well documented.[53,79,80,138,183,189,190,198] The irreversible loss of auditory sensitivity in humans begins at the high frequencies and progresses gradually to the lower frequencies.[53,79,80,131,132,138,198,240] ARHL also occurs in various animal species, including dogs[22,80,108,109,183,189,190,211,259,282,284] and ARHL in dogs starts at the age of 8-10 years and although the entire frequency range is affected, losses are most pronounced and progress most rapidly in the middle- to high-frequency range (8-32 kHz).[284]

The most widely referenced framework for describing histopathological changes in the cochlea associated with aging divides ARHL into 6 types: sensory (predominant loss of outer hair cells [OHCs]), neural (loss of afferent neurons), metabolic (atrophy of stria vascularis), cochlear conductive, mixed (sensory, neural and metabolic) and indeterminate (no morphological

findings).[22,80,108,120,183,189,233] Although >25% of cases are classified as indeterminate, in most aging humans there is a mixture of histopathological changes.[22,80,138,189,233]

Age-related loss of hair cells and spiral ganglion cells (SGCs) has been reported in several animal species, including dogs.[22,108,109,132,183,211,240,259] There are histological changes in the cochlea of a 20-year old dog[183] and loss of SGCs in a group of deaf dogs in which auditory thresholds were completely absent.[132] There is a varying degree of SGC loss, and atrophy of the organ of Corti and the stria vascularis in all dogs over 12 years of age, predominantly in the basal turn of the cochlea.[240]

The latter findings would seem to imply a high-frequency hearing loss of the mixed type in dogs, comparable to that found in human ARHL, but hearing thresholds were not determined in these dogs. No relation between cochlear lesions and auditory thresholds has been reported thus far in dogs. The aim of this study was to test the hypothesis that cochlear histopathology in dogs with ARHL is similar to that in the mixed type of ARHL in humans. It is further hypothesized that the location and severity of histological changes are reflected in and can be deduced from the audiograms obtained with BERA using tonebursts.

Materials and Methods

The protocol of this study was approved by the Animal Experimentation Committee of the Faculty of Veterinary Medicine, Utrecht University, the Netherlands (DEC III.08.113).

Animals

This study was carried out in a group of 10 aged dogs (6 mixed-breed intact females and 4 Beagle dogs, of which 3 were intact females and 1 was an intact male) that were clinically healthy. Their mean body weight was 15.8 kg (range, 13.2-21.5 kg) and their mean age was 12.7 years (median, 13.3 years; range, 11.0-14.0 years), and all were considered to have become geriatric according to the Goldston classification.[284] Three healthy 9-month-old, intact male Beagle dogs, having a mean body weight of 14.1 kg (range, 13.5-14.8 kg), were used as a control group. None of the 13 dogs had any clinical signs of neurological or otological disease. Otoscopic examination under anesthesia verified that the external ear canal and tympanic membrane was normal in all ears.

Auditory testing

Auditory thresholds were determined by BERA under a light plane of anesthesia, as described previously.[282,284] For each ear, hearing was assessed by

delivering toneburst stimulations (1-32 kHz), beginning at 80 dB SPL and decreasing in intensity in steps of 10 dB until a threshold was reached.[282,284] The threshold intensity was defined as 5 dB above the loudest intensity that first failed to evoke a recognizable brainstem wave V. When no response was observed at 80 dB SPL, the intensity of the stimulus was increased in steps of 10 dB to a maximum of 100 dB SPL. If still no response was seen at 100 dB SPL, then 100 dB was arbitrarily assigned as the threshold value.

Tissue preparation

The following procedure was performed in each of the 13 dogs. Immediately after determination of the hearing threshold, anesthesia was deepened to and maintained at a surgical level by isoflurane[a] inhalation. The dog was placed in dorsal recumbency and the skin of the neck was prepared as for surgery. The skin was incised on both sides to expose the common carotid artery and jugular vein. Each of the 4 vessels was catheterized with a 16G IV catheter. The dog was then euthanized by IV administration of T61[b]. The left and right carotid arteries and jugular veins were ligated immediately caudal to the inserted catheters. Isotonic saline solution[c] containing 5000 IU heparin[d]/L was infused via the catheters in the carotid arteries. Perfusion was continued until the effluent was clear. Tri-aldehyde fixative[e] was then infused until the facial soft tissues became stiff. The temporal bones were removed en bloc and were immersed overnight in tri-aldehyde fixative at 4 °C. On the following day, the specimens were rinsed for at least 2 hours in buffer[f] , followed by opening of the tympanic bulla and removal of the tympanic and squamous parts of the temporal bone. After immersion in decalcifying solution[g] for 7-14 days at room temperature, residual petrous bone was removed and the cochlea was divided in half along a midmodiolar plane and post-fixed with a reduced OsO_4 solution[h] for 2 hours at 4 °C. Dehydration was performed in an ascending graded ethanol series and propylene oxide. Next, the specimens were gradually infiltrated with fresh Spurr's low-viscosity resin under constant rotation and were polymerized overnight at 70 °C.[297] Semithin (1 µm) sections were cut with a diamond knife on a Reichert-Jung 2050 microtome (Leica Microsystems; Rijswijk, The Netherlands), collected on gelatin-coated glass slides and stained with 1% methylene blue and 1% azur II in 1% sodium tetraborate. They were examined under a Zeiss Axiophot light microscope (Carl Zeiss; Sliedrecht, The Netherlands).

Hair cell counts

Hair cells were counted in midmodiolar sections of both cochleas of each dog. The numbers of inner hair cells (IHCs) and OHCs in one midmodiolar plane were counted at 6 different locations along the basilar membrane, at half-turn

intervals (B1-A2, see Fig. 6.1A) and were expressed as the percentage of control hair cells remaining.

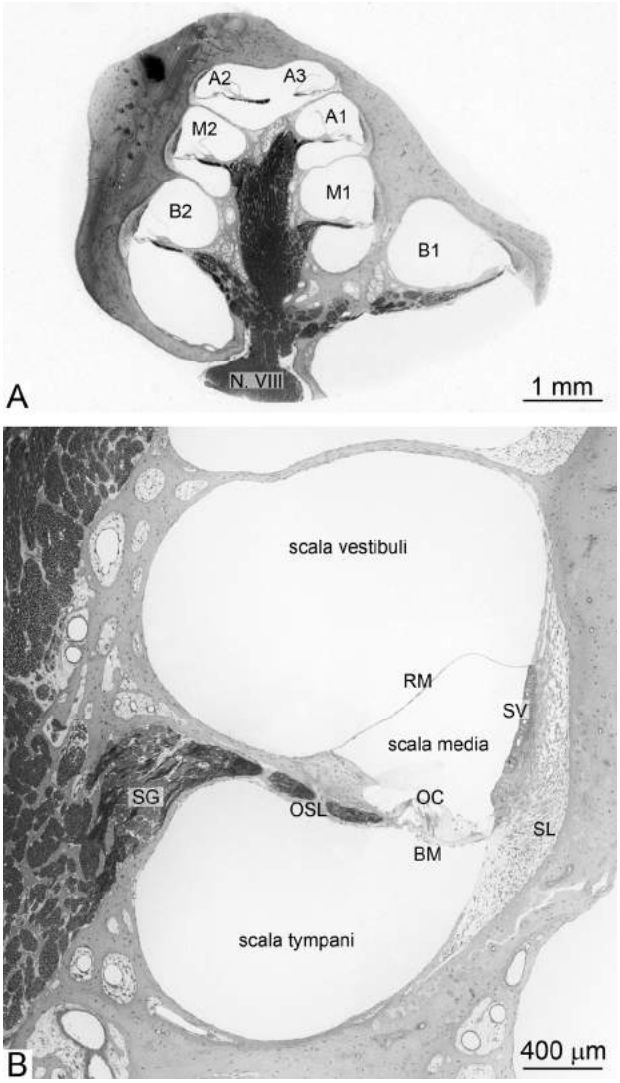


Fig 6.1.*(A) Low-power light micrograph of a midmodiolar section through the cochlea of a young dog: (B1) lower basal turn, (B2) upper basal turn, (M1) lower middle turn, (M2) upper middle turn, (A1) lower apical turn, (A2) upper apical turn, (A3) helicotrema region, (N.VIII) cochlear nerve. (**B**) High-magnification view of the lower middle turn (M1) demonstrating the organ of Corti (OC) with basilar membrane (BM) and osseous spiral lamina (OSL), Rosenthal's canal with spiral ganglion (SG), Reissner's membrane (RM), and lateral wall consisting of the stria vascularis (SV) and spiral ligament (SL).*

Spiral ganglion cell packing densities

SGC packing densities were determined in midmodiolar sections of both cochleas of each dog. Digitized images of the spiral ganglia at 6 locations (Fig. 6.1A) were imported into the NIH Image program. The bony boundaries of Rosenthal's canal were outlined and its cross-sectional area in mm^2 was calculated.[297] The number of perikarya was counted at each location. SGC packing density was calculated by dividing the number of perikarya by the cross-sectional area of Rosenthal's canal and expressed as the number of SGCs/mm^2.[297]

Stria vascularis cross-sectional area

SVCA was determined in midmodiolar sections of both cochleas. Digitized images of the stria vascularis at 6 locations (Fig. 6.1A) were imported into the NIH Image program, and its cross-sectional area in μm^2 was calculated.[297]

Statistical Analysis

A logistic regression model[i] was used to determine the effects of the factors "age group", "ear", and "cochlear turn" on OHC and IHC counts. "Dog" was taken as the random effect to model the dependency of the observations within a dog, which was assumed to be normally distributed. The maximum mumber of OHC's (3) was used as the denominator. In both models, the independent factors were "age group", "ear" and "cochlear turn", with 2-way interactions between them.

A linear mixed model[j] was used to determine the effects of the factors "age group", "ear", and "cochlear turn" on SGC packing density and SVCA. SGC packing densities were assumed to be normally distributed but log transformation was applied for normalization of the SVCA data. In both models the independent factors were "age group", "ear", and "cochlear turn" and the 2-way interactions between them. In both models the random effect was "dog" to model the dependency of the observations within a dog. The residuals were examined to ascertain whether the assumptions for the models were met.

To determine the effects of the histopathological parameters on the electrophysiological data, the same regression models were used[j]. OHC and IHC counts (logistic regression), SGC packing densities and SVCA (mixed model) for the basal, middle, and apical turns were used as the dependent variable, while "ear" and "BERA-thresholds" for the different frequencies were used as the independent factors. "Dog" was taken again as the random effect.

In all models the method of maximum likelihood was used to estimate the effects. Akaike's Information Criterion was used to select the best model. The models were analyzed in R version 2.5.1 (R Development Core Team 2007). $P \leq 0.05$ was considered significant.

Results

Cochlear function

Brainstem-evoked responses had the characteristic 5-7 positive-peak pattern after high-intensity toneburst stimulation.[282,284] In all dogs, as the intensity of the stimulus decreased, the amplitude of the evoked response decreased, while peak latency increased until the threshold was reached.[282,284] The mean auditory thresholds of the 10 geriatric dogs were reported previously in a cross-sectional study on hearing (Fig. 6.2).[284] In the 3 young dogs auditory thresholds were normal over the entire frequency range (Fig. 6.2). The combined results showed that the thresholds in the geriatric dogs were substantially higher at all tested frequencies. However, the increase in thresholds in the middle- to high-frequency area (8-32 kHz) was considerably greater than that in the low-frequency area (1-4 kHz). The relation between the mean frequency-specific hearing loss and the cochlear lesions is shown in Figure 6.5. The relation between the audiograms of 4 selected geriatric dogs and their cochlear lesions is shown in Figure 6.6.

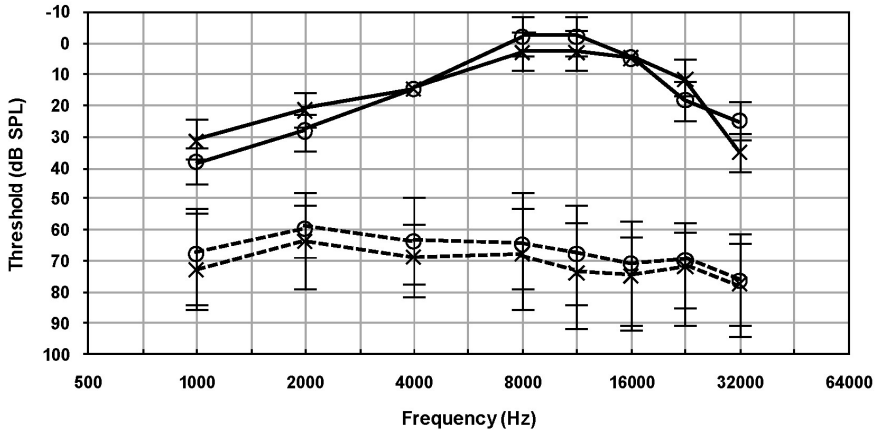


Fig. 6.2. *Tone audiogram showing mean threshold values of 10 geriatric dogs, mean age = 12.7 years (dashed lines) and 3 young dogs, mean age = 9 months (solid lines). Standard deviations (shown in the figure) for the geriatric dogs ranged from 11.0 dB to15.6 dB for 1-32 kHz and from 0 dB to 5.8 dB for 1-32 kHz for the young dogs. X = left ear, O = right ear.*

General histological findings

Hair cell counts, SGC packing density, and SVCA could be determined in nearly all cochleas for all transections from B1 to A2. SGC packing densities could not always be determined for the most apical location (A2), due to tangential sectioning of Rosenthal's canal at this level. Lesions were observed in the cochleas of all geriatric dogs and even though the severity differed, in all there was a mixed type of ARHL combining loss of hair cells (OHCs and IHCs), SGCs, strial lesions, and a reduction in SVCA.

Organ of Corti

In all 3 young dogs, the IHCs and OHCs (1 and 3, respectively, for each transection) were present in all cochlear turns (Fig. 6.3A). There was variable hair cell loss in the geriatric dogs with a radial gradient, the 1st and 2nd rows of OHCs being more severely affected than the 3rd row. There was also loss of IHCs but not as pronounced as that of OHCs. In the most severe cases, all OHCs were missing and were replaced by supporting cells (Fig. 6.4A).

Hair cell counts revealed a longitudinal gradient in the degeneration from base to apex, the hair cell loss being predominantly in the basal turn (Fig 6.5). Statistical analysis revealed a significant loss of 42% of OHC in the basal turn (B1 and B2) (95% CI; 24-64%). OHC loss was significantly greater in the basal turn than in the middle turn (M1 and M2, $P < 0.001$) and the apical turn (A1 and A2, $P < 0.001$). Differences between the right and left ears ($P = 0.9$) and between the lower and upper parts within a cochlear turn (A1 versus A2, M1 versus M2, B1 versus B2) were not significant. The loss of IHC in the basal turn was 21% (95% CI; 62-90%). IHC loss was significantly higher in the basal than in the middle turn ($P = 0.03$).

Spiral ganglion

Appearance and distribution of the SGCs and nerve fibers were normal in all young dogs (Fig. 6.1B and Fig. 6.3B). A decrease in SGC packing density, indicating a loss of SGCs (Fig. 6.4B), was observed in all cochlear turns in the geriatric dogs (Fig. 6.5), but the greatest losses were in the basal turn. The loss of SGC was associated with a loss of nerve fibers in the spiral ganglion (Fig. 6.4B) and the osseous spiral lamina (Fig. 6.4A). In the remaining SGCs there were abundant lipofuscin inclusions, intracellular vacuoles, and shrinkage of the perikarya (Fig. 6.4B). Statistical analysis revealed that SGC packing density in all turns in the young dogs (mean 403 SGCs/mm^2) was significantly higher than in the geriatric dogs (95% CI 290-515, $P < 0.001$). SGC packing densities in the basal turns were significantly lower (mean 323 SGCs/mm^2) than in the apical turns (95% CI 216-290, $P < 0.001$). None of the differences between ears, between middle and apical turns, or between individual transections of the basal, middle, and apical turns were significant.

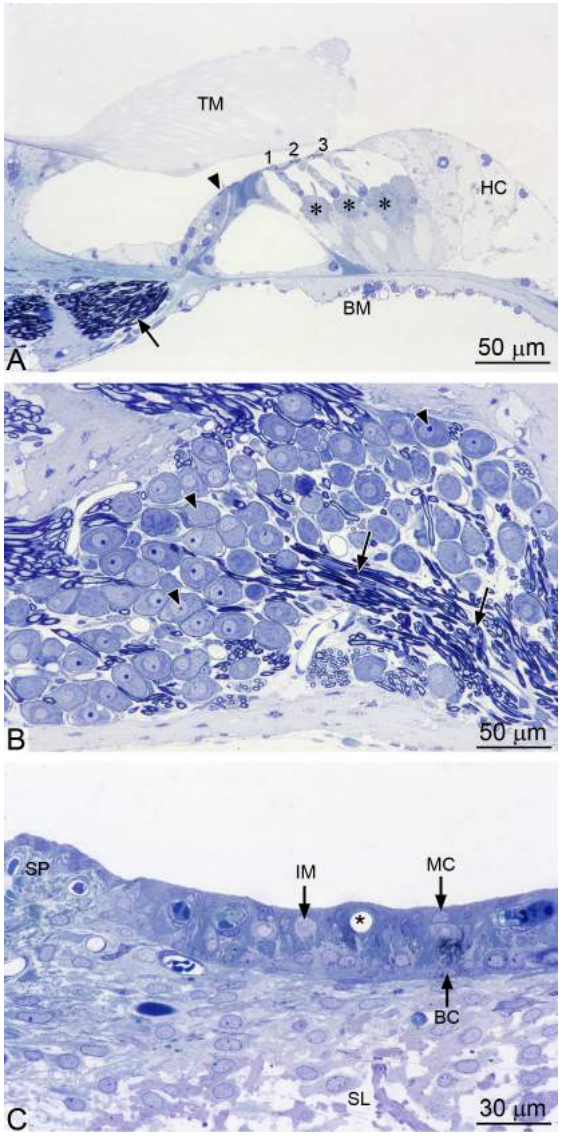


Fig. 6.3. *Midmodiolar section through the lower middle turn (M1) of the cochlea of a young dog. (**A**) In the organ of Corti the OHCs are arranged in 3 separate rows (1- 3) and the IHCs (arrowhead) are present as a single row. The arrow points to nerve fibers in the osseous spiral lamina. (TM) tectorial membrane, (BM) basilar membrane, (HC) Hensen's cells. Asterisks indicate Deiters' cells. (**B**) Rosenthal's canal, in its normal appearance, containing the spiral ganglion with SGCs (arrowheads) and nerve fibers (arrows). (**C**) The lateral wall consists of the spiral ligament (SL), the spiral prominence (SP), and the stria vascularis consisting of marginal cells (MC), intermediate cells (IM), basal cells (BC) and strial capillaries (asterisk).*

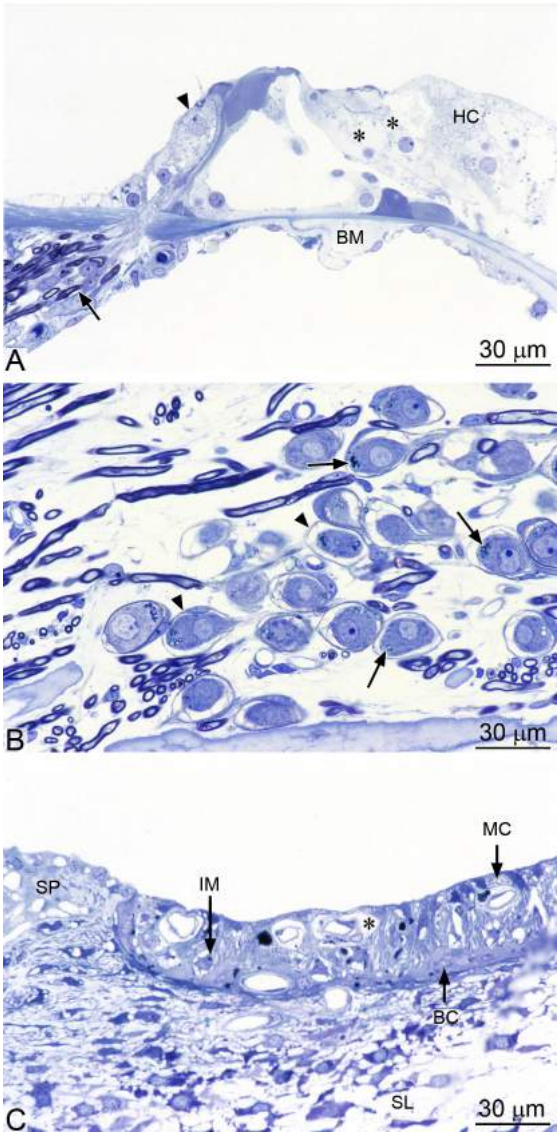


Fig 6.4. *Midmodiolar section through the upper basal turn (B2) of the cochlea of a geriatric dog. (**A**) In the organ of Corti the OHCs have been replaced by supporting cells (asterisks). The IHC (arrowhead) is still present. Note that the number of nerve fibers in the osseous spiral lamina is reduced (arrow). (BM) basilar membrane, (HC) Hensen's cells. (**B**) In the spiral ganglion there is obvious loss of SGCs and nerve fibers. In the remaining SGCs there is shrinkage of the perikarya (arrowheads), intracellular vacuolation, and lipofuscin inclusions (arrows). (**C**) In the stria vascularis there is extracellular edema (asterisks), shrinkage of the intermediate cells (IM), and vacuolation of the marginal cells (MC). (BC) basal cells, (SL) spiral ligament, (SP) spiral prominence.*

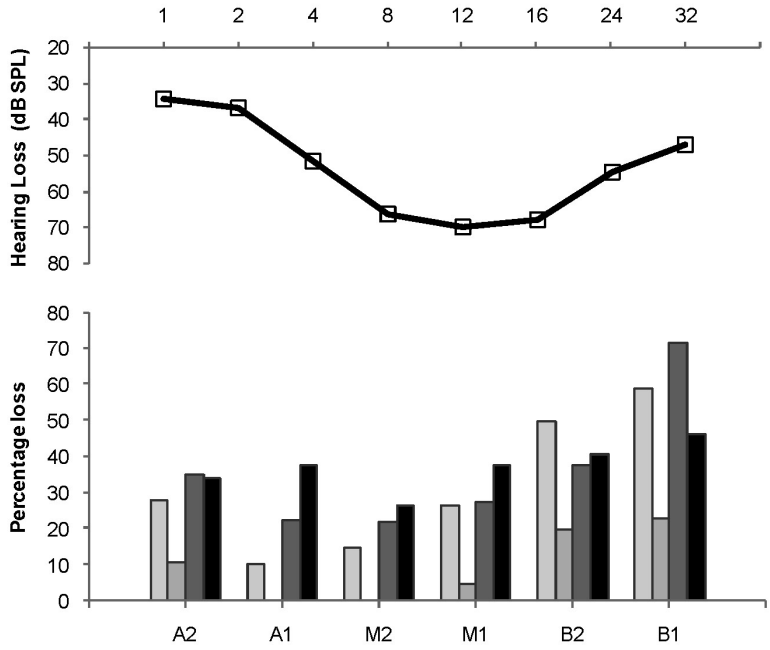


Fig. 6.5. *Cochlear lesions in dogs with ARHL. The graph shows the mean frequency-specific hearing loss in the 10 geriatric dogs (mean BERA threshold difference between geriatric and young dogs). The histogram shows the mean percent loss of OHCs (light grey bars) and IHCs (medium grey bars) and the mean percent reduction in SGC packing density (SGC; dark grey bars) and SVCA (black bars) in 10 geriatric dogs at 6 locations. SGC packing density and SVCA in the geriatric dogs are expressed as percent of the mean values in young dogs. Standard deviations of the values for all turns ranged from 0 to 44% for the IHCs, 25 to 48% for the OHCs, 10 to 23% for SGC packing density, and 13 to18% for SVCA.*

Lateral wall

Histological appearance of the stria vascularis in all young dogs was normal (Fig. 6.1B and Fig. 6.3C). The SVCA in the geriatric dogs was reduced in all cochlear turns (Fig. 6.5). In the most severe cases, the lesions in the stria vascularis included shrinkage of the intermediate cells, vacuolation of the marginal cells, and intercellular edema (Fig. 6.4C). Statistical analysis revealed that SVCA was significantly larger (1.62 x) in all cochlear turns in the young dogs than in the geriatric dogs (95% CI 1.43-1.84, $P < 0.001$). SVCA was significantly larger in the basal turn than in the middle and apical turns (1.21 x and 1.43 x, respectively, $P < 0.001$), but the differences between turns were not significantly larger in the geriatric dogs than in the young dogs.

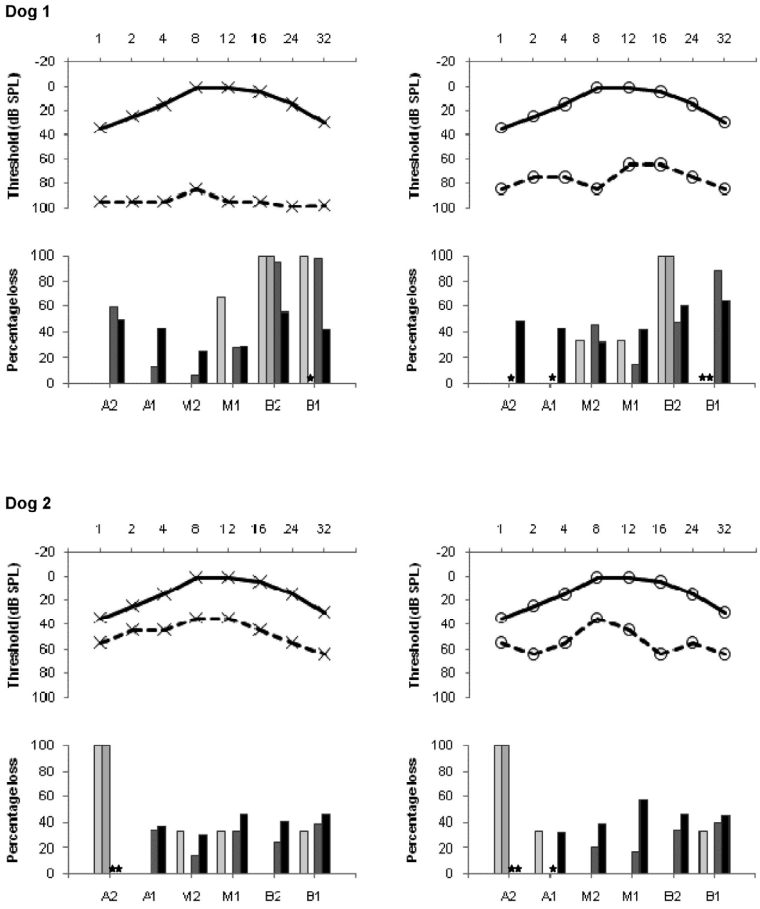


Fig. 6.6. *Correlation of the histological findings and BERA data: composite individual tone audiograms of 4 selected geriatric dogs (1, 2, 3, and 4) at octave frequencies from 1 to 32 kHz related to the histological changes at 6 locations. The figures on the left correspond to the left ears and those on the right to the right ears. In each figure the graph shows the frequency-specific BERA thresholds (X = left ear, O = right ear) of the selected geriatric dogs (dashed lines) compared with the mean thresholds of the young dogs (solid lines). The histogram shows the percent loss of OHCs (light grey bars), IHCs (medium gray bars), and SGCs (dark grey bars), and the percent reduction in SVCA (black bars) at 6 locations. (*) data not determined.*

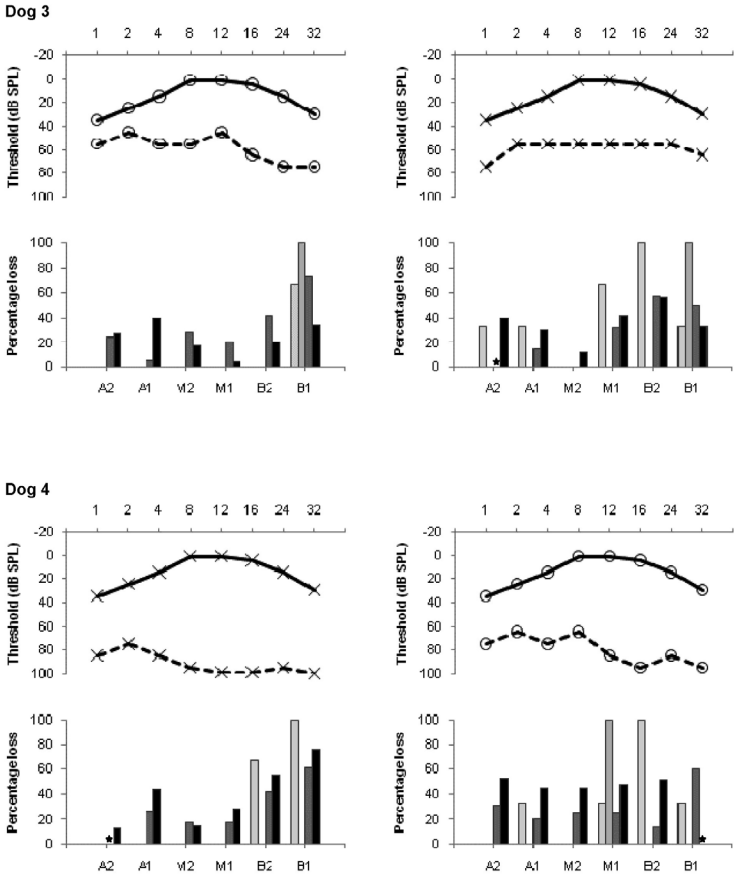


Fig. 6.6. Continued

Correlation of histological findings with BERA data

Neither the mean increase in the threshold at specific frequencies nor the individual thresholds were significantly related to either histological parameter. However, the histological changes were greatest in the basal turn of the cochlea, coinciding with the largest shifts in threshold in the middle- to high-frequency region. Frequency-specific hearing loss shown by BERA was therefore consistent with the cochlear localization of morphological damage. In all of the geriatric dogs there was a mixture of cochlear lesions of varying severity (Figs. 6.5 and 6.6). The severity of the histological changes in

individual dogs was usually reflected in the audiogram (see Dogs 1 and 2, Fig. 6.6), but in no case was the correlation between threshold value and specific histological parameter significant. The mean BERA thresholds were most closely correlated with losses of OHCs and SGCs.

Discussion

In the present study we found auditory thresholds in geriatric dogs to be dramatically higher than those in young dogs. The thresholds in the 3 young dogs included in the present study were very similar to those in young dogs (1.9 years of age; range, 0.9-3.4) reported previously.[284] The previous study showed that functional losses in the geriatric dogs were significantly greater at middle and high frequencies (8-32 kHz) than at low frequencies (1-4 kHz).[284] ARHL, or presbycusis, in its broadest sense reflects the cumulative effects of hereditary factors, disease, noise, ototoxic agents, and probably other environmental and dietary factors, superimposed on those of the aging process itself.[80,183] This increasingly common form of sensorineural hearing loss in humans has been studied extensively and its incidence, prevalence, pure-tone thresholds, and BERA characteristics have been described in detail.[22,53,79,80,138,183,189,190,198] Age-related changes in auditory thresholds also occur in various animal species, and have recently been reported in the dog.[22,80,108,109,131,132,183,211,240,259,284] In studying ARHL in dogs we have used BERA with toneburst stimuli to assess hearing and determine auditory thresholds. The advantages and disadvantages of this technique have been discussed extensively in previous reports.[282,284]

It is unclear how histological changes in the cochlea relate to BERA data in humans with ARHL, for there are some serious limitations in applying the findings to cellular mechanisms underlying ARHL.[22,309-311] Optimal preservation of the temporal bones for histological examination requires that they be processed immediately after death, which is not always possible.[47,110] In addition, temporal bones have often been studied from persons for whom there was little or no audiometric information available.[22,309-311] Consequently, specific information about the pattern of degeneration and the histological changes in the aging cochlea has required studies in laboratory animals.[108,109,211,259] These studies have shown the necessity of high-quality histological material. Adequate perfusion and the correct composition of the fixation fluid are essential to avoid artifacts and to facilitate histological interpretation of cochlear abnormalities.[5,47,120] In this study we have combined primary fixation with a tri-aldehyde fixative and post-fixation with a reduced OsO_4 solution to preserve cochlear tissues, a method that has given optimal preservation of the cochleas in guinea pigs[47] and rats.[27]

Well-documented age-related changes in the auditory system of humans and animals include the progressive degeneration of sensory, neural, strial, and supporting cells in the cochlea, as well as alteration and plasticity of central neural processing.[22,183,189,190,233,309-311,313] In most aged human and animal cochleas there is a mixture of lesions affecting many cochlear tissues.[233] The current view is that "pure" forms may exist but ARHL in humans is usually a combination of sensory, neural, and strial abnormalities. The one-fourth of cases in which none of these are found are now classified as indeterminate ARHL.[108,233] There have been few reports concerning age-related cochlear disease in dogs. Knowles et al. found a loss of SGCs in a group of deaf dogs in which auditory thresholds were completely absent.[132] The greatest losses of SGCs occurred in the upper and lower parts of the basal turn (56 and 85% reduction, respectively). Abnormalities in the organ of Corti or stria vascularis were not discussed. The largest study reported describes a variable loss of SGCs, and atrophy of the organ of Corti (loss of OHCs and IHCs) and of the stria vascularis, in all dogs over 12 years of age, predominantly at the base of the cochlea.[240] Hearing thresholds were not determined in these dogs, however, and there was only a limited description of the abnormalities in the basal turn.

Similar to Shimada et al.[240] we found cochlear lesions in all geriatric dogs, aged 11-14 years. In contrast to Knowles et al.[132], we found significant loss not only of SGCs, but also of OHCs and IHCs and a reduction in SVCA. The abnormalities we found were primarily in the basal turn, very similar to the findings of Shimada et al.[240] Considering the limited degree of IHC loss, the SGC degeneration we observed must have been primary and not secondary to the IHC loss. This is similar to what has been found in both humans and animals.[22,297] SVCA was lower than in the young dogs, in all cochlear turns from the base to the apex. This might explain the increase in thresholds over the entire frequency range, especially in view of the recent finding that in gerbils raised in quiet environments, degeneration of the stria vascularis is an early event in ARHL, usually beginning at both the base and apex and extending to midcochlear regions with advancing age.[80,183,236,259] Based solely on the light microscopic evaluations we can conclude that the ARHL in this small group of geriatric dogs is similar to the mixed type of ARHL in humans.

The geriatric dogs in this study were housed lifelong in kennels in which they were exposed to loud barking. Comparable sound pressure levels would not have caused noise-induced alterations in humans, but the possible effect of frequent high levels of ambient noise on hearing thresholds in dogs is unknown.[284] Hence we cannot exclude the possibility that noise-induced cochlear damage contributed to the age-related changes in the geriatric dogs.

Schuknecht proposed that the form of ARHL can be determined from the shape of the audiogram[189], but this is not supported by a recent review and a recent study.[35,182] Ohlemiller points out that lack of correspondence between the

audiogram and the type of ARHL should not be surprising, for even if different traumas are additive, many different combinations could produce the same result.[189] An additional obstacle to comparison of audiometric data and cochlear histopathology in dogs is that tonotopic mapping of the cochlea has not been done in this species. To enable comparisons to be made in our study, the high frequencies (16-32 kHz) were arbitrarily assigned to the basal turn of the cochlea, the middle frequencies to the middle turn, and the low frequencies (1-4 kHz) to the apical turn. Several studies have failed to show a close correspondence between audiometric data and cochlear pathology and our study is no exception.[309-311] The auditory thresholds found in these dogs did not indicate whether histological abnormalities were primarily sensory, neural, or strial. This is most likely due to the fact that there were mixed lesions in all cases, even though the individual contribution of OHC, IHC, and SGC loss and reduction in SVCA varied. For example, Dogs 1 and 4 (Fig. 6.6) had similar audiograms of advanced hearing loss, but in Dog 1 it was due to loss of both hair cells and SGC in the basal turn, whereas in Dog 4 the loss of hair cells was much greater than the loss of SGCs. Overall, cochlear pathology correlated better with absolute thresholds (Fig. 6.2) than with threshold differences (hearing loss) between the geriatric and young dogs (Fig. 6.5). For absolute thresholds, the hearing loss is best explained by the loss of OHCs and SGCs, especially in the middle- to high-frequency region. While the reduction in SVCA in all turns could explain the loss of hearing sensitivity over the entire frequency range, it cannot explain the difference between low- and middle- to high-frequency loss. A mixture of lesions thus seems to be the best explanation for the audiometric results.

Individual audiograms did, however, reflect the severity and location of cochlear lesions in all cases. In general, cochlear lesions were more extensive in dogs with more advanced hearing loss (Fig. 6.6, Dogs 1-4). Also, the predominance of lesions in the basal turn of the cochlea is consistent with the occurrence of the largest threshold shifts and lowest absolute thresholds in the middle- to high-frequency regions. Tone audiograms are thus necessary for the diagnosis and characterization of ARHL in dogs, for they not only indicate the severity of hearing loss but also the extent and location of cochlear lesions. This information is necessary in planning treatment using hearing aids or middle ear or cochlear implants.

We conclude that the cochlear lesions in these dogs with ARHL are similar to those in the mixed type of ARHL in humans. OHC and IHC loss, reduced SGC packing density, and reduced SVCA occurred primarily in the basal turn of the cochlea. Also, although tone audiograms do not reveal the relative contribution of sensory, neural, or strial lesions to the hearing loss, they do provide information about the extent of functional hearing loss and the location and severity of mixed histological changes in the cochlea.

Acknowledgements

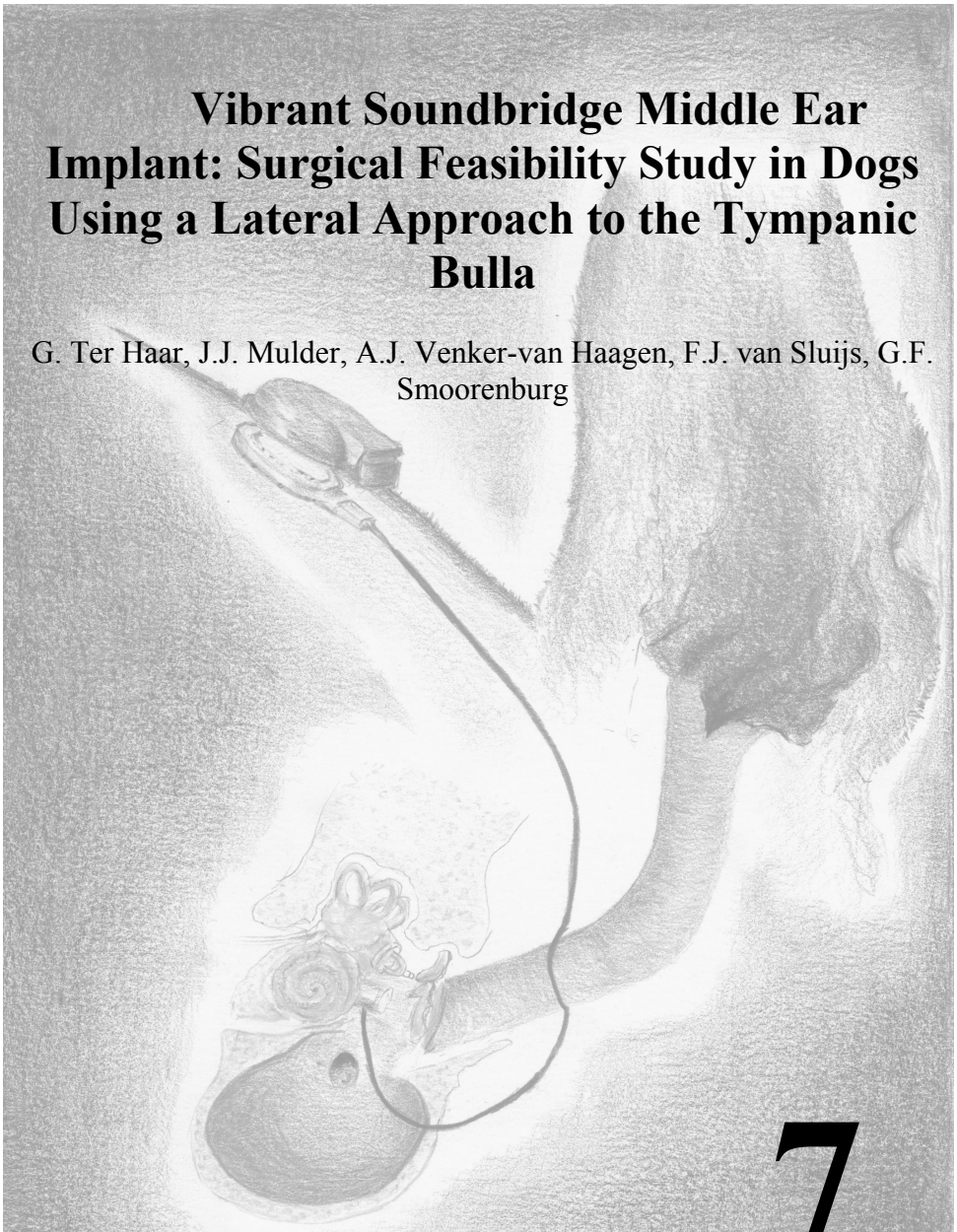
The authors are grateful for the assistance of the supporting technicians, Dr. J.C.M. Vernooij for the statistical analysis of the data, and Dr. B.E. Belshaw for editing.

Footnotes

[a] Isoflurane, Abbott Laboratories Ltd., Maidenhead, Berkshire, England
[b] T61, Intervet International, Boxmeer, the Netherlands
[c] Saline = 0.9% sodium chloride in distilled water
[d] Heparin, 5000 IU/ml, LEO Pharma BV, Breda, The Netherlands
[e] Tri-aldehyde fixative: 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, and 2.5% DMSO in 0.08 M sodium cacodylate-HCl buffer, pH 7.4 [20]
[f] 0.1 mol/l sodium cacodylate-HCl buffer, pH 7.4
[g] 10% EDTA.2Na, pH 7.4
[h] 1% OsO_4 containing 1% $K_4Ru(CN)_6$ in distilled water
[i] Douglas Bates (2007). lme4: Linear mixed-effects models using S4 classes. R package version 0.99875-9.
[j] Jose Pinheiro, Douglas Bates, Saikat DebRoy and Deepayan Sarkar, the R Core team (2007). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-86.

Vibrant Soundbridge Middle Ear Implant: Surgical Feasibility Study in Dogs Using a Lateral Approach to the Tympanic Bulla

G. Ter Haar, J.J. Mulder, A.J. Venker-van Haagen, F.J. van Sluijs, G.F. Smoorenburg



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Submitted to Veterinary Surgery

Abstract

Elderly people with age-related hearing loss (ARHL) benefit greatly from middle ear implants to improve hearing when they do not benefit from conventional hearing aids. ARHL in dogs is increasingly recognized and yet there have been no reports of treatment with implants. We hypothesized that it was technically possible to implant the Vibrant Soundbridge (VSB) middle ear implant successfully in dogs. We examined the feasibility of using a lateral approach to the tympanic bulla (TB) to insert the floating mass transducer (FMT) of the VSB and then to place it in the round window niche by manipulation through the acoustic bony meatus after reflecting the tympanic membrane, all by means of an operating microscope. The procedure was safely executed unilaterally in three dogs without intraoperative complications and with computer-tomographic (CT) evidence of correct placement of the FMT. We conclude that the VSB can be successfully implanted in dogs without intraoperative complications.

Introduction

ARHL or presbycusis may very well be the most common form of acquired hearing loss in dogs[48,277], as common as it is in humans[80,112,226,313], yet little is known about the prevalence, etiology, or therapeutic options in dogs. Age-related changes in auditory thresholds revealed by brainstem-evoked response audiometry have been reported in dogs.[282,284] Significant hearing loss was found to have begun at around 8-10 years of age in all dogs studied.[284] Cochlear pathology in dogs with ARHL was found to be similar to the mixed type of presbycusis in humans.[285]

Presbycusis is receiving growing attention in humans because of its detrimental effects on the affected individual's psychosocial situation, including social isolation, depression, and loss of self-esteem.[80,112,226,313] Hearing impairment has also been implicated as a cofactor in senile dementia.[80] The psychosocial effects of hearing impairment in dogs are not known, but undoubtedly hearing loss contributes to the lethargy, depression, and lack of interest in interaction with the environment that is commonly observed in old dogs.

Remediation of presbycusis is therefore an important contributor to quality of life in geriatric medicine. For hearing-impaired people, modern hearing aids, assistive listening devices, middle ear implants, cochlear implants, and brainstem implants provide valuable aids to communication.[7,80,112,226,241,313] Elderly people with mild to severe sensorineural hearing loss (SNHL) who do not benefit from conventional external amplification greatly benefit from

implantable auditory prostheses.[66,72,149,252,268,300] There have been no reports on the use of implants in hearing-impaired dogs. Of the available options, fully implantable or semi-implantable devices are of particular interest, since loss of the device, occlusion of the ear canal, and induction of otitis externa are potentially associated with conventional, nonimplanted hearing aids.[7,66,72] The VSB is an active, semi-implantable, middle ear hearing device that has been available in Europe for several years and there have been many reports of its effectiveness in treating people with mild to severe SNHL.[66,72,149,231,252,268,290,300]

Several surgical approaches have been described in which coupling of the transducer of the VSB to the middle ear ossicles, usually via the incus, provides a "direct drive" of the ossicular chain.[66,72,253] Recently, placement of the transducer of the VSB in the round window niche to bypass a damaged ossicular chain was reported as an alternative to clipping the FMT to the incus.[36]

There have been no reports on the use of the VSB in dogs and thus there has been no indication of the most suitable surgical approach in dogs or the means of either coupling the transducer to the incus or placing it in the round window niche. The aim of this study was to test the hypothesis that it is surgically feasible to implant the VSB in the dog using a lateral approach to the middle ear cavity and fixation of the FMT in the round window niche.

Materials and methods

The protocol of this study was approved by the Animal Experimentation Committee of the Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands (DEC III.08.113).

Animals

This feasibility study was carried out in three mesaticephalic dogs: an intact male Mixed Breed dog, 15 years of age and weighing 21 kg, and two Beagles, one being 11 years of age and weighing 16.8 kg and the other being 12 years of age and weighing 17.4 kg. None of the three had clinical signs of neurologic or otic disease and otoscopic examination under anesthesia verified a normal external ear canal and normal tympanic membrane in all ears. Computed tomography of the external, middle, and inner ears 3-6 weeks prior to surgery revealed no abnormalities.

Middle Ear Implant

The two main components of the Vibrant Soundbridge Middle Ear Implant (MED-EL Corp.) are the Audio Processor (AP) and the Vibrating Ossicular Prosthesis (VORP). The AP is the external part of the VSB, worn on the head. It is held over the implant by magnetic attraction and converts sound to a signal

that is transmitted to the implant. The VORP is surgically implanted and contains a magnet within a receiving coil, a demodulator, a conductor link, and the Floating Mass Transducer (FMT) (Fig. 7.1). The VORP receives the signal from the AP to drive the FMT, which mechanically stimulates the ossicles or the round window membrane, the stimuli being perceived as sound. The titanium clamp of the FMT was removed with fine scissors prior to implantation and fixation of the FMT in the round window niche (Fig. 7.1).

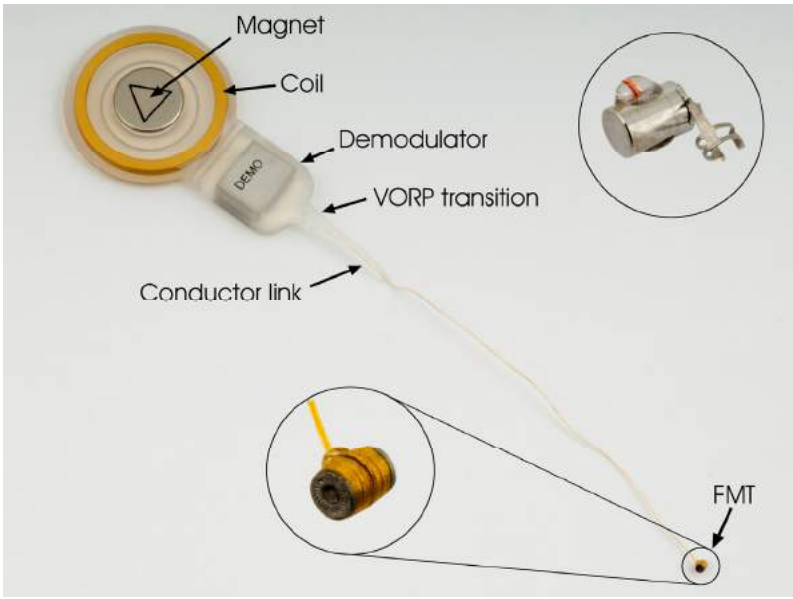

Fig. 7.1. *The implanted part of the Vibrant Soundbridge middle ear implant (vibrating ossicular prosthesis or VORP) consists of a magnet, a coil, and a demodulator. It is connected to the floating mass transducer (FMT) via a conductor link. The insets show the FMT in greater detail. For implantation of the FMT in the round window niche, the titanium clamp (upper inset) was removed.*

Surgical Feasibility Study

All three dogs were premedicated with methadone (0.5 mg/kg IV; Methadone HCl, Eurovet Animal Health) and then anesthetized with propofol (1 mg/kg IV; Propovet, Abbott Laboratories Ltd.). The dogs were intubated and surgical anesthesia was maintained by isoflurane inhalation (<1% end-tidal concentration in 50% air and 50% O2; Isoflo, Abbott Laboratories Ltd.) and fentanyl (5-30 µg/kg IV; Fentanyl, Bipharma) with atropine (0.05 mg/kg IV; Atropine sulfate, Pharmachemie B.V.). Rectal temperature was maintained at 36.5 to 37.5°C by means of circulating water heating pads placed under the dog.

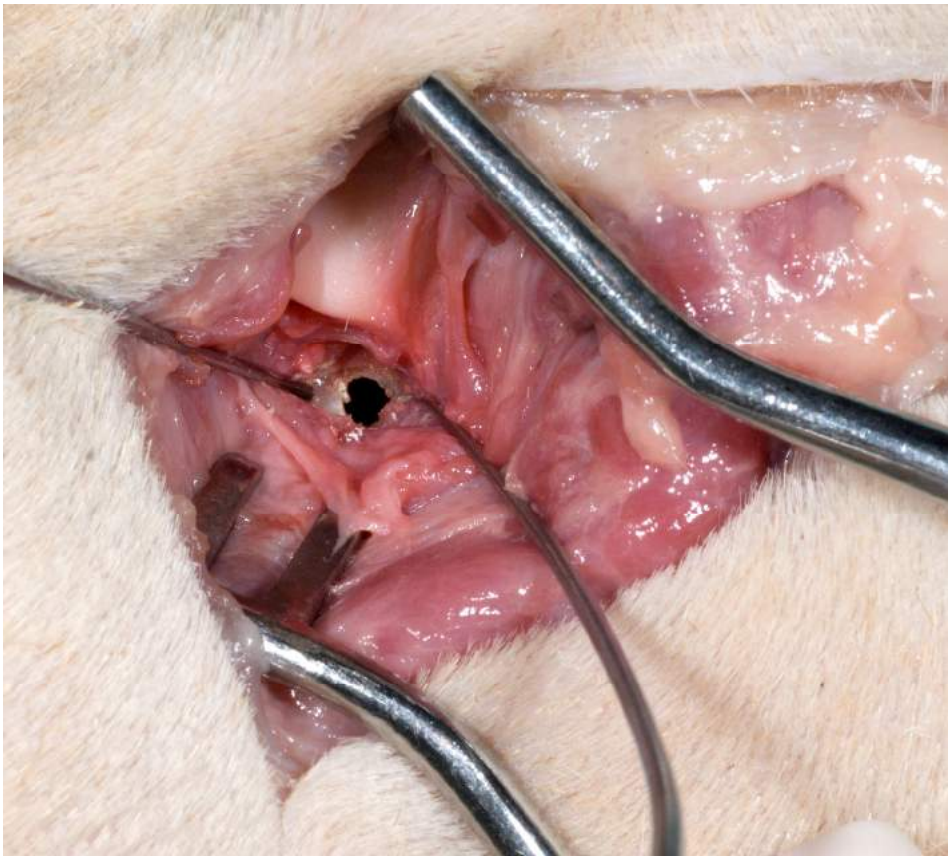

Fig. 7.2. *A small hole was drilled in the caudodorsal quadrant of the tympanic bulla for introduction of the FMT.*

The left side of the head and anterior cervical region were prepared for aseptic surgery. The dogs were placed in lateral recumbency with the head tilted to position the mandibula slightly higher than the maxilla. The rostral part of the head was raised to an angle of 40° from the table. The first surgical site was created by a curvilinear skin incision from the lateral wing of the atlas to the transition between the vertical (dorsal-to-ventral) part of the external ear canal and the horizontal (lateral-to-medial) part, and then diagonally approximately 5 cm over the horizontal part. After transection of the platysma muscle and the parotidoauricularis muscle, blunt dissection was employed until the junction of the parotid salivary gland and cartilage of the ear canal was encountered. The horizontal part of the ear canal was freed from the surrounding tissues and followed to the bony acoustic meatus, using sharp dissection and taking care to

avoid the facial nerve. The caudodorsal quadrant of the tympanic bulla was freed from its periosteum over an area 7-8 mm in diameter. Using a metal ear speculum to protect the surrounding tissues, a small hole was drilled in the tympanic bulla, to be used later for introduction of the FMT of the Vibrant Soundbridge middle ear device (Fig. 7.2).

Under microscopic guidance the fibrous attachment of the ear canal to the bony acoustic meatus, together with the overlying epithelium, was sharply dissected from the bony acoustic meatus in the medial direction, toward the tympanic membrane, leaving the external ear canal intact. This dissection involved the caudoventral part of the attachments. Parts of the caudoventral bony rim of the external acoustic meatus were removed with a rongeur to facilitate further sharp dissection of the tympanic membrane and the epithelium inside the bony acoustic meatus from the bony wall, to improve access to the round window niche. In opening the tympanic bulla, the tympanic membrane was reflected cranially to allow manipulation within the caudodorsal part of the bulla through the bony acoustic meatus.

The second surgical site was created by an incision cranial to the auricle in the skin overlying the cranial border of the parietal bone, starting 1-1.5 cm caudal to the frontal process of the zygomatic bone and 1 cm lateral to the external sagittal crest. The fascia of the temporal muscle was incised in the same direction and a pocket was created within the muscle to incorporate the VORP of the implant (Fig. 7.3). A deep subcutaneous tunnel was created between the two surgical sites for the wire connecting the VORP to the FMT. The fascia of the temporalis muscle was closed over the VORP in an interrupted pattern with PDS 3-0 to hold the implant in place during and after surgery.

Again under microscopic guidance, the FMT of the VSB was inserted through the hole drilled in the tympanic bulla and manipulated via the bony acoustic meatus to place it in position near the round window niche. A portion of the most inner part of the mastoid process of the petrous part of the temporal bone was removed with a small drill when more space was needed in the dorsocaudal direction to tilt the FMT into position in the round window niche (Fig. 7.4). The FMT was positioned perpendicular to the round window and in direct contact with its membrane. An air-tight seal was achieved with tissue glue (Tissucol, Baxter B.V.) after inserting and encapsulating the FMT with small fragments of dried autogenous muscle fascia harvested from the incision site. The tympanic membrane and ligament with epithelial lining were then carefully folded back into position within the bony acoustic meatus. The subcutaneous tissues were closed routinely with care not to twist or damage the wire connecting the FMT to the VORP. The two skin incisions were closed with continuous subdermal sutures of monocryl 4-0.

CT scan

Computed tomography (CT) of the petrous bone, tympanic bulla, and ear canal was performed postoperatively in each dog with a single-slice helical CT scanner (Philips Secura, Philips N.V.). Contiguous 1 mm slices were made with the dog in sternal recumbency using 120 kV, 160 mA, and 1 s.

At the end of the procedure, the dog was euthanized by intravenous administration of T61 (Intervet International).

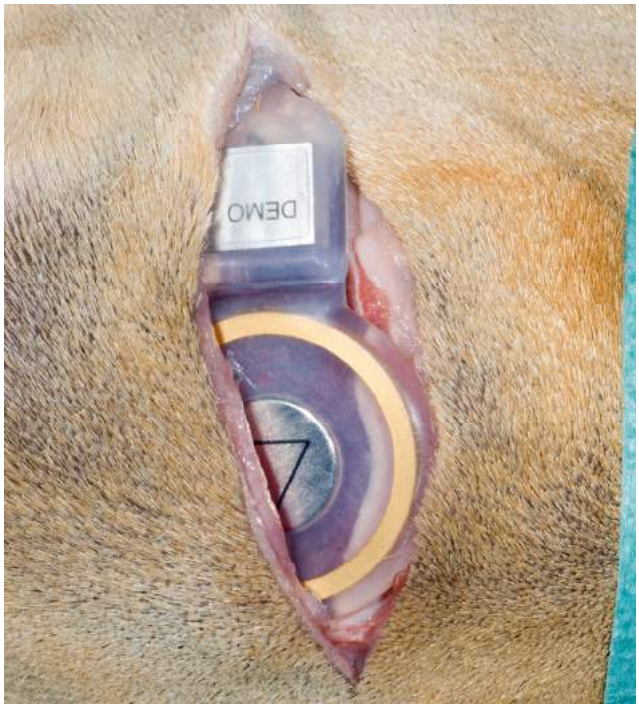

Fig. 7.3. *The fascia of the temporal muscle was incised to create a pocket in the muscle to hold the VORP.*

Results

Anesthesia was uneventful in all three dogs and the entire procedure lasted 265, 233, and 219 minutes, respectively. Surgical times were 210, 179, and 168 minutes, respectively, and there were no surgical complications. In all three dogs the middle ear cavity was exposed from the lateral approach without accidental opening of the external ear canal, excessive blood loss, or trauma to the facial nerve.

In the first dog, however, sharp dissection of the epithelium inside the bony acoustic meatus caused a small perforation of the tympanic membrane. This was avoided in the other two dogs by removing more bone from the ventral rim of the external bony meatus with a rongeur to provide better visibility. In the first dog, it was difficult to draw the FMT from its point of entry into the tympanic bulla toward the hole in the bony meatus. In the other two dogs this was resolved by drilling the hole in the caudodorsal part of the tympanic bulla slightly closer to the ridge of the external acoustic meatus. The amount of bone that had to be removed from the mastoid process in order to tilt the FMT into the correct position in the round window niche was different in all three dogs. In the largest dog there was nearly sufficient space in the dorsal mesotympanum so that scarcely any drilling was required. In the two smaller dogs more bone had to be removed to ensure perpendicular positioning of the implant on the round window membrane. Even though the manipulations were technically demanding, under microscopic vision the FMT could be placed accurately in the round window niche and could be attached to the membrane satisfactorily with autogenous soft tissue and Tissucol in all three dogs.

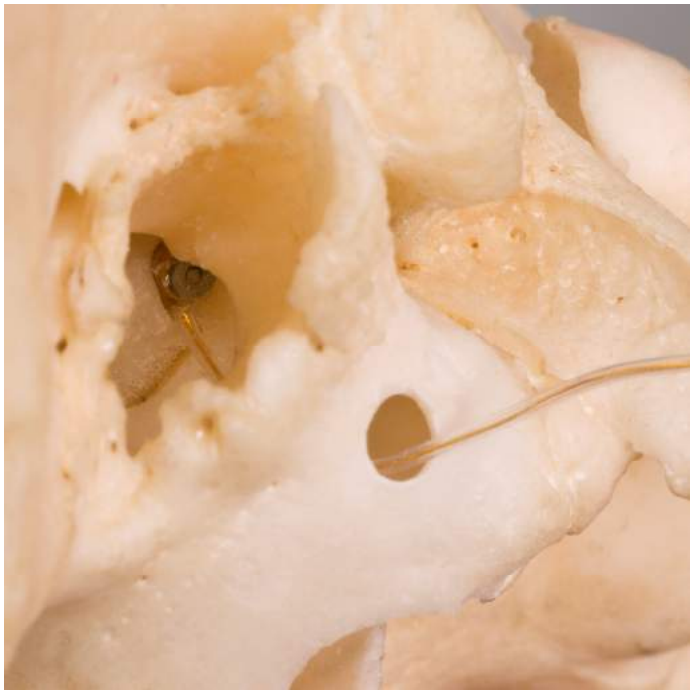

Fig. 7.4. *A skull showing where the hole was drilled in the left tympanic bulla for introduction of the FMT and the position of the FMT in the round window niche viewed through the bony acoustic meatus.*

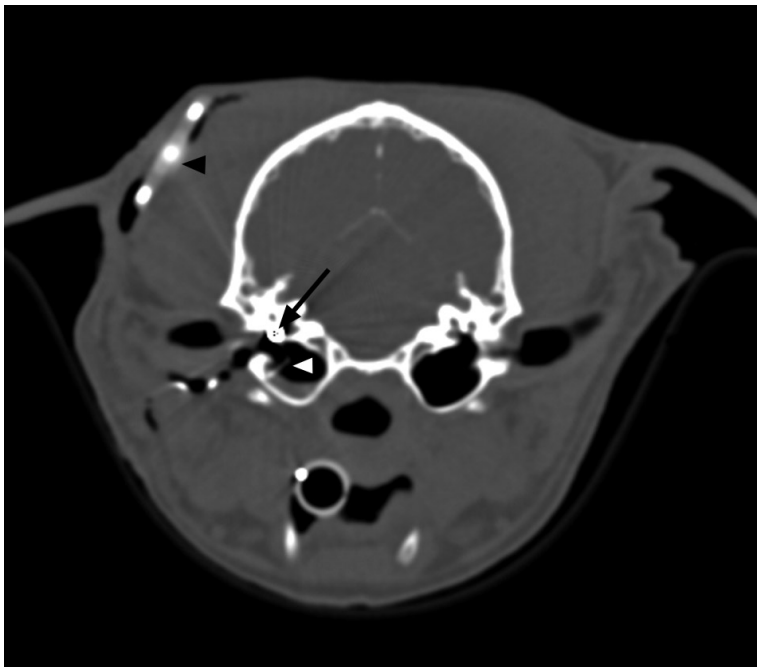

Fig. 7.5. *Postoperative CT showing the FMT in the round window niche on the left side (black arrow), some fluid in the tympanic bulla, the conductor wire (white arrowhead), and the VORP (black arrowhead).*

The directly postoperative CT scans revealed some free fluid in the middle ear cavity as a result of blood loss during the surgery, but most of the tympanic cavity was filled with air. The hole in the tympanic cavity, the conductor link, the VORP, and the FMT in correct position in the round window niche were visible in all three dogs (Fig. 7.5).

Discussion

The objective of this study was to determine the technical feasibility of implanting the VSB in dogs without intraoperative complications. It was shown to be possible via the lateral approach to the middle ear cavity. After the FMT was inserted into the middle ear cavity it could be manipulated through the bony acoustic meatus under microscopic guidance in order to position and attach the transducer in the round window niche. Although technically demanding, this was achieved satisfactorily in all three dogs and the technique can now be evaluated clinically in dogs with ARHL.

Hearing deficiencies constitute one of the most prevalent chronic health problems among the elderly and many millions of people over the age of 65 experience hearing loss.[80,112,226,313] It is estimated that ARHL affects over 40% of the population above 75 years of age.[80,112,313] In dogs the prevalence of the disease is unknown, but ARHL is the most common form of acquired SNHL in dogs for which owners seek veterinary advice.[48] In addition, in a study of ARHL in dogs, brainstem-evoked response audiometry revealed that all of those above 10 years of age had significantly elevated auditory thresholds over the entire frequency range.[284] The anatomical, physiological, and audiological changes exhibited by the aging auditory system have been well characterized in humans.[80,112,226,313] The histopathological changes related to changes in auditory thresholds in dogs have also been described recently.[285] It was concluded that ARHL in dogs is of the mixed sensorineural type, similar to that in humans. There is progressive degeneration of the outer and inner hair cells and the spiral ganglion cells, and reduction of the cross-sectional area of the stria vascularis, mainly in the basal region of the cochlea.[285]

In humans with mixed SNHL in which average hearing thresholds reach 40 dB on the audiogram, amplification is usually indicated. Amplification is primarily accomplished with a conventional hearing aid, which remains the choice of treatment in humans because it is noninvasive and less expensive than the use of implants. Despite great progress in technology and the clear advantages of digital hearing aids over the conventional analogue types, not everyone can benefit from them. In addition, problems with hearing aids can limit their use in humans as well as in dogs. They are subject to acoustic feedback, limited gain due to anatomical factors, sound and voice distortion, otitis externa associated with occlusion of the ear canal, and the need for frequent servicing and maintenance, besides being unsightly, uncomfortable, and costly.[66,72,149] The use of conventional hearing aids in dogs has been mentioned anecdotally but has not met with great success. In-the-ear devices are not well accepted by dogs, causing continuous shaking of the head until the device is dislodged and may then be eaten by the dog.[155] In addition, dogs are prone to develop otitis externa and possibly otitis media as a result of the occlusion of the ear canal.

Implantable hearing devices—cochlear and middle ear implants—have been developed to address the shortcomings of traditional hearing aids in those who do not benefit from them. Persons with moderate to severe SNHL are usually not considered to be good candidates for cochlear implantation because their relatively good residual hearing may be damaged by the procedure.[149] Hence these implants are only indicated in patients with bilateral severe-to-profound hearing loss that is not improved by other means.[80] A bone-anchored hearing aid (BAHA) is a surgically implantable system for treatment of hearing loss that works through direct bone conduction, and has been approved by the

U.S. Food and Drug Administration (FDA) for treatment of both conductive and mixed and unilateral SNHL.[254,256] The BAHA was mainly developed to help people with conductive hearing loss, chronic ear infections, inoperable congenital external auditory canal atresia, and unilateral deafness.[184,254] The BAHA consists of three parts: a titanium implant, an external abutment, and a sound processor. The sound processor transmits sound vibrations through the external abutment to the titanium implant. The vibrating implant sets up vibrations within the skull and inner ear that stimulate the nerve fibers of the inner ear, resulting in hearing. For patients with unilateral SNHL, the BAHA device is placed on the side of the deaf ear and sound is conducted through bone to stimulate the cochlea of the contralateral normal ear.[184] Newer devices have higher gain and the BAHA Cordelle can be used in persons with severe SNHL [254], but the bilateral and exclusively sensorineural character of hearing loss in elderly dogs make this device less suitable for treatment of ARHL in dogs.

Implantable middle ear prostheses have been used successfully in persons with mild to severe SNHL who do not benefit sufficiently from conventional hearing aids and whose hearing loss is not severe enough for consideration of cochlear implantation. Unlike the BAHA, middle ear implants transmit vibrations only through the ossicular chain and not through the skull. This requires less power than the BAHA, while avoiding the problems listed above for conventional hearing aids.[7] The lack of success of conventional hearing aids in dogs led us to consider the feasibility of middle ear implants.

The Vibrant Soundbridge middle ear implant is the only middle ear implantable hearing device currently available commercially with FDA approval and with which there is significant clinical experience in Europe.[7] There have been several reports of successful short-, medium- and long-term use of the implant in human patients.[66,72,149,172,231,268,290,300] For these reasons the VSB implant was chosen for this feasibility study in dogs.

Three surgical techniques have been described for implantation of the VSB in humans, one incision usually being sufficient both to insert the FMT into the middle ear cavity and fix it to the incus or stapes or in the round window niche, and to implant the VORP.[66] Some surgeons prefer an endaural or maximal retroauricular approach after which an implant bed can be burred for the VORP in the temporal bone. Others prefer a minimal retroauricular incision after which only a bony bed for the demodulator package has to be drilled, because the coil and magnet portion are then held in position by the tight subperiosteal pocket created with this technique.[66] Slight modifications and minimal invasive techniques for the approach to the middle ear cavity have been described.[71,116,289] However, in all of these techniques the place where the VORP is implanted and thus where the AP is worn externally on the head is the same, i.e., posterosuperior (caudodorsal) to the pinna, where it can be comfortably worn and is covered by hair. It was decided that the best location

for the AP in dogs would be either caudodorsal or craniodorsal to the pinna. A ventral location was considered inappropriate because inadvertent loss of the AP would seem more likely from this location. It is recommended that the total thickness of the flap over the magnet of the VORP be less than 7 mm for adequate magnetic attraction.[66,72] The limited length of the conduction wire and the necessity for strong fixation to either bone or in a subfascial pocket being covered by a flap of less than 7 mm led us to choose the craniodorsal area for implantation of the VORP. The relatively thin temporal muscle and firm fascia in this location proved to make it excellent for firm implantation of the entire VORP in a subfascial pocket without the necessity to burr a bed in the relatively thin bone of the skull of the dog. However, because access to the tympanic cavity could not easily be obtained from this location, a second incision was required for introduction of the FMT into the middle ear cavity.

In humans, a mastoidectomy is performed after endaural or retroauricular exposure and is followed by posterior tympanotomy for introduction and manipulation of the FMT in the middle ear cavity and to expose the long process of the incus. Dogs do not have a mastoid bone and either a lateral or a ventral approach could be used for sterile introduction of the FMT into the middle ear cavity. The lateral approach was chosen to enable the surgery to be performed in one session without repositioning of the patient. In dogs, the lateral approach to the middle ear cavity is usually employed for total ear canal ablation (TECA) for chronic otitis externa and media or to explore fistulas and remove epithelial remnants within the external bony meatus following TECA.[105,248] Although the lateral approach to the bulla without TECA has been described in the dog[10], only one clinical case has been reported using this approach. In that case, the left tympanic bulla was exposed through a caudal auricular incision to remove a cholesteatoma.[42] The bulla was exposed by removing the caudodorsal tympanic portion of the temporal bone with a burr. A transverse incision was also made in the proximal part of the ear canal to remove a polyp and to increase surgical exposure and working space in the tympanic cavity.

In order to lessen fibroplasia in the tympanic bulla after bulla osteotomy, which could interfere with functioning of the implant, we decided to make the opening just large enough to introduce the FMT and to work through the external bony meatus to manipulate it and fix it in position. However, maintaining sterility required that the ear canal not be opened during surgery. Although technically demanding, it was possible to elevate the epithelium within the external bony meatus up to the level of the tympanic membrane, after which the ventrocaudal part could be detached and reflected to provide sufficient workspace. This required microscopic guidance and meticulous technique. In the first dog a small perforation of the tympanic membrane occurred during dissection of the epithelium near the internal bony ring. In the

other two dogs, removal of part of the ventral rim of the external bony meatus with a rongeur, after carefully detaching the overlying epithelium, greatly improved visibility and avoided similar problems. At the close of surgery, the detached epithelium could be gently replaced and normal healing could be expected. The FMT could also have been introduced into the tympanic cavity through the external bony meatus, but in that case the conductor link would have to lie subepithelially, with the potential for infection and wire extrusion through the skin of the outer ear canal.[289]

Once placed in the tympanic cavity, rigid coupling of the FMT is of the utmost importance to achieve the maximal gain of the device. In humans, fixation is usually achieved by crimping the titanium clamp of the FMT around the long process of the incus, with or without bone cement.[253] This could not be duplicated in the dog because of the restricted size of the epitympanic recess and the size and shape of the incus. Fixation of the FMT in the round window niche was described in a human patient with combined conductive and sensorineural hearing loss to bypass a damaged ossicular chain.[14,36] Animal studies have confirmed the utility of round window stimulation[50,260,306] and we therefore decided to use this technique for fixation of the FMT in dogs.

The titanium attachment clip can be removed with fine scissors but to fit the transducer to the round window in humans, the anterior and posterior margins of the bony lip of the round window must be removed. In dogs the FMT was found to fit exactly without removing the lip, but to ensure perpendicular positioning of the FMT on the membrane, in all but the largest dog part of the mastoid process of the petrous part of the temporal bone just dorsolateral to the round window had to be removed using a small drill. Extreme care must be taken to avoid damage the round window membrane or the facial nerve coursing through this part of the bone. The base of the FMT was placed in contact with the window membrane and stabilized with tissue glue, making an air-tight fit by interposing a small fragment of muscle fascia.

Correct positioning of the implant in all three dogs was confirmed by postoperative CT. CT is the preferred method for this purpose and for diagnostic imaging of the skull in patients with magnetic implants.[287]

Conclusions

Implantation of the VSB using a lateral approach to the TB is feasible in dogs but is technically demanding. The technique described here can now be evaluated in dogs with ARHL to determine their acceptance of the device and the incidence of postoperative complications such as delayed healing, infection, or breakage of conductor link wire, and most importantly, to evaluate the efficacy of the procedure in improving hearing.

The surgical approach to the middle ear cavity described here, which avoids opening or sacrificing the external ear canal, may itself have additional application in the treatment of primary secretory otitis media, for taking large biopsies of middle ear mucosal abnormalities, and for placement of cochlear implants.

Acknowledgements

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Treatment of Age-related Hearing Loss in Dogs with the Vibrant Soundbridge Middle Ear Implant: Short Term Results in 3 Dogs

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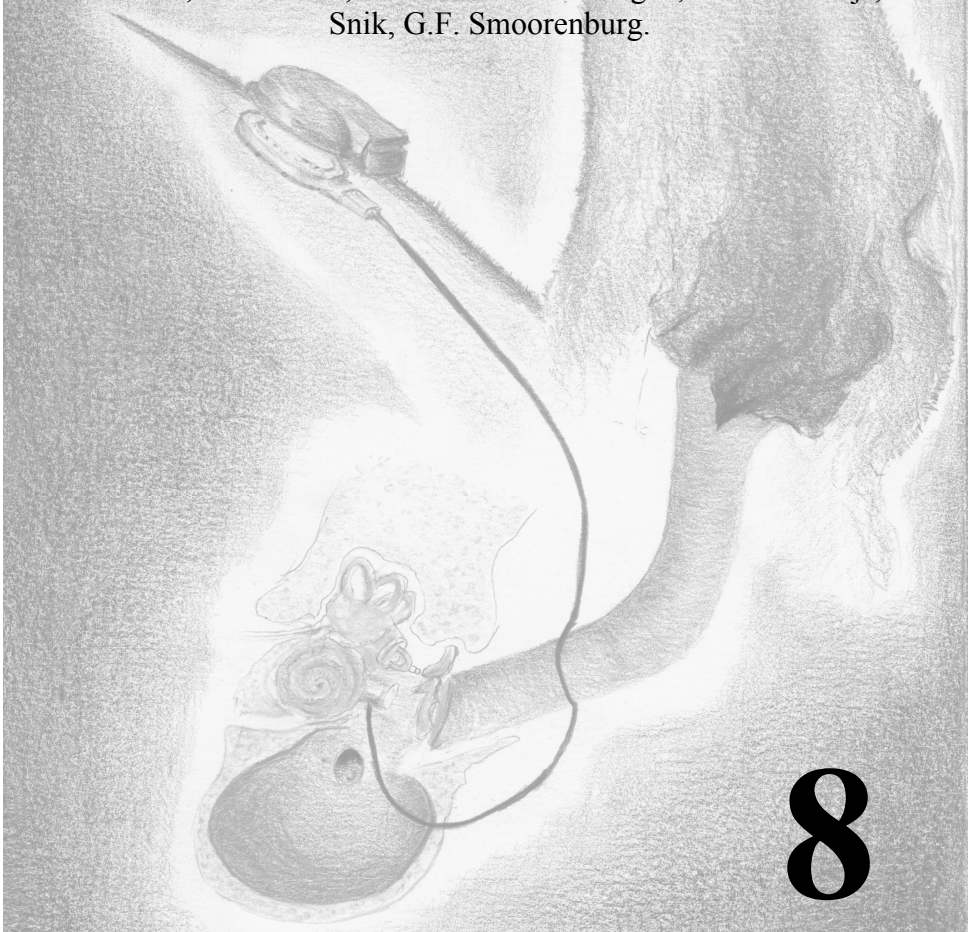

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Abstract

Age-related hearing loss (ARHL) is the most common form of acquired sensorineural hearing loss (SNHL) in dogs. Middle ear implants have been used successfully in people with ARHL who cannot benefit from conventional hearing aids. We hypothesized that audibility improves in dogs with ARHL after implantation of the Vibrant Soundbridge (VSB) middle ear implant. This study was carried out in 3 Beagle dogs with ARHL, mean age 11.1 years. The dogs were assessed pre- and postoperatively by brainstem-evoked response audiometry (BERA) using tone bursts, by otoscopy, and by CT scans of the ears. A VSB middle ear implant was implanted unilaterally with placement of the transducer (FMT) in the round window niche. Three months later the functionality of the implants was assessed by auditory steady-state responses (ASSRs), after which the dogs were euthanized for histopathological examination. The VSB was implanted successfully in all 3 dogs. Recovery from surgery was uneventful, except for transient facial nerve paralysis in 2 of the 3 dogs. The implantation procedure did not affect residual hearing as measured by BERA. ASSRs showed improved hearing with a maximum mean decrease in threshold of 20.7, 13, and 16.3 dB at 1, 2, and 4 kHz, respectively. We concluded that implantation of the VSB with the FMT positioned in the round window niche resulted in lower ASSR thresholds, but only at the higher gain settings of the audioprocessor. As in humans, a more powerful audioprocessor is required to treat SNHL exceeding 20 dB in dogs.

Introduction

Active middle ear implants and implantable hearing aids in general are well-accepted for treatment of mild to severe SNHL in persons who do not benefit from conventional hearing aids.[72,149,252,290] The most common form of hearing loss in humans is ARHL, which is mainly sensorineural, appears from the age of 60 years onward, and initially affects high frequencies but gradually encompasses the entire frequency range and progresses steadily.[24,80,138,198] The principle of middle ear implants is to augment residual hearing and transmission of sound energy by coupling a vibratory element (implanted transducer) directly to the middle ear ossicular chain or to the round window membrane.[72,128,241] Of the two available systems, more clinical experience in humans has been obtained with the MED-EL Vibrant Soundbridge and many reports on its efficacy have been published.[66,72,149,172,231,252,268,290,292,300]

The VSB consists of an external audio processor (AP) and an implantable part (Fig. 8.1), the vibrating ossicular prosthesis (VORP).[72,128,241] The active vibratory element of the VSB is a small electromagnetic element,

called the floating mass transducer (FMT). It is usually coupled to the long process of the incus but alternatively it can be coupled to the stapes footplate if this is mobile and the oval window niche is accessible and sufficiently large.[128] A second alternative is coupling of the FMT to the round window membrane for direct stimulation of the cochlea.[14,36,128] Round window stimulation can be regarded as a reversal of the normal pathway of activation, but animal studies have confirmed its effectiveness.[50,260,306]

ARHL is increasingly recognized in dogs.[284,285] It is sensorineural, occurs from a mean age of 8-10 years onward, and eventually encompasses the entire frequency range but occurs initially and is greatest at middle to high frequencies (8-32 kHz).[284] There have been no reports of the clinical application of the VSB in dogs with ARHL. We performed a feasibility study on implantation of the VSB in dogs using a lateral approach to the tympanic bulla and concluded that the VSB can be implanted successfully by placing the FMT in the round window niche.[286]

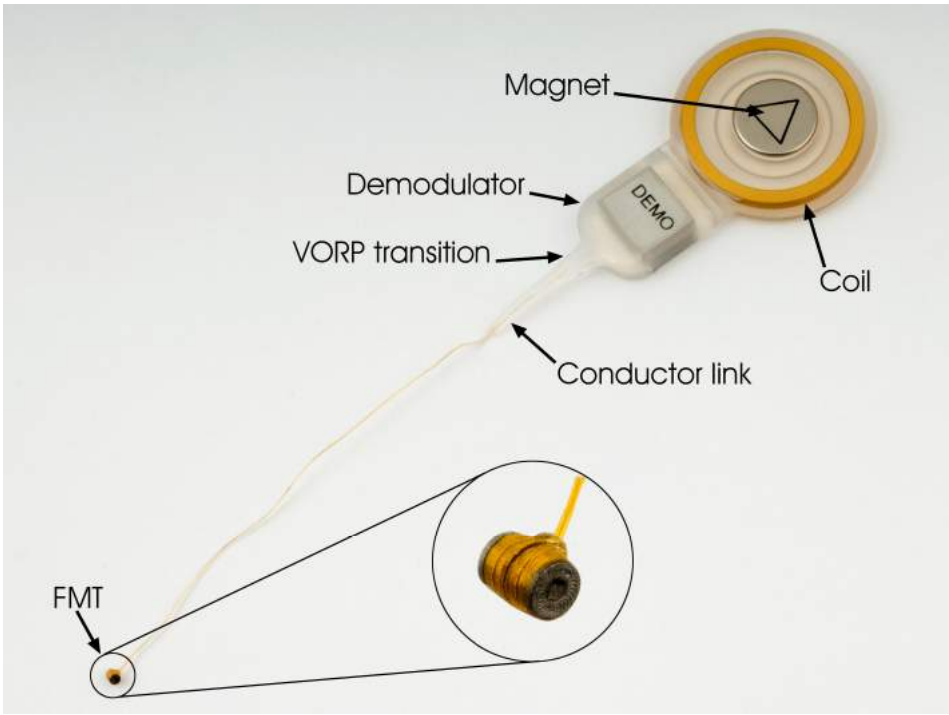


Fig. 8.1. *The implanted part of the Vibrant Soundbridge middle ear implant (vibrating ossicular prosthesis or VORP) consists of a magnet, a coil, and a demodulator. It is connected to the floating mass transducer (FMT) via a conductor link. The inset shows the FMT in greater detail.*

Implant surgery in humans is usually uneventful, but there have been occasional reports of postoperative infection, delayed wound healing, vertigo, facial nerve paralysis, alterations in perception of taste due to damage to chorda tympani, tinnitus, and pain at the implant site.[14,36,66,72,128,149,172,231,268,292,300] The surgery does not negatively influence normal air-conducted hearing in the implanted ear and thus unaided hearing thresholds (residual hearing) remain unchanged in almost all patients.[14,36,66,72,128,149,231,268,292,300] The postoperative gain with the transducer coupled to the incus and the device activated is frequency dependent and can be as high as 35 dB.[72,128,149] The gain after placement of the FMT in the round window niche usually does not exceed 20 dB, but this is the method of choice when the ossicular chain cannot be used for coupling as a result of malformation or because of technical difficulties.[14,36,286]

The aims of this study were to examine the clinical use of the VSB in dogs with ARHL, including postoperative follow-up and possible complications related to the implant surgery, and to evaluate the effects of the implantation surgery on residual hearing and the benefits for audibility of implantation of the FMT in the round window niche.

Materials and methods

The protocol of this study was approved by the Animal Experimentation Committee of the Faculty of Veterinary Medicine, Utrecht University, The Netherlands (DEC III.08.113).

Animals

This study was carried out in three Beagle dogs that had spent their entire lives in our research facilities. The first dog (A) was a neutered female, 10.5 years of age and weighing 15 kg, the second (B) was a castrated male, 12.7 years of age and weighing 14 kg, and the third (C) was a 10-year-old neutered female weighing 13 kg. They had no clinical signs of neurologic or otic disease and otoscopic examination under anesthesia verified normal external ear canals and normal tympanic membranes in all ears.

Preoperative evaluation

Preoperative evaluation 3-6 weeks prior to surgery consisted of a computed tomography (CT) scan of the skull and assessment of hearing by BERA, in one anesthesia session. CT scanning of the petrous bone, tympanic bullae, and outer ear canals was performed with a single slice helical CT scanner[a]. With the dog in sternal recumbency, 1-mm-thick contiguous slices were made using 120 kV, 160 mA, and 1 s scanning time.

Auditory thresholds were determined by BERA under light anesthesia, as described previously.[282] Hearing was assessed in each ear by delivering tone burst stimulations (1-32 kHz), beginning at 100 dB SPL and decreasing in intensity in steps of 10 dB until a threshold was reached. The threshold was defined as the intensity 5 dB above the first decreasing level at which no visually recognizable brainstem wave V was elicited.[282]

Anesthesia and postoperative medications

For implant surgery and immediate postoperative CT scanning, each dog was premedicated with methadone[b] (0.5 mg/kg IV) and then anesthetized with propofol[c] (1 mg/kg IV). The dog was intubated and surgical anesthesia was maintained by isoflurane[d] inhalation (<1% end-tidal concentration in 50% air and 50% O2), and fentanyl[e] (5-30 µg/kg IV) with atropine[f] (0.05 mg/kg IV). Rectal temperature was maintained at 36.5 to 37.5°C by means of circulating water heating pads placed under the dog. Antimicrobial prophylaxis was provided by amoxicillin with clavulanic acid[i] (20 mg/kg IV) administered during induction of anesthesia and repeated 3 hours later. Antibiotic therapy was continued after surgery with amoxicillin with clavulanic acid[j] orally (12.5 mg/kg twice daily) for 14 days. Analgesia was provided for 7 days postoperatively with carprofen[g] (2 mg/kg PO twice daily) and buprenorphine[h] (20 µg/kg SC three times daily).

VSB implant surgery

Each dog received a VSB middle ear implant on the left side, via the lateral approach to the tympanic cavity described previously.[286] After placement and fixation of the FMT in the round window niche, the tympanic membrane and ligament with their epithelial lining were carefully folded back in position within the bony acoustic meatus. Surgical gauze impregnated with chloramphenicol ointment[k] was inserted into the external ear canal with light pressure, to promote reattachment of the epithelial lining to the bone and to prevent postoperative infection. Correct positioning of the FMT in the round window niche was confirmed by a CT scan made with the same settings as for preoperative evaluation.

After recovery from surgery, each dog was fitted with an Elizabethan collar and was then monitored daily until the postoperative evaluation 3 months later.

Postoperative evaluation

Three months after surgery, auditory thresholds were determined by BERA using tone burst stimulation, and a CT scan of the skull was performed, both under light anesthesia as described for the preoperative evaluation. A generalized linear model for binomial data was used to analyze the clustered

BERA-derived thresholds using the statistical program R, version 2.7.0 (R Development Core Team 2008).[l]

In the same session, auditory steady-state responses (ASSR) were measured as described below to evaluate functionality of the implant. After completion of the postoperative evaluation, the dogs were euthanized by IV administration of T61[m]. The implanted VORP was removed and tissue specimens were obtained for histopathologic examination to evaluate wound healing associated with the implant and the location and type of adhesion of the FMT to the round window membrane.

ASSR recording

The ASSR equipment was described in detail previously.[296] The equipment and the procedure for measurements in adult humans were used with minor adaptations in these dogs. Our procedure was similar to that reported by others for ASSR recording in dogs.[152] In short, for stimulation, test tones of 1, 2, and 4 kHz, presented with 100% amplitude modulation and 20% frequency modulation were applied.[206] Modulation frequencies of the three test tones were 94.2, 96.3, and 98.3 Hz, respectively. The three modulated test tones were presented simultaneously to the implant via a standard audiometer[n]. Responses to the stimuli were recorded by three-channel EEG with high-quality amplifiers[o]. The three recording needle electrodes were placed SC over the mastoid (inverting), the inion (inverting), and the vertex (noninverting). The ground electrode needle was placed near the right elbow. The recorded EEG signals were analogue filtered from 20 Hz (6 dB/octave) to 300 Hz (12 dB/octave). A gain of 100,000 was used.

For the ASSR, EEG signals were recorded for 4.4 minutes using software functionally similar to the MASTER software.[117] The EEG was sampled at 8 kHz per channel (256 epochs of 1.024 seconds each), averaged over intervals of 16 epochs, and Fast Fourier Transformed. Frequency resolution was 0.061 Hz. For each modulated test tone, F values were calculated by comparing the signal power of the response at the modulation frequency with the power averaged over 60 frequency bins on either side of it, assumed to be noise. Responses for which $P = 0.025$ were considered significantly above the noise level in a one-sided F-test ($F = 3.75$) and a response was considered to be present if significant in at least one of the three recording channels after 4.4 minutes of recording time.

A standard VSB audioprocessor (AP)[p] was used for stimulation via the VSB implant. The output transducer of the audiometer was an insert earphone (Ear tone 3A). Its acoustical output was fed to the microphone of the VSB AP by a plastic tube, the end of which was glued to the opening of the microphone.[299] The AP was programmed in linear amplification mode and the maximum output limiter was deactivated. Special options (noise reduction and

speech enhancement features) were turned off. The gain of the AP was initially set at 20, on a scale of 0–70. Its linearity was studied with an implant-in-the-box device[q] with the AP being driven by the audiometer and the gain at which clipping of the output signal occurred being recorded at each test frequency.

ASSR thresholds were measured for all three test frequencies, starting at the gain just below amplifier saturation. Whenever a significant ASSR response was observed, the stimulation level was lowered by 5 dB. If after lowering the stimulus level no response was observed in any of the three EEG channels, the stimulation was increased by 5 dB. This procedure was repeated at 1, 2, and 4 kHz, independently. After determination of the threshold, the gain of the AP was increased to 45, 60, and then 70 and ASSR thresholds were determined for all gain settings, as described above. For calibration, the ASSR thresholds of the contralateral ear were determined in the classical manner with the output of the earphone coupled to the ear by a foam ear plug. The resulting thresholds were compared with the known BERA thresholds for that ear. Calibration factors for ASSR were thereby derived to enable direct comparison of ASSR thresholds with BERA thresholds.

Results

Preoperative evaluation

No abnormality was found on the preoperative CT scan of the skull of any of the dogs. The preoperative BERA with tone burst stimulation revealed the characteristic 5-7 positive peaks at 100 dB SPL in all three dogs.[282] In all dogs as the intensity of the stimulus decreased, the amplitude of the evoked response decreased while peak latency increased until the threshold was reached. Hearing thresholds across the entire frequency range in all three dogs were increased in comparison with the mean values in healthy young dogs and were similar to those in geriatric dogs with ARHL.[284] The hearing loss differed among the three dogs, ranging from 30-50 dB in the low-frequency range (1-4 kHz) to 40-60 dB in the middle- to high-frequency range (Fig. 8.2).

Surgery

Anesthesia and implantation of the VSB were uneventful in all three dogs. There were no problems in placing the FMT in the round window niche via a lateral approach to the tympanic bulla as previously described[286] and postoperative CT scans (Fig. 8.3) confirmed correct positioning of the FMT in all three dogs. There was some fluid, most likely from intraoperative bleeding, in the tympanic bulla in all three dogs.

Clinical recovery and follow-up

The recoveries were uneventful. On the day after surgery the dogs were alert, ate and drank normally, and did not object to being touched on the head. The surgical gauze impregnated with chloramphenicol ointment was removed from the external ear canal. Dogs B and C had left-sided facial paralysis, which disappeared in 5 weeks in one dog and 7 weeks in the other There was no wound infection and there was complete healing and regrown of hair by the time of the 3-month postoperative evaluation (Fig. 8.4).

Postoperative evaluation

The left ears were examined otoscopically 3 months postoperatively. In all dogs the epithelium had reattached to the bony meatus, there were no signs of otitis externa, and a clear, taut tympanic membrane was visible. In dog A, the caudoventral part of the pars tensa protruded slightly toward the ear canal and appeared fibrotic but was intact and without evidence of infection or inflammation. The postoperative CT scans of the skull revealed the implants to be in correct position and the tympanic bullae to be filled with air and free of fluid (Fig. 8.3). The ear canals were also filled with air and the tympanic membranes were in normal position.

Auditory thresholds of the left ears determined postoperatively using BERA with tone burst stimulation were not significantly different from those determined before surgery (P = 0.763, 95% CI; 0.27-0.80), demonstrating that mean residual hearing was unchanged in the implanted ears (Fig. 8.2). Five of the 24 thresholds were worse after surgery and six were better.

The AP was fitted (Fig. 8.4) and activated with the MED-El software, after which aided ASSR thresholds (at AP gains of 20, 45, 60, and 70, Fig. 8.2) were determined. Progressively lower thresholds were observed with increasing gain in all three dogs and the mean decrease in threshold was linearly correlated with the increase in gain, demonstrating that the implants functioned correctly in all three dogs. Only the ASSR thresholds at gain settings of 60 and 70 were lower than the postoperative BERA thresholds.

Pathology

In all three dogs the pocket in the temporal muscle around VORP and its conduction wire (Fig. 8.5) was lined by a thin layer of fibrous connective tissue with occasional focal infiltration by mononuclear inflammatory cells. Within the tympanic bulla the conduction wire leading to the round window niche and the FMT were covered by similar fibrous tissue with mild focal infiltration of mononuclear inflammatory cells. The FMT and the surrounding fibrous tissue were firmly attached to the round window membrane.

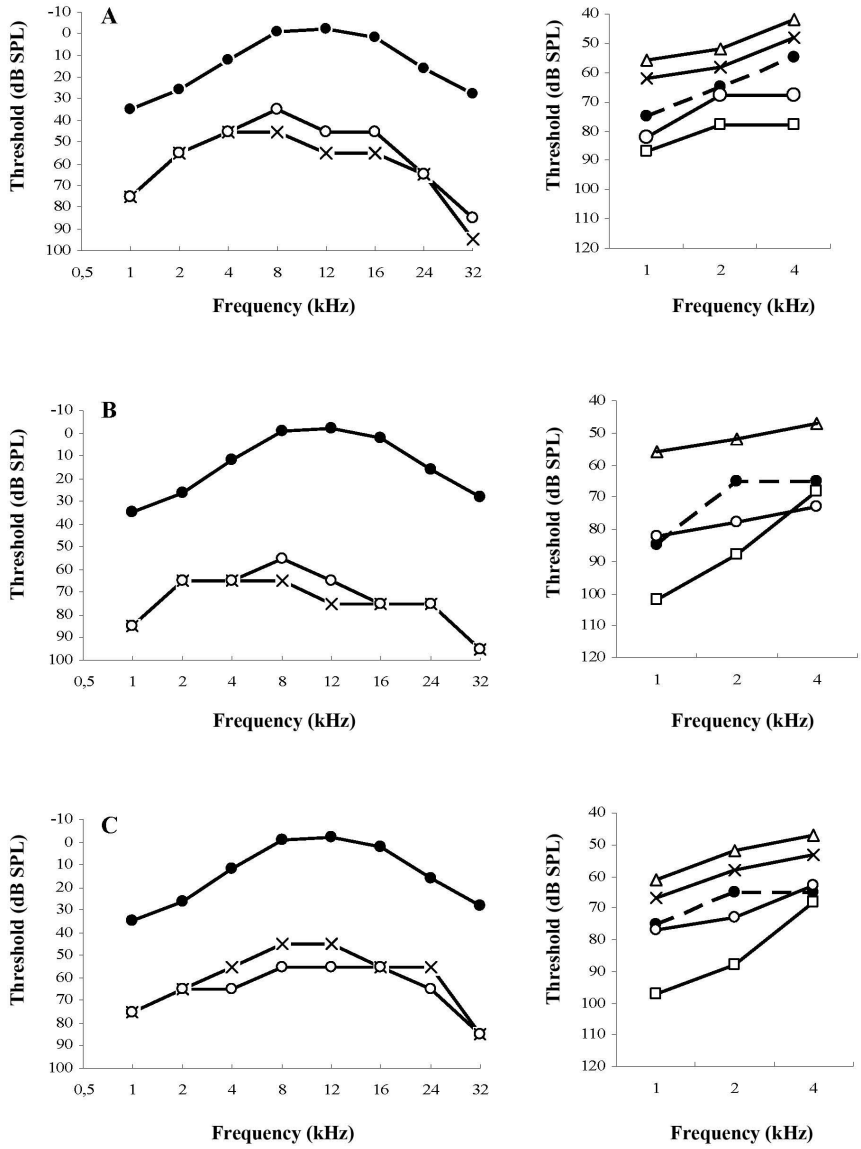


Fig. 8.2. *(left) Tone audiograms of dogs A, B, and C . Within each audiogram are the auditory thresholds at octave frequencies from 1 to 32 kHz determined by BERA. X = preoperative, O = postoperative, ● = mean threshold values in 10 young dogs with a mean age of 1.9 years (Ter Haar et al., 2008). (right) Postoperative aided ASSR thresholds at octave frequencies from 1 to 4 kHz at AP gain settings of 20 (□), 45 (O), 60 (X), and 70 (Δ).*

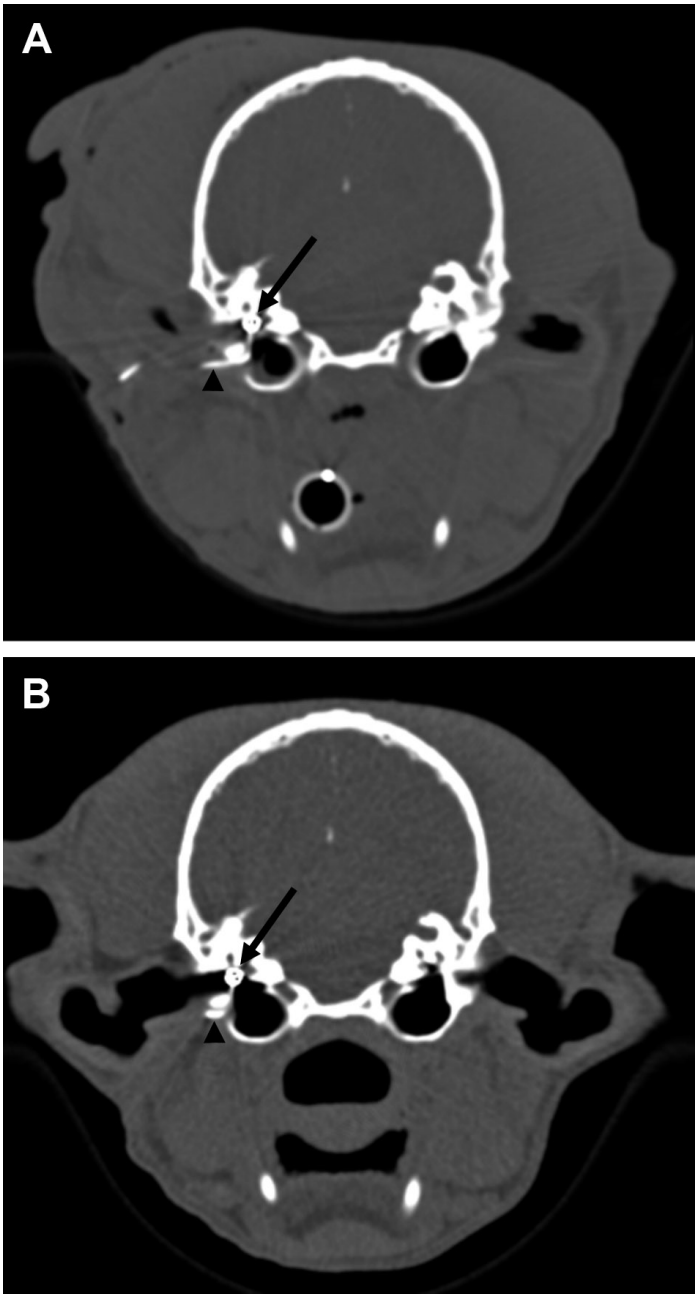


Fig. 8.3. *Postoperative CT scan of dog C, directly after surgery (left) and 3 months later (right). Both scans show the FMT in the left round window niche (black arrow) and part of the conducting wire (black arrowhead). The small amount of fluid in the tympanic bulla directly after surgery has disappeared 3 months later.*

Fig. 8.4. *Dog A with external audio processor held in position by the attraction of the magnet in the VORP implanted in the temporal muscle.*

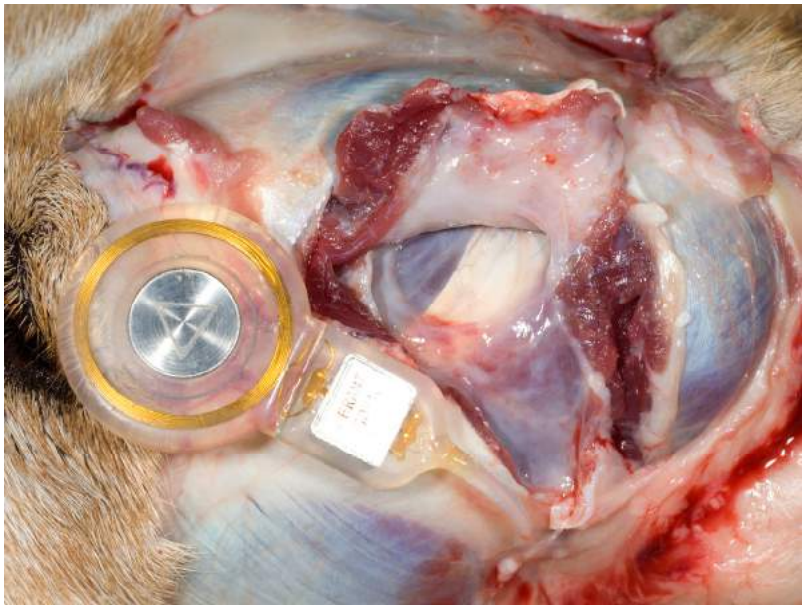


Fig. 8.5. *Removal of the implanted VORP after euthanasia. Note the smooth fibrous lining of the pocket in the temporal muscle and the absence of exudate or other signs of inflammation.*

Discussion

There was postoperative left-sided facial nerve paralysis in two of the three dogs, characterized by drooping of the left cheek and inability to close the left lower eyelid. The facial nerve paralysis could have been caused by damage to the nerve at the level of the stylomastoid foramen where the nerve exits the skull and passes around the proximal ear canal close to the tympanic bulla, during the drilling of the hole in the caudodorsal quadrant of the bulla. The nerve could also have been damaged during removal of the part of the mastoid process of the petrous temporal bone dorsolateral to the round window, since the facial nerve runs in this area. That the nerve was probably damaged by heat from the drilling or by blunt trauma and was not severed is indicated by disappearance of the facial paralysis a few weeks later. Facial nerve paralysis has also been reported in humans after VSB implantation and is usually transient.[72,289] It has been attributed to reactivation of a virus residing in the geniculate ganglion, possibly induced by surgery[72], or trauma to the nerve during mastoidectomy.[289] Implantation of the VSB in people has occasionally been complicated by postoperative infection, delayed wound healing, vertigo, abnormal taste as a result of damage to chorda tympani, tinnitus, or pain at the implant site, but the overall incidence of complications is less than 3%.[14,36,66,72,128,149,172,231,268,292,300] Furthermore, placement of the FMT on the round window membrane does not cause additional complications in humans.[36]

Wound healing in these dogs was uncomplicated. There was no evidence of vertigo. The occurrence of tinnitus cannot be evaluated in dogs. Taste is affected, usually temporarily, in 2–15% of human patients with damage to the chorda tympani.[66,268] We observed no abnormal eating or drinking behavior in these dogs, but perception of taste could not be evaluated specifically. We conclude that implantation of the VSB had no significant side effects in these dogs in the period of 3 months after surgery, other than the above-mentioned transient facial paralysis.

Our main objective was to evaluate the effects of the VSB implantation procedure on residual hearing. Ideally, normal air conduction hearing should remain unchanged after implantation, or decrease with ageing as expected. Comparison of the hearing thresholds determined by BERA indicated that residual hearing was not significantly affected by the implantation procedure.[282,284] The postoperative thresholds differed by no more than 10 dB from corresponding preoperative values. These changes were not significant and were within the expected variability for repeated measurements of BERA.[282,284] Although there are no clearly defined limits for significant reduction in residual hearing measured as unaided pure-tone averages, there is consensus among otologists and audiologists that a threshold change of less than 10 dB is not significant.[149]

The average difference between pre- and postoperative air-conduction thresholds at all frequencies tested in humans is reported to be between 2 and 8 dB. In general, postoperative thresholds are higher than preoperative thresholds.[66,72,149,231,290,300] Our results are similar to those reported by Fraysse et al., who found postoperative thresholds to be 6.3–8.8 dB higher than before surgery in 5 patients, and 11.3 dB lower in one.[72] Sterkers et al. reported similar findings of higher postoperative thresholds in 3 patients (10, 14, and 20 dB) and lower thresholds in 2 (10 and 11 dB).[268] Fisch et al. observed that the frequencies most susceptible to change are very low (250–500 Hz) and high (4, 6, and 8 kHz) frequencies and that patients with significant changes in the implanted ear did not have changes of the same degree in the nonimplanted ear.[66] They therefore concluded that the observed threshold changes could not be attributed to systematic influences such as anesthesia, measurement error, or patient-specific influences, but were most likely the result of the implant procedure itself. In most cases the change was sensorineural and was attributed to damage incurred in opening of the antrum during mastoidectomy.[66] Similar threshold changes were found after FMT placement in the round window niche when air conduction thresholds could be determined pre- and postoperatively. On average, postoperative thresholds were 7-8 dB higher across frequencies, with no statistically significant frequency dependencies in these increases.[36] Because of anesthesia-time restrictions, the nonimplanted ear was not tested in these dogs. No trends in frequency-specific susceptibility were observed, although this could be the result of the small number of dogs used. Nevertheless, we conclude that residual hearing was not significantly affected by the procedure, as also has been reported in humans. Histopathological examinations revealed no problems with the implants. The ossicular chain was uninterrupted and free of abnormalities, and tissue reaction to the implant was limited to a modest layer of fibrous tissue and only mild focal mononuclear inflammation. There was firm contact of the FMT with an intact round window membrane in all three dogs. Nevertheless, these findings do not rule out functional disturbances and the short period of postoperative follow up does not exclude the possibility of long-term complications of the implant surgery.

This study was designed to evaluate the benefits of the implant to audibility by measuring auditory thresholds at different gain settings of the AP. In humans the hearing improvement obtained with the implant is measured using the technique of auditory steady state evoked potentials or ASSRs.[206,251,252,253,299] ASSRs are preferred over other techniques for evaluating the actual functioning of hearing aids because they are frequency specific and time effective.[206] Compared to transient stimuli, these stimuli are much less likely to be distorted by amplification in a hearing aid.[206] ASSRs to amplitude modulation tones with modulation frequencies between 80 and 105 Hz can be recorded when multiple stimuli are presented simultaneously through a sound-

field speaker and amplified using a hearing aid. Picton et al. demonstrated their use in determining aided thresholds and showed that the actual gain of the aid cannot only be measured but also adjusted on the basis of the ASSR measurements.[205] The feasibility of using ASSRs in experimental assessment of hearing in dogs, using modulation rates around 100 Hz, to obtain frequency-specific ASSR-derived thresholds was reported recently.[152] Even though the audible frequency range is broader in dogs than in people (high frequency cutoffs in dogs are slightly above 40 kHz)[282] and the lowest thresholds have been found to be around 12-16 kHz in normal hearing dogs[282], ASSR-derived thresholds have not been reported for frequencies above 8 kHz, since these carrier frequencies were not available in the aforementioned study[152]. The VSB middle ear implant used in this study was developed for humans and has a frequency response between 250 Hz and 7 kHz, which allows amplification of all frequencies necessary for understanding normal speech. Even though this implant can therefore not compensate for the middle- to high-frequency losses in dogs with ARHL[284], its augmentation of the less, yet also significantly, affected low frequencies was considered to be important to improve communication between dog and owner. The characteristics of the implant eliminate the need for testing frequencies of 8 kHz and above. A slight modification of the ASSR technique used and validated in people, similar to that used by Markessis et al.[152], was used to determine the functionality of the implants in this study.[252,253,299]

The present study demonstrates that implants with the FMT placed in the round window niche function satisfactorily, since progressively lower ASSR thresholds were observed with increasing gain settings of the AP. Direct comparison of BERA thresholds with aided ASSR-derived thresholds is difficult, but comparing pre- and postoperative BERA-derived thresholds with ASSR thresholds revealed that at the highest gain settings (60 and 70) of the AP, thresholds were lower after than before surgery and hence audibility had improved. The mean maximum decrease in thresholds was 20.7, 13, and 16.3 dB at 1, 2, and 4 kHz, respectively, similar to what has been reported for round window stimulation in humans.[14,36,73,145]

Colletti et al. were the first to report on the use of the VSB without coupling to the incus, in human patients with mixed or conductive hearing loss.[36] They instead attached the FMT in the round window niche in close contact with the membrane, and demonstrated that the cochlea could be stimulated effectively in this way. The use of round window stimulation can be regarded as a reversal of the normal pathway of activation, but animal studies had confirmed the utility of this type of stimulation.[50,260,306]

The implant-associated threshold varied among the 7 patients described, even approaching 30 dB improvement in 2 patients with mixed hearing loss.[36] Since this original report appeared, several research groups have reported new

applications of the round window approach in patients with conductive or mixed hearing loss, yet these reports suggest that less gain is to be expected.[14,73,145] In patients with SNHL receiving a VSB implant with coupling of the transducer to the incus, a maximum mean gain decrease in threshold of 35 dB for 0.5, 1, 2, and 4 kHz can be expected.[66,72,149,253,290] Other studies have reported on the less effective energy transfer with round window stimulation by the FMT.[14,145]

Beltrame et al. recently reported on 12 patients with mixed hearing loss with a 40 dB sensorineural component and measured aided thresholds for comparison with bone-conduction thresholds.[14] They found a mean decrease in threshold of -20, 0, 15, and 5 dB at 0.5, 1. 2, and 4 kHz, respectively. Linder et al. described 4 patients with mixed hearing loss also having a mean sensorineural component of 40 dB, and found a mean decrease in threshold of -8, 5, 15, and 10 dB at these 4 frequencies.[145] These results suggest that in humans placement of the FMT in the round window niche is considerably less effective than the classic coupling of the FMT to the incus in an intact ossicular chain. Our results show that a gain can indeed be expected in dogs with ARHL after placement of the FMT in the round window niche. However, as in humans, a more powerful AP is required for round window stimulation than for a direct drive of the ossicular chain, to compensate for more severe SNHL. The use of a more powerful AP for implants placed in client-owned dogs with ARHL will be the subject of further research. Important points of interest that need to be addressed in these follow-up studies are acceptance of the externally worn AP by client-owned dogs, the incidence of accidental loss of the AP, and most importantly, evaluation of the improvement in patient-owner communication as a result of improved hearing.

We conclude that in this small group of dogs with ARHL implantation of the VSB with placement of the FMT in the round window niche was a safe surgical procedure and caused no significant side effects or change in residual hearing. The implants functioned correctly and produced a definite improvement in hearing. Several important implant-related aspects, mainly concerning the externally worn AP, require further research before the VSB can be routinely used in client-owned dogs with ARHL.

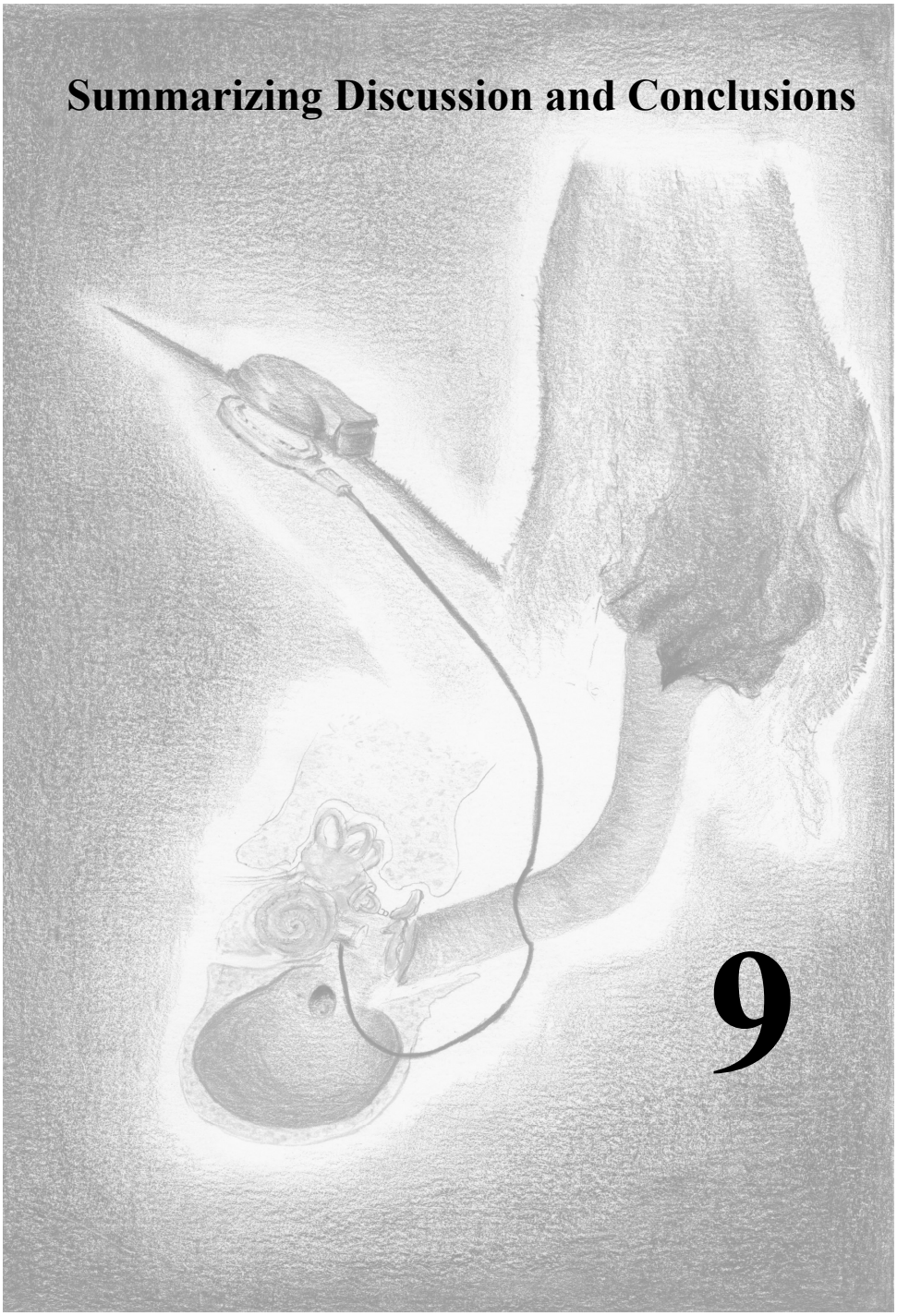
Acknowledgements

The authors are grateful for the assistance of supporting technicians of the Department of Anesthesiology and the Biophysical Laboratory of the Department of Clinical Sciences of Companion Animals (UU), and Mr J. Noten and Mr. B. Luijten of the Department of Otorhinolaryngology (Nijmegen) for their assistance in performing the ASSR measurements. We also thank MED-EL corporation for generous assistance and advice, Dr. E.J.B. Veldhuis-Kroeze, DECVP, for the photomicrograph in Figure 6, and Dr. B.E. Belshaw for editing.

Footnotes

[a] Philips Secura, Philips NV, Eindhoven, The Netherlands.
[b] Methadone HCl, Eurovet Animal Health, Bladel, The Netherlands
[c] Propovet, Abbott Laboratories Ltd., Maidenhead, Berkshire, England
[d] Isoflo, Abbott Laboratories Ltd., Maidenhead, Berkshire, England
[e] Fentanyl, Bipharma, Weesp, The Netherlands
[f] Atropine sulfate, Pharmachemie B.V., Haarlem, The Netherlands
[g] Carporal, AST Farma BV, Oudewater, The Netherlands
[h] Buprecare, AST Farma BV, Oudewater, The Netherlands
[i] Augmentin, GlaxoSmithKline, Zeist, The Netherlands
[j] Synulox, Pfizer BV, Capelle a/d IJssel, The Netherlands
[k] Chlooramphenicol, Sanofi Santé BV, Maassluis, The Netherlands
[l] R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.
[m] T61, Intervet International, Boxmeer, the Netherlands
[n] Interacoustics AD 229, Interacoustics, Assens, Denmark
[o] Ekida, Jager-Tonnies, Helmstadt, Germany
[p] Type 404, Med-El, Innsbruck, Austria
[q] Audio Processor Adaptor, Med-El, Innsbruck, Austria

Summarizing Discussion and Conclusions



9

Acquired sensorineural hearing loss (SNHL) is a very common cause of hearing impairment in dogs, second only to congenital SNHL[277], yet it has received little attention in the veterinary literature. The classification of hearing loss in dogs, its clinical signs and social implications, and the aims of this thesis are outlined in the general introduction (***chapter 1***). The diagnosis of acquired, frequency-specific hearing impairment exemplified by ototoxicity, noise-induced hearing loss (NIHL), or age-related hearing loss (ARHL), requires objective electrophysiological tests that evaluate the entire audible frequency range in dogs. The technique most commonly used in veterinary medicine for hearing assessment has been brainstem-evoked response audiometry (BERA) using click stimuli (CS).[314] However, clicks stimulate a large portion of the cochlea and are therefore not frequency specific, which renders this technique unsuitable for the diagnosis of acquired SNHL. One of the aims of this thesis was to modify the BERA technique to obtain an objective, complete hearing test for dogs and to use this to determine frequency-specific thresholds over the entire audible frequency range, as well as to collect reference values for BERA characteristics in normal dogs (***chapter 4***). A second aim was to investigate and document the audiometric (***chapter 5***) and histological (***chapter 6***) characteristics of ARHL in dogs. In addition, the clinical feasibility (***chapter 7***) and applicability (***chapter 8***) of treatment of this typical form of hearing loss with hearing aids in the form of middle ear implants was examined. Background information on the embryology and the macroscopic, microscopic, and computed tomographic anatomy, and the physiology of the dog's ear is described in ***chapter 2***, which can be used as a reference for the later chapters on surgery. BERA as a diagnostic tool in veterinary medicine is reviewed in ***chapter 3***, together with current knowledge about NIHL and ARHL in dogs. This includes the first four original articles in this thesis (chapters 4-7), which will be summarized and discussed hereafter.

A method was developed in our laboratory for frequency-specific assessment of the cochlea in dogs using BERA with tone burst stimulation (TS). Tone bursts ranging from 1 to 32 kHz were created and used in this study (***chapter 4***). Our results demonstrate that the greatest sensitivity of the dog's ear is at 12 and 16 kHz, which is in very close agreement with results of behavioral tests.[98] The advantages and disadvantages of using BERA with TS for determination of frequency-specific thresholds are discussed in detail in ***chapters 3, 4, and 5***. Various methods could have been used to test frequency-specific areas of the cochlea, such as the use of CS with high-pass noise or notch-noise masking, or the use of direct stimulation with tone bursts. The latter was selected for this study because it had been shown to be easy to perform and to yield reliable information about pure tone thresholds.[45,63,133,188,263] A concern in using tone bursts is spectral splatter.[208,243] This means that the BERA

threshold obtained using tone bursts is not solely determined by the response of the neurons at the nominal frequency but also that of neurons at the side lobe frequencies. In patients with hearing loss, this could lead to an underestimation of the loss at the nominal frequency of the tone burst.[243] Based on our results, however, and their close agreement with results of behavioral tests, we concluded that reproducible information on frequency specificity of the canine cochlea can be obtained by TS and that this report provides a normative database with stimulus variables needed to evaluate frequency-specific hearing loss in dogs.

Another technique was reported recently for measuring frequency-specific thresholds in dogs, using auditory steady-state evoked potentials.[152] This appears to be a valid method but frequencies above 8 kHz were not tested. It may be found to be applicable when data at higher carrier frequencies are available. In addition to BERA, the use of otoacoustic emissions (OAEs) has been advocated to assess cochlear function in humans.[122,258,303] OAEs can not only be obtained quickly but can also be applied very objectively to evaluate outer hair cell integrity. They can, for example, identify ototoxicity earlier than BERA.[258] In dogs, valid spontaneous and evoked OAEs have been recorded, but the reports are scarce and the clinical applicability and usefulness in frequency-specific hearing assessment in dogs has yet to be demonstrated.[246,257,258]

It is generally assumed that the hearing of dogs becomes impaired with advancing age, yet there are few reports to support this. ARHL is claimed to be the most frequent cause of acquired SNHL in dogs[48,277] and reports of decreased hearing in aged dogs have been documented by auditory brainstem-evoked responses, but using CS or one stimulus level only.[131,132,240] No thresholds have been reported in aged dogs with impaired hearing by use of either CS or TS. We hypothesized that hearing in dogs, as in humans, becomes impaired with ageing across the entire frequency range, but primarily in the high-frequency area, and can be assessed quantitatively by BERA using TS. In ***chapter 5***, the results verifying this hypothesis are presented in the form of a cross-sectional and a longitudinal study of age-related changes in audiograms in dogs obtained by the technique described in chapter 4. The most important conclusion of our cross-sectional study is that auditory thresholds at all frequencies tested were significantly higher in geriatric than in young and middle-aged dogs and were likely to be the result of presbycusis. Although differences were significant at all frequencies, the most dramatic increase in thresholds was seen at middle to high frequencies (8-32 kHz), which is similar to that in human presbycusis.[171,219]

The audiograms of the dogs included in the longitudinal study show a progressive increase in thresholds associated with ageing, starting around 8-10 years of age and being most pronounced in the middle- to high-frequency region (8-32 kHz). Thresholds were significantly higher at a mean age of 12 years than

at a mean age of 6 years for 8, 12, 16, 24, and 32 kHz ($P < 0.05$). The average increase between 10 and 12 years of age was around 10 dB at 1, 2, and 4 kHz, and ranged from 15 dB at 8 kHz to over 22 dB at 12, 16, 24, and 32 kHz. This difference between low and high frequencies is also observed in longitudinal studies in presbycusis in humans.[53,138] From these studies, it appears that a significant reduction in hearing capacity occurs from the age of 60 years onward and begins at the high frequencies (6-16 kHz), but gradually encompasses the entire frequency range. Furthermore, between the ages of 70 and 80 years the reduction in hearing amounts to 1-2 dB per year, depending on the specific frequency tested.[53,138] The threshold increases which we observed in dogs are 10 times greater than those reported in humans. This may in part be explained by technical differences between studies (for practical purposes we decreased stimulus intensity in steps of 10 dB), and in part by differences between species. Dogs having a much shorter lifespan, any age-related deterioration might occur at a more rapid rate than in man.

The cross-sectional study also revealed a significantly higher threshold in the middle-aged dogs than in the young dogs at 4 kHz, while the thresholds at other frequencies did not differ. Kennel noise exposure might be responsible for this finding. The middle-aged dogs had been housed in our facilities during their entire lives and were thus exposed to the noise of their own barking. NIHL in humans typically occurs in the 2-6 kHz range, with initial changes at 6 kHz and the greatest increase in threshold at 4 kHz.[138,198] This suggests that the increased threshold in the middle-aged dogs at 4 kHz could have been the result of noise damage. These dogs had been used in the first study (chapter 4) to establish normative data. In retrospect, they cannot be considered to be healthy, normal-hearing dogs, since at 4 kHz they had a significantly higher threshold than a group of very young dogs. However, since there was a significant difference in auditory thresholds between the geriatric and young dogs, and over time within the group of middle-aged dogs, we conclude that presbycusis does exist in dogs, as in humans. The impairment can be demonstrated and its progression can be followed using BERA with frequency-specific, tone burst stimulation of the cochlea.

Our observations and conclusions are derived from a small group of medium-sized dogs and therefore not necessarily applicable to all dogs. Future studies should be carried out on a larger scale in client-owned dogs, not housed in kennels and not exposed to loud recreational and environmental noise, to determine the actual prevalence of this disorder. To demonstrate or exclude differences in effects of ageing on hearing between dogs of different sizes, with different lifespans, several more groups would have to be included in these studies. In future studies it would also be advisable to combine BERA with measurements of otoacoustic emissions, as discussed above.

Schuknecht proposed that the histological form of ARHL can be determined from the shape of the audiogram.[233] Age-related loss of hair cells and spiral ganglion cells (SGCs) has been reported in dogs.[118,132,234,240] Knowles et al. found loss of SGCs in a group of deaf dogs in which auditory thresholds were completely absent[132] and Shimada et al. reported varying degrees of loss of SGCs, and atrophy of the organ of Corti and the stria vascularis, in all dogs over 12 years of age, predominantly in the basal turn of the cochlea.[240] Although the latter findings would seem to imply a high-frequency hearing loss of the mixed type in dogs, comparable to that found in most cases of human ARHL, frequency-specific hearing thresholds were not determined. Having found audiometric proof of frequency-specific age-related changes in hearing in dogs, we further hypothesized that cochlear lesions in dogs with ARHL would be similar to those in humans as well, and that the severity of the histological changes would be reflected in tone audiograms.

In ***chapter 6*** the results are presented of a study on age-related changes in the cochlea in relation to BERA-derived thresholds. Cochlear lesions were found in all 10 geriatric dogs studied. Not only was significant loss of SGCs found in this study, but also of outer hair cells (OHCs) and a reduction in stria vascularis cross-sectional area (SVCA). Histological abnormalities were found primarily in the basal turn, consistent with the occurrence of the largest threshold shifts and highest absolute BERA-derived thresholds in the middle- to high-frequency regions (8-32 kHz). It was concluded that the degeneration of the OHCs and SGCs observed in the basal turn was primarily responsible for the elevated hearing thresholds, similar to findings in humans.[80,183] The SVCA was smaller in all cochlear turns in the geriatric dogs, similar to gerbils, where degeneration of the stria vascularis is an early event in ARHL, usually beginning at both the base and apex and extending to midcochlear regions with advancing age.[259] While the reduction in SVCA in all turns could explain the loss of hearing sensitivity over the entire frequency range in the geriatric dogs, it could not explain the difference between low- and middle-to-high-frequency loss. A mixture of lesions—OHC loss, SGC loss, and reduction in SVCA—thus seemed to be the best explanation for the audiometric results. Our hypothesis that cochlear lesions would be similar to those found in humans with ARHL, which is most often the mixed type[189], was therefore accepted.

Despite Schuknecht's statements, several studies have failed to show a close correspondence between audiometric data and cochlear pathology and this study was no exception.[35,182] The auditory thresholds found in these dogs did not indicate whether the histological abnormalities were primarily sensory, neural, or strial in nature. This is most likely due to the fact that there were mixed lesions in all cases, with variable degrees of OHC loss, IHC loss, SGC loss, and reduction in SVCA. Individual audiograms did, however, reflect the severity and location of cochlear lesions in all cases. In general, cochlear lesions

were more extensive in dogs with more advanced hearing loss. It was concluded that tone audiograms could therefore be used to diagnose and characterize ARHL in dogs, for they not only indicated the severity of hearing loss but also the extent and location of the mixed cochlear lesions.

Electron microscopic examination of the stria vascularis and the SGCs will be needed to determine the exact nature and importance of the observed microscopic abnormalities in the cochleas of the geriatric dogs, such as the abundant lipofuscin inclusions and intracellular vacuoles in the remaining SGCs. Furthermore, although there is consensus that the cochlea is the site of ARHL[146], the brain is the ultimate organ of hearing and perception, and the central auditory system is also known to be affected by ageing.[312] Neurophysiological studies of cochlear nucleus neurons have shown lower glycine-mediated inhibition, reflected in increased firing rates in cochlear nucleus neurons from old animals relative to young adults. In addition, anatomical reductions in neurons of the cochlear nucleus and their output pathways can occur due to ageing changes in the brain, as well as due to age-dependent plasticity of the cochlear nucleus in response to the age-related loss of inputs from the cochlea, particularly from the basal, high-frequency regions.[312] Nerve cell loss, astrogliosis, and ubiquitin deposition were found in cochlear nuclei of dogs over 10 years of age.[240] Age-related alterations in GABA synthesis and release are found in the auditory midbrain (superior olivary complex and inferior colliculus) and primary auditory cortex of humans.[29] It is not known whether they are primarily the result of ageing of the central pathways or secondary to peripheral pathology, nor whether they also occur in dogs.

Effective treatment of presbycusis is important for quality of life in geriatric medicine, and modern hearing aids, middle ear implants, and cochlear implants are valuable aids to communication for elderly people with hearing loss.[7,80] Most people with ARHL are fitted with a conventional hearing aid, but the use of these in dogs has been mentioned only anecdotally[155], and has not met with much clinical success. People with mild to severe SNHL who do not benefit from conventional external amplification benefit greatly from implantable auditory prostheses.[72] There have been no reports on the use of implants in hearing-impaired dogs. Of the available options, fully implantable systems are of particular interest because there is less likelihood of loss of the device, occlusion of the ear canal, or induction of otitis externa with these implants than with nonimplanted hearing aids.[7,72] However, fully implantable devices were not commercially available at the start of this study and semi-implantable systems had to be used. Nevertheless it is expected that continuing technological innovations will enable conversion to fully implantable systems in the foreseeable future.

The Vibrant Soundbridge (VSB) is an active, semi-implantable, middle ear hearing device that has been available in Europe for several years and there have been many reports of its effectiveness.[252,268,290] The VSB consists of an external audio processor (AP) and an implantable part, the vibrating ossicular prosthesis (VORP). The active vibratory element of the VSB is a small electromagnetic element, called the floating mass transducer (FMT). Several surgical approaches for implantation have been described, usually with coupling of the transducer of the VSB to the long process of the incus, to provide a "direct drive" of the ossicular chain.[66,72] This could not be duplicated in dogs because of the restricted size of the epitympanic recess and the size and shape of the incus. In humans, placement of the transducer of the VSB in the round window niche has also been reported, as an alternative to clipping the FMT to the incus.[36] We hypothesized that it was technically possible to implant the Vibrant Soundbridge (VSB) middle ear implant successfully in dogs using a lateral approach to the tympanic bulla (TB). The results of this feasibility study are described in ***chapter 7***. The experimental technique was safely executed unilaterally in three dogs without intraoperative complications, after which computer tomography of the temporal bone was used to ascertain correct placement of the FMT and the dogs were then euthanized. The hypothesis that the implant surgery was technically feasible without intraoperative complications using a lateral approach to the TB was accepted. A lateral approach to the middle ear cavity was used rather than the ventral approach which is commonly used in dogs because it would enable the FMT to be introduced into the tympanic bulla and maneuvered into the round window niche, as well as fixation of the VORP, in one session without repositioning of the patient.

It was further hypothesized that the technique would also be feasible in dogs with ARHL, that the incidence of postoperative complications such as delayed wound healing, wound infection, or breakage of conductor link wire would be low, that residual hearing would be unaffected, and that it would be possible to demonstrate improved hearing in the implanted ear. To verify this hypothesis, three geriatric dogs with ARHL were implanted unilaterally with a VSB implant and were allowed to recover. The early clinical results of this study with 3 months follow-up are described in ***chapter 8***. No intraoperative or postoperative complications occurred and recovery was uneventful in all three dogs, except for transient facial nerve paralysis in 2 of them. As in people, differences in air conducted pre- and postoperative BERA-derived thresholds were not significant, demonstrating that residual hearing was unaffected by the surgery. The functionality of the implants was assessed using auditory steady state evoked potentials. This technique is used to measure the gain of the implant in humans[206,251,255] because auditory steady-state responses (ASSRs) can

be evoked by amplitude-modulated tones, which are frequency specific and time effective and, compared with transient stimuli, are much less likely to be distorted by amplification in a hearing aid.[206] The feasibility of using ASSRs in dogs, with carrier frequencies up to 8 kHz for experimental assessment of hearing, was reported recently[152] and was therefore used in this study to determine aided thresholds. Since the VSB middle ear implant, developed for human use, has a frequency response between 250 Hz and 7 kHz, there was no need to test frequencies of 8 kHz and above. The VSB implant amplifies all frequencies necessary for normal speech understanding in humans, and this implant could therefore not be used to substitute for the middle- to high-frequency losses found in dogs with ARHL. However, augmentation of the less, yet also significantly, affected low frequencies was considered to be more important, since our goal was to improve communication between dog and owner. ASSR thresholds determined at 1, 2, and 4 kHz were found to decrease progressively with increasing gain of the AP in all three dogs, demonstrating correct functioning of the implants. However, the thresholds determined using the maximal gain settings of the AP were lower than the postoperative BERA-derived thresholds. Depending on the frequency and dog tested, the maximum decrease in threshold was 20 dB. The decrease in threshold reported for the VSB in humans with coupling of the FMT to the incus can be as high as 35 dB[251,255], but our results are similar to those in humans in whom the FMT is also implanted in the round window niche.[14,145] We therefore accepted our hypothesis and concluded that in this small group of dogs with ARHL, implantation of the VSB with placement of the FMT in the round window niche was a safe surgical procedure, without significant side effects or degradation of residual hearing. The implants functioned satisfactorily and produced a clear improvement in hearing using the maximal gain setting of the AP.

The three Beagle dogs used for this study appeared to fully accept the externally worn AP and made no attempts to remove it by shaking the head or pawing at it. However, since these dogs had been housed in our kennel facilities during their entire lives and had not been trained to respond to vocal commands, it was not possible to determine on the basis of their behavior whether their hearing was improved after surgery. How well client-owned dogs with ARHL will accept wearing the AP remains to be seen. Since it is held on the dog's head only by magnetic attraction to the implanted VORP, there may be problems in wearing it in a household environment that were not encountered in the kennel and, if so, a remedy will have to be found. Preventing complete loss of the AP by means of a collar containing a magnet sufficient to catch an AP dislodged from the head is one of the options that have been considered. The current cost of the implant, the surgery, and postoperative follow-up including hearing tests is approximately 10,000 Euros. A definite increase in audibility

leading to a substantial improvement in patient-owner communication will have to be demonstrated in future studies to justify a financial investment of this magnitude. Finally, as in humans, an AP with a greater gain than currently available is required for dogs with SNHL exceeding 20 dB.

Additional applications to surgery in the dog's ear

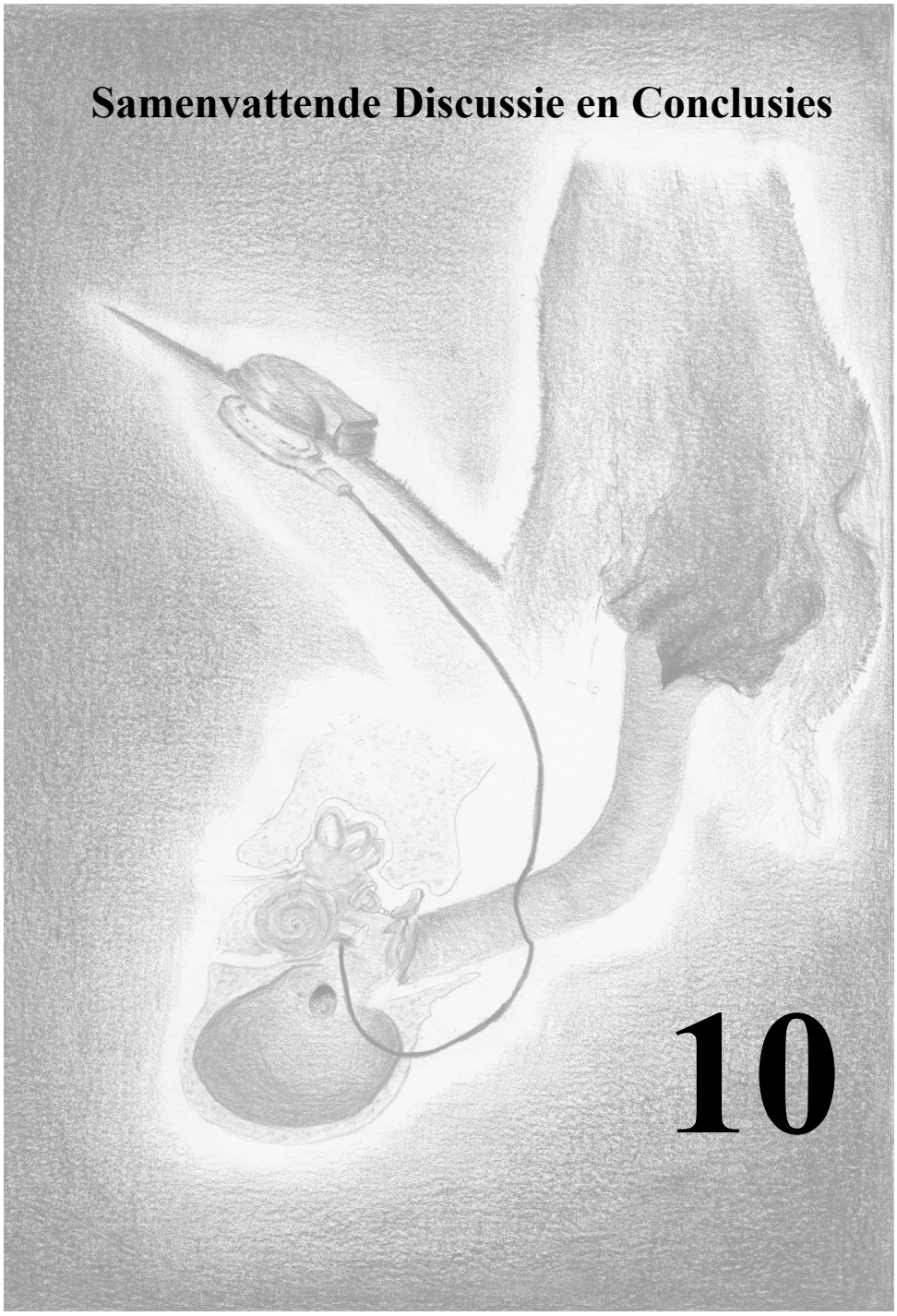
The surgical approach to the middle ear cavity described in this thesis, which avoids opening or sacrificing the external ear canal, may itself have additional applications in small animal surgery for treatment of such conditions as the primary secretory otitis media commonly found in the Cavalier King Charles Spaniel, for taking large biopsies of middle ear mucosal abnormalities, and for reconstruction of ossicular chain abnormalities. The accessibility of the round window niche provided by this approach will also allow placement of cochlear implants.

The VSB middle ear implant can now be evaluated in clinical trials in dogs. Beyond this, patients requiring bone-anchored hearing aids, cochlear implants, or brainstem implants will undoubtedly be identified when appropriate diagnostic techniques are applied. Considering the great similarities in audiometric and histological characteristics between dogs and humans with ARHL, dogs would make an excellent model for future studies of ARHL, and possibly other forms of SNHL, in humans. Not only would this research considerably raise the knowledge and standard of treatment of SNHL in dogs to a level comparable to that now possible in humans, but the relative ease with which histopathological evaluation of treatment can take place in dogs would benefit the development of appropriate treatment protocols in humans as well.

Conclusions

ARHL is the most common form of acquired SNHL in dogs, yet had received little attention in the veterinary literature. This thesis describes the diagnosis and audiometric characteristics of ARHL using BERA with frequency-specific tone burst stimulations. Furthermore, the age-related histopathological characteristics of the inner ear are described and related to the BERA-derived auditory thresholds. The feasibility of treatment of ARHL with the VSB middle ear implant was explored and an experimental trial using this implant was conducted.

Samenvattende Discussie en Conclusies



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Verkregen perceptief gehoorverlies (PGV) is een zeer frequent voorkomende oorzaak van slechthorendheid of doofheid bij honden, alleen aangeboren PGV komt nog vaker voor.[277] Toch heeft dit probleem tot nu toe weinig aandacht gekregen in de diergeneeskundige literatuur. De classificatie van gehoorverlies bij honden en de klinische verschijnselen en sociale gevolgen van aangeboren en verkregen gehoorverlies alsmede de doelstellingen van dit proefschrift zijn beschreven in de algemene inleiding (***hoofdstuk 1***). Voor de diagnose van verkregen, frequentiespecifiek gehoorverlies, zoals die veroorzaakt door ototoxiciteit, lawaai-slechthorendheid (LSH) of leeftijdgerelateerd gehoorverlies (LGGV), is een objectieve elektrofysiologische gehoortest nodig die het hele hoorbare frequentiebereik bij de hond test. De meest gebruikte methode om het gehoor te testen in de diergeneeskunde is hersenstamaudiometrie (BERA), waarbij de "respons" op een klik, een korte akoestische stimulus, toegediend aan het gehoororgaan, als elektrische potentiaal wordt geregistreerd. Deze potentialen worden opgewekt in de hersenstam.[314] Klikken stimuleren een groot deel van de cochlea en zijn niet frequentiespecifiek, waardoor deze techniek niet bruikbaar is voor de diagnose van verkregen PGV. Een van de doelstellingen van dit proefschrift was om de klik-BERA dusdanig aan te passen dat een objectieve, volledige gehoortest gedaan zou kunnen worden bij honden om frequentiespecifieke gehoordrempels te kunnen bepalen in het gehele frequentiebereik en om referentiewaarden te verzamelen voor hersenstamrespons karakteristieken bij normale honden (***hoofdstuk 4***). Een tweede doelstelling was de audiometrische (***hoofdstuk 5***) en histologische (***hoofdstuk 6***) karakteristieken van LGGV in deze diersoort te onderzoeken en documenteren. Tevens werd de klinische uitvoerbaarheid (***hoofdstuk 7***) en toepasbaarheid (***hoofdstuk 8***) onderzocht van behandeling van deze typische vorm van slechthorendheid met elektrische gehoorprothesen in de vorm van middenoor implantaten. Informatie over de embryologie, macroscopische, microscopische en computer-tomografische anatomie en fysiologie van het hondenoor is beschreven in ***hoofdstuk 2***. Dit hoofdstuk dient tevens als referentie bij de beschrijvingen van de chirurgische benadering van het middenoor in dit proefschrift. Een overzicht van de BERA als diagnostische methode in de diergeneeskunde, alsmede van de huidige stand van kennis over LSH en LGGV bij de hond wordt gegeven in ***hoofdstuk 3***. De resultaten van de eerste vier hoofdstukken van dit proefschrift (hoofdstuk 4-7) worden hierin samengevat en besproken.

In ons laboratorium werd een methode ontwikkeld om toneburst stimuli (TS) aan de cochlea aan te kunnen bieden met een frequentiebereik van 1-32 kHz voor frequentiespecifieke evaluatie van het gehoor bij honden (***hoofdstuk 4***). Onze resultaten toonden aan dat de hoogste gevoeligheid van het hondenoor

ligt bij 12 en 16 kHz, wat sterk overeenkomt met de resultaten gevonden met gedragobserverende gehoortesten.[98]

De voor- en nadelen die inherent zijn aan het gebruik van de BERA met TS voor de bepaling van frequentiespecifieke gehoordrempels worden in detail bediscussieerd in ***hoofdstukken 3, 4 en 5***. Bij aanvang van dit onderzoek waren er verschillende mogelijkheden beschikbaar waaruit gekozen kon worden om frequentiespecifieke gebieden van de cochlea te testen, bijvoorbeeld door gebruik te maken van KS met maskering met "high-pass noise" of "notch noise" of door directe stimulatie met tonebursts. Aangezien van deze laatste methode was aangetoond dat deze relatief eenvoudig uitgevoerd kon worden en bovendien betrouwbare informatie over frequentiespecifieke gehoordrempels opleverde[45,63,133,188,263], werd deze methode gebruikt voor dit proefschrift. Een punt van zorg bij het gebruik van tonebursts is een fenomeen dat bekend staat als "spectral splatter".[208,243] Tengevolge hiervan zijn de BERA-afgeleide gehoordrempels bepaald met TS niet volledig bepaald door de respons van de neuronen van de centrale frequentie, maar ook door die van de "side-lobe" frequenties. Bij patiënten met gehoorverlies zou dit kunnen leiden tot een onderschatting van het gehoorverlies bij de nominale frequentie van de gebruikte toneburst.[243] Echter, afgaande op onze bevindingen en op de grote overeenkomst met de bevindingen van gedragobserverende gehoortesten, kunnen we concluderen dat betrouwbare en herhaalbare informatie over de frequentiespecificiteit van de cochlea bij honden verkregen kan worden met TS en deze publicatie omvat een database met parameters die nodig zijn om frequentiespecifiek gehoorverlies bij de hond te kunnen evalueren.

Een andere methode voor bepaling van frequentiespecifieke gehoordrempels bij de hond waarbij gebruik gemaakt wordt van "auditory steady-state potentials" werd recent beschreven.[152] Deze techniek lijkt een valide alternatieve methode te zijn voor het verkrijgen van frequentiespecifieke gehoordrempels bij honden. Echter, frequenties hoger dan 8 kHz werden in deze studie niet getest. Deze techniek kan mogelijk in de toekomst in de diergeneeskunde toegepast worden, indien de benodigde draagfrequenties beschikbaar zijn. Bij mensen wordt aangeraden om naast het meten van de hersenstamrespons voor evaluatie van het gehoor, ook otoacoustische emissies (OAEs) te meten.[122,258,303] OAEs zijn niet alleen eenvoudig te meten, maar tevens kunnen metingen van OAEs gebruikt worden als een objectieve methode voor evaluatie van de integriteit van de buitenste haarcellen. Met OAEs is ototoxiciteit eerder op te sporen dan met meting van de hersenstamrespons.[258] Bij honden zijn spontane en opgewekte OAEs gemeten en beschreven, maar er zijn niet veel studies beschikbaar en de klinische toepasbaarheid en bruikbaarheid van OAEs voor bepaling van frequentiespecifieke gehoordrempels moet in honden nog aangetoond worden.[246,257,258]

In het algemeen wordt aangenomen dat het gehoor van honden vermindert met toename van de leeftijd, er zijn echter niet veel studies beschikbaar die dit aantonen. Van LGGV wordt aangenomen dat het de meest voorkomende oorzaak van verkregen PGV is bij honden.[48,277] Er zijn studies beschikbaar die gehoorverlies bij oude honden beschrijven, maar de onderzoekers maakten gebruik van BERA met KS of met slechts een enkel stimulatieniveau.[131,132,240] Er zijn geen gepubliceerde data over gehoordrempels bij geriatrische honden met gehoorverlies gebruik makend van ofwel KS of TS. Wij stelden als hypothese dat het gehoor van honden bij het toenemen van de leeftijd verslechtert met toename van de leeftijd over het gehele frequentiebereik, zoals bij mensen; dat dit met name optreedt in het hoogfrequente gebied en dat dit kwantitatief kan worden aangetoond met BERA gebruik makend van TS. De bevindingen in dit proefschrift ondersteunen deze hypothese. In ***hoofdstuk 5*** worden de resultaten gepresenteerd van een dwarsdoorsnedenonderzoek en een longitudinaal onderzoek naar de leeftijdsgerelateerde veranderingen in de audiogrammen van honden, gebruik makend van dezelfde meetmethode als beschreven in hoofdstuk 4.

De belangrijkste conclusie die getrokken kon worden uit het dwarsdoorsneden-onderzoek was dat in de audiogrammen de gehoordrempels bij alle geteste frequenties significant hoger waren bij de geriatrische honden dan bij de jonge honden en honden van middelbare leeftijd en dat dit het meest waarschijnlijk het gevolg was van LGGV. Alhoewel de verschillen significant waren voor alle frequenties, werd de meest dramatische toename van gehoordrempels gezien bij de midden tot hoge frequenties (8-32 kHz), wat vergelijkbaar is met de bevindingen bij mensen met LGGV.[171,219] De audiogrammen van de honden in de longitudinale studie tonen eveneens een progressieve toename aan van de gehoordrempels bij het toenemen van de leeftijd. Het gehoorverlies begint significant te worden vanaf een leeftijd van 8-10 jaar en is het meest uitgesproken in het midden- tot hoogfrequente gebied (8-32 kHz). De gehoordrempels voor 8, 12, 16, 24 en 32 kHz, bleken op een gemiddelde leeftijd van 12 jaar significant ($P < 0.05$) hoger te zijn dan die op een gemiddelde leeftijd van 6 jaar, wat aantoont dat LGGV begint in het midden- tot hoogfrequente gebied bij honden. De gemiddelde toename van de gehoordrempels tussen de leeftijd van 10 en 12 jaar was ongeveer 10 dB voor 1, 2 en 4 kHz en varieerde tussen de 15 dB voor 8 kHz tot meer dan 22 dB bij 12, 16, 24 en 32 kHz. Dit verschil tussen lage en hoge frequenties wordt ook gezien in longitudinale studies naar LGGV bij mensen.[53,138] Uit deze studies blijkt dat een significant gehoorverlies optreedt vanaf een leeftijd van 60 jaar, dat begint in de hoge frequenties (6-16 kHz bij mensen) en zich geleidelijk uitbreidt over het hele frequentiegebied. Tussen de leeftijd van 70 en 80 jaar bedraagt het gehoorverlies ongeveer 1-2 dB per jaar, afhankelijk van de specifieke frequentie die getest wordt.[53,138] De toenamen in gehoordrempels die wij vonden bij

honden zijn een factor 10 groter dan die beschreven bij mensen. Dit zou ten dele kunnen worden verklaard door technische verschillen tussen de studies (om praktische redenen gebruikten wij stappen van 10 dB bij het bepalen van de gehoordrempels), en ten dele door verschillen tussen diersoorten. Vergeleken met mensen hebben honden een veel kortere levensverwachting. Het is daarom niet onaannemelijk dat een leeftijdgerelateerde vermindering van het gehoor bij honden sneller verloopt dan bij mensen.

Het dwarsdoorsnedenonderzoek toonde ook een significant hogere gehoorsdrempel aan bij 4 kHz in de groep middelbare honden, vergeleken met de groep met jonge honden. De gehoordrempels bij andere frequenties waren niet verschillend tussen deze twee groepen. Het lawaai in de kennels waar de honden gehouden werden tijdens de studie speelt hier mogelijk een rol in. Deze honden werden gedurende hun hele leven door hun eigen geblaf blootgesteld aan een hoog niveau van omgevingslawaai. LSH bij mensen treedt typisch op in het frequentiebereik tussen 2-6 kHz, waarbij de eerste veranderingen zich voordoen bij 6 kHz en de grootste gehoordrempelstijging uiteindelijk bij 4 kHz gezien wordt.[138,198] Dit suggereert dat de toename in de gehoordrempel bij 4 kHz in de groep met middelbare honden het gevolg zou kunnen zijn geweest van lawaaischade. Deze middelbare honden waren gebruikt voor het bepalen van normatieve data in het eerste onderzoek (hoofdstuk 4). Terugblikkend kunnen we deze honden niet langer beschouwen als gezonde, normaal horende honden, aangezien een significant hogere gehoordrempel werd gevonden bij 4 kHz vergeleken met de groep jonge honden. Echter, aangezien er een statistisch significant verschil gevonden werd voor alle frequenties tussen de geriatrische en jonge honden en in verloop van de tijd ook binnen de groep met middelbare honden, kunnen we concluderen dat LGGV voorkomt bij honden en dat het vergelijkbaar is met LGGV bij mensen. Het gehoorverlies kan aangetoond worden en de progressie van het verlies kan gevolgd worden door gebruik te maken van BERA na frequentiespecifieke toneburst stimulatie van de cochlea.

Onze conclusies zijn afkomstig van observaties bij een kleine groep middelgrote honden en zijn daarom niet noodzakelijkerwijs van toepassing op alle honden. Toekomstig onderzoek op grotere schaal bij honden, die niet gehuisvest zijn geweest in kennels of zijn blootgesteld aan lawaai, zal moeten worden uitgevoerd om de werkelijke prevalentie van deze aandoening te kunnen bepalen. Honden van grote rassen hebben een kortere levensduur dan honden van kleine rassen. Om verschillen in leeftijdsgebonden effecten op het gehoor tussen deze beide groepen te kunnen aantonen of uitsluiten, zullen vertegenwoordigers van zowel grote als kleine rassen moeten worden onderzocht. Voor toekomstig onderzoek is het wenselijk om niet alleen het gehoor te evalueren met BERA, maar ook met OAEs, zoals bovenstaand besproken.

Volgens Schuknecht kan de histologische vorm van LGGV afgelezen worden aan de vorm van het audiogram.[233] Leeftijdgerelateerd verlies van haarcellen en spirale ganglioncellen (SGCs) is beschreven bij honden.[118,132,234,240] Knowles et al. vonden verlies van SGCs in een groep dove honden waarbij gehoordrempels volledig afwezig waren[132] en Shimada et al. beschreven variabele verliezen van SGCs, en atrofie van het orgaan van Corti en de stria vascularis bij alle door hen onderzochte honden ouder dan 12 jaar, met name in de basale winding van de cochlea.[240] Hoewel deze laatste bevindingen suggereren dat leeftijdgerelateerd gehoorverlies van hoge frequenties bij honden berust op een gemengde histologische pathologie, vergelijkbaar met wat meestal wordt gevonden bij mensen met LGGV, werden frequentiespecifieke gehoordrempels niet bepaald. Aangezien wij met audiometrie bewijs hadden gevonden voor frequentiespecifieke leeftijdgerelateerde veranderingen in de gehoordrempels bij honden, stelden we in de vervolg hypothese dat de histologische veranderingen in de cochlea bij honden met LGGV ook vergelijkbaar zouden zijn met die bij mensen en dat de ernst van de histologische afwijkingen af te leiden zou zijn van de audiogrammen.

In ***hoofdstuk 6*** worden de resultaten van een onderzoek naar leeftijdsgerelateerde veranderingen in het slakkenhuis gerelateerd aan BERA-afgeleide gehoordrempels beschreven. Cochleaire afwijkingen werden bij alle bestudeerde geriatrische honden gevonden (n = 10). Niet alleen werd een significant verlies van SGCs gevonden in dit onderzoek, maar ook werden een verlies van buitenste haarcellen (BUHCs) en een reductie in de oppervlakte van de stria vascularis dwarsdoorsnede (SVDO) gevonden. De histologische afwijkingen speelden zich voornamelijk af in de basale windingen, wat consistent is met de bevinding dat de grootste gehoordrempel verschuivingen en de hoogste absolute door BERA bepaalde gehoordrempels werden gevonden in het midden- tot hoogfrequente gebied (8-32 kHz). Wij concludeerden dat de in de basale winding waargenomen degeneratie van de BUHCs en SGCs primair verantwoordelijk was voor de hogere gehoordrempels, vergelijkbaar met de situatie bij mensen.[80,183] De SVDO bij geriatrische honden bleek kleiner te zijn in alle windingen van de cochlea, wat vergelijkbaar is met de bevindingen bij gerbils, waar degeneratie van de stria vascularis vroeg optreedt in het kader van LGGV en meestal begint aan zowel de basis als de punt van de cochlea en zich met toenemende leeftijd geleidelijk aan uitstrekt naar het middengebied van de cochlea.[259] Alhoewel een reductie in SVDO in alle windingen zou kunnen verklaren waarom we gehoorverlies bij geriatrische honden vonden in het gehele frequentiebereik, kan het niet het verschil verklaren tussen de mate van verlies in het lage- versus het midden- tot hoogfrequente gebied. Gemengde pathologie van zowel BUHC verlies, SGC verlies en reductie in SVDO lijkt dan ook een betere verklaring te zijn voor de audiometrische bevindingen. Onze

hypothese dat de cochleaire afwijkingen vergelijkbaar zouden zijn met die bij mensen met LGGV, bij wie in het merendeel van de gevallen een gemengd histologisch type wordt gevonden[189], werd daarom aangenomen.

Ondanks de stelling van Schuknecht dat de histologische vorm van LGGV kan worden afgelezen aan de vorm van het audiogram[233], is het tot op heden ondanks veelvuldig onderzoek niet mogelijk gebleken om een nauwe samenhang tussen audiometrische data en histologische afwijkingen in de cochlea aan te tonen.[35,182] De resultaten van ons onderzoek zijn hiermee in overeenstemming. De gehoordrempels die we in onze honden vonden, gaven niet aan of de histologische afwijkingen primair de haarcellen betrof, de spirale ganglioncellen of the stria vascularis. Dit wordt uiteraard mede veroorzaakt door het feit dat er in alle gevallen een gemengd verlies gevonden werd. In het algemeen kon uit de individuele audiogrammen echter wel afgelezen worden wat de ernst en de lokalisatie van de afwijkingen in de cochlea waren. In het algemeen waren de afwijkingen in de cochlea het meest uitgebreid bij honden met het ernstigste gehoorverlies. We concludeerden daarom dat audiogrammen met door BERA bepaalde gehoordrempels, gebruik makend van TS, gebruikt kunnen worden voor de diagnose en karakterisering van LGGV bij honden. Zij geven niet alleen een indicatie van de ernst van het gehoorverlies, maar ook van de uitbreiding en lokalisatie van de gemengde histologische afwijkingen in de cochlea. Deze informatie is essentieel voor de planning van de behandeling van LGGV met bijvoorbeeld hoortoestellen of middenoor- of binnenoorimplantaten.

Gedetailleerd elektronenmicroscopisch onderzoek van de stria vascularis en de SGCs zal moeten worden uitgevoerd om verder licht te werpen op het exacte karakter van de gevonden microscopische afwijkingen in de cochlea's van onze geriatrische honden, zoals bijvoorbeeld de overvloedige lipofuscine insluitsels en intracellulaire vacuoles in de overgebleven SGCs en om het belang daarvan de bepalen. Bovendien mag niet vergeten worden dat de hersenen en daarmee het centrale gehoorsysteem ook leeftijdgerelateerde veranderingen ondergaan[312], alhoewel er wel consensus is dat de primaire leeftijdgebonden afwijkingen die leiden tot LGGV zich afspelen in de cochlea.[146] Neurofysiologisch onderzoek van de neuronen van de nucleus cochlearis (in de pons) hebben aangetoond dat sprake is van een reductie in glycine-gemedieerde inhibitie, wat zich uit in een verhoogde vuurfrequentie van deze neuronen bij oude dieren in vergelijking met die van jong volwassen dieren. Daarnaast is ook aangetoond dat het aantal neuronen in de cochleaire kern kan verminderen tengevolge van veroudering van de hersenen zelf, alsmede door verlies van input vanuit de cochlea door leeftijdgerelateerde veranderingen in met name de basale windingen van de cochlea.[312] Vergelijkbaar hiermee werd bij honden ouder dan 10 jaar neuronen verlies in de cochleaire kernen gevonden, alsmede astrogliosis en afzetting van ubiquitine in deze kernen.[240] Daarnaast zijn ook nog leeftijdgerelateerde veranderingen in

GABA synthese en afgifte gevonden in hogere hersencentra.[29] Of deze veranderingen het gevolg zijn van veroudering van de centrale delen zelf of secundair zijn aan afwijkingen in de perifere organen en of ze ook voorkomen bij honden, zal in toekomstig onderzoek bekeken moeten worden.

De behandeling van LGGV is belangrijk voor de levenskwaliteit van oudere mensen en daarom zijn moderne hoortoestellen, middenoor- en binnenoorimplantaten ontwikkeld om de communicatie mogelijkheden van ouderen met gehoorverlies te verbeteren.[7,80] De meeste mensen met LGGV kunnen adequaat geholpen worden met een conventioneel gehoorapparaat, het gebruik hiervan bij honden is slechts anekdotisch vermeld[155] en is klinisch niet erg succesvol gebleken. Mensen met mild tot ernstig PGV die geen voordeel kunnen behalen met een conventioneel gehoorapparaat, kunnen soms sterk geholpen worden met implanteerbare gehoorprothesen.[72] Tot nu toe zijn er geen studies gedaan naar het gebruik van dergelijke implantaten bij honden met gehoorverlies. Van de beschikbare mogelijkheden lijken de volledig implanteerbare modellen het meest geschikt te zijn, aangezien problemen als verlies van het apparaat, afsluiting van de uitwendige gehoorgang en het induceren van gehoorgangontsteking minder waarschijnlijk optreden met deze implantaten dan met niet geïmplanteerde en in de gehoorgang geplaatste modellen.[7,72] Bij aanvang van dit onderzoek waren volledig implanteerbare modellen echter niet beschikbaar, waardoor semi-implanteerbare modellen moesten worden gebruikt. Het is overigens wel te verwachten dat in de nabije toekomst deze semi-implanteerbare modellen verder zullen worden ontwikkeld tot volledig implanteerbare modellen.

De Vibrant Soundbridge (VSB) is een actieve, semi-implanteerbare, middenoor gehoorprothese die al enkele jaren beschikbaar is in Europa en waarover al verschillende studies zijn gepubliceerd die zijn effectiviteit aantonen.[252,268,290] De VSB bestaat uit verschillende onderdelen: een uitwendige audioprocessor (AP) en een implanteerbaar deel, genaamd “vibrating ossicular prosthesis” (VORP). Het actieve vibrerende onderdeel van de VORP is een kleine electromagnetische transducer, genaamd de “floating mass transducer” (FMT). Verschillende chirurgische benaderingen voor implantatie van de VSB zijn beschreven, waarna meestal koppeling van deze FMT plaatsvindt aan het lange been van de incus om een directe aansturing van de gehoorbeentjes keten te krijgen.[66,72] Het bleek echter onmogelijk te zijn om bij honden deze koppeling te bewerkstelligen, aangezien er in vergelijking met mensen te weinig ruimte voor de FMT is in het dorsale epitympanum. Daarnaast was door de grootte en vorm van de incus een betrouwbare permanente koppeling met het bestaande titanium klemmetje niet mogelijk. Plaatsing van de FMT op het ronde venstermembraan was echter bij mensen beschreven, als een alternatief voor koppeling aan de incus.[36] Dierproeven hebben de bruikbaarheid van stimulatie

via het ronde venster bewezen[260] en om die reden werd besloten om bij honden te onderzoeken wat de uitvoerbaarheid was van implantatie van de VSB met fixatie van de FMT op het ronde venstermembraan. Wij stelden als hypothese dat het technisch mogelijk moest zijn om de VSB te implanteren in honden door gebruik te maken van een laterale benadering naar de middenoorholte.

De resultaten van dit onderzoek naar de uitvoerbaarheid van de procedure worden beschreven in ***hoofdstuk 7***. De experimentele procedure kon veilig eenzijdig worden uitgevoerd in drie honden. Er traden hierbij geen intra-operatieve complicaties op en met een postoperatieve CT-scan kon in alle drie de honden worden aangetoond dat de FMT zich op de juiste plaats in de ronde vensternis bevond, waarna deze honden werden geeuthanaseerd. De hypothese dat de implantatie procedure technisch mogelijk was bij honden zonder intra-operatieve complicaties werd daardoor aangenomen. Er was gekozen voor een laterale benadering naar de middenoorholte, in tegenstelling tot de meer gebruikelijke ventrale benadering bij honden, omdat met deze benadering de implantatie van de FMT in de middenoorholte en de fixatie van de VORP in de spierfascie in één procedure uitgevoerd konden worden zonder herpositionering van de patiënt.

We stelden als vervolg hypothese dat de beschreven techniek ook uitvoerbaar zou zijn bij honden met LGGV, dat de incidentie laag zou zijn van postoperatieve complicaties zoals vertraagde wondgenezing, wondinfectie, het breken van de verbindingsdraad, dat het rest-gehoor niet zou verminderen door de procedure en dat het mogelijk zou zijn om een verbetering van het gehoor aan te kunnen tonen. Om deze hypothese te verifiëren, kregen drie honden met LGGV eenzijdig een VSB geïmplanteerd en werd het postoperatieve verloop gedurende drie maanden bestudeerd. De resultaten van deze trial zijn beschreven in ***hoofdstuk 8***. Er traden geen intra-operatieve of postoperatieve complicaties op, en het postoperatieve herstel verliep probleemloos bij alle drie de honden, met uitzondering van een tijdelijke nervus facialis paralyse bij twee honden. Zoals ook bij mensen beschreven, konden er geen significante verschillen gevonden worden tussen de via luchtgeleiding bepaalde pre- en post-operatieve met BERA bepaalde gehoordrempels, waarmee aangetoond werd dat de implantatie zelf geen negatief effect had gehad op het gehoor. De werkzaamheid van de implantaten werd geëvalueerd met de techniek van auditory steady-state evoked potentials. Bij mensen wordt de versterking door het implantaat gemeten met deze techniek[206,251,255] omdat auditory steady-state responses (ASSRs) opgewekt kunnen worden met simpele AM tonen, die niet alleen frequentiespecifiek, maar stabiel in de tijd zijn en vergeleken met korte, voorbijgaande stimulaties, minder gestoord worden door de versterking in het implantaat.[206] De toepasbaarheid van ASSRs bij honden voor beoordeling van het gehoor is recent beschreven, waarbij gebruik gemaakt werd van

draagfrequenties tot 8 kHz.[152] Deze techniek werd daarom in dit onderzoek gebruikt om de door het implantaat verbeterde gehoordrempels te bepalen. Omdat het voor gebruik bij de mens ontwikkelde VSB middenoor implantaat een frequentiebereik heeft van 250 Hz tot 7 kHz, was het voor het bepalen van de werkzaamheid niet noodzakelijk om frequenties van 8 kHz of hoger te testen. Dit implantaat laat versterking toe van alleen die frequenties die in het spraakverstaan voor mensen van belang zijn en moet dus niet gezien worden als een vervanging voor de verliezen in het midden tot hoog frequente gebied zoals gevonden bij honden met LGGV. Echter, versterking van het eveneens significant verminderd gehoor van lage frequenties, werd gezien als belangrijkste doel van dit onderzoek, aangezien hiermee de communicatie tussen hond en eigenaar zou kunnen verbeteren. ASSR-afgeleide gehoordrempels werden bepaald voor 1, 2 en 4 kHz, en deze bleken significant progressief te verbeteren met toenemende versterking van de AP, bij alle drie de honden, wat de juiste werkzaamheid van de implantaten aantoont. Echter, alleen de gehoordrempels die bepaald werden met de maximale versterkingsstanden van de AP, zijn lager dan de postoperatieve BERA-afgeleide gehoordrempels. Afhankelijk van de frequentie en de hond die werd getest, kon een maximale drempelverlaging van 20 dB gevonden worden. Hoewel de drempelverlaging bij mensen niet zo groot is als na koppeling van de FMT aan de incus, en maximaal zo'n 35 dB kan bedragen[251,255], zijn onze resultaten vergelijkbaar met die bij mensen na implantatie van de FMT op het ronde venstermembraan.[14,145] Wij accepteerden daarom onze hypothese en concludeerden dat implantatie van de VSB met plaatsing van de FMT op het ronde venstermembraan bij deze kleine groep honden met LGGV kan worden beschouwd als een veilige chirurgische methode zonder significante complicaties of veranderingen in het postoperatieve overgebleven gehoor. De implantaten bleken alle goed te functioneren en bewerkstelligden bovendien een duidelijke verbetering in het gehoor wanneer de maximale versterking van de AP gebruikt werd.

De drie Beagles die dit laatste onderzoek ondergingen, accepteerden allen zonder problemen de uitwendig gedragen AP en deden geen pogingen om deze te verwijderen door te schudden met de kop of er met de poten aan te krabben. Deze honden hadden echter hun gehele leven in de kennels van onze onderzoeksinstelling doorgebracht en waren niet gesocialiseerd. Het was daarom niet mogelijk om op basis van evaluatie van hun gedrag te bepalen of het gehoor door de implantatie was verbeterd, aangezien zij nooit hadden geleerd op vocale bevelen te reageren. Of in huis gehouden dieren met LGGV de AP zullen accepteren, zal in vervolgonderzoek moeten worden bekeken. Of deze dieren geneigd zijn de AP te verliezen, die alleen door magnetische aantrekkingskracht op de kop wordt gefixeerd, tijdens normaal gebruik in een natuurlijke omgeving en eventuele oplossingen voor dit probleem indien dit

zich voordoet, dient ook nader onderzocht te worden. Een van de mogelijke oplossingen zou kunnen liggen in het door het dier laten dragen van een halsband met een grote magneet die de AP zou kunnen aantrekken en fixeren indien deze van zijn plaats op de kop afkomt, zodat hij niet in de omgeving verloren gaat. De huidige kosten van het implantaat, de implantatie, postoperatieve controles van het gehoor en aanpassingen van de AP liggen in de orde van grootte van 10.000 euro. Een duidelijk verbeterd gehoor leidend tot een substantiële verbetering in eigenaar-patiënt communicatie zal moeten worden aangetoond om een dergelijke financiële investering door de eigenaar te rechtvaardigen. Tenslotte zal voor het verkrijgen van bevredigende resultaten bij honden met een PGV groter dan 20 dB, een krachtigere AP gebruikt moeten worden dan op dit moment beschikbaar is.

Verdere vooruitgang in de ontwikkeling van de chirurgie aan het hondenoor

De in dit proefschrift beschreven chirurgische benadering van de middenoorholte, waarbij de gehoorgang zelf niet geopend of opgeofferd wordt, kan op zichzelf ook toepassing vinden in de gezelschapsdieren chirurgie, bijvoorbeeld voor de behandeling van primaire secretoire middenoorontsteking bij de Cavalier King Charles Spaniël, voor het nemen van grote biopsieën van afwijkingen in het middenoor of voor reconstructie van afwijkingen aan de middenoorbeentjes keten. De bereikbaarheid van het ronde venster op deze wijze maakt het ook mogelijk om binnenoorimplantaten te plaatsen met dezelfde benadering van de middenoorholte.

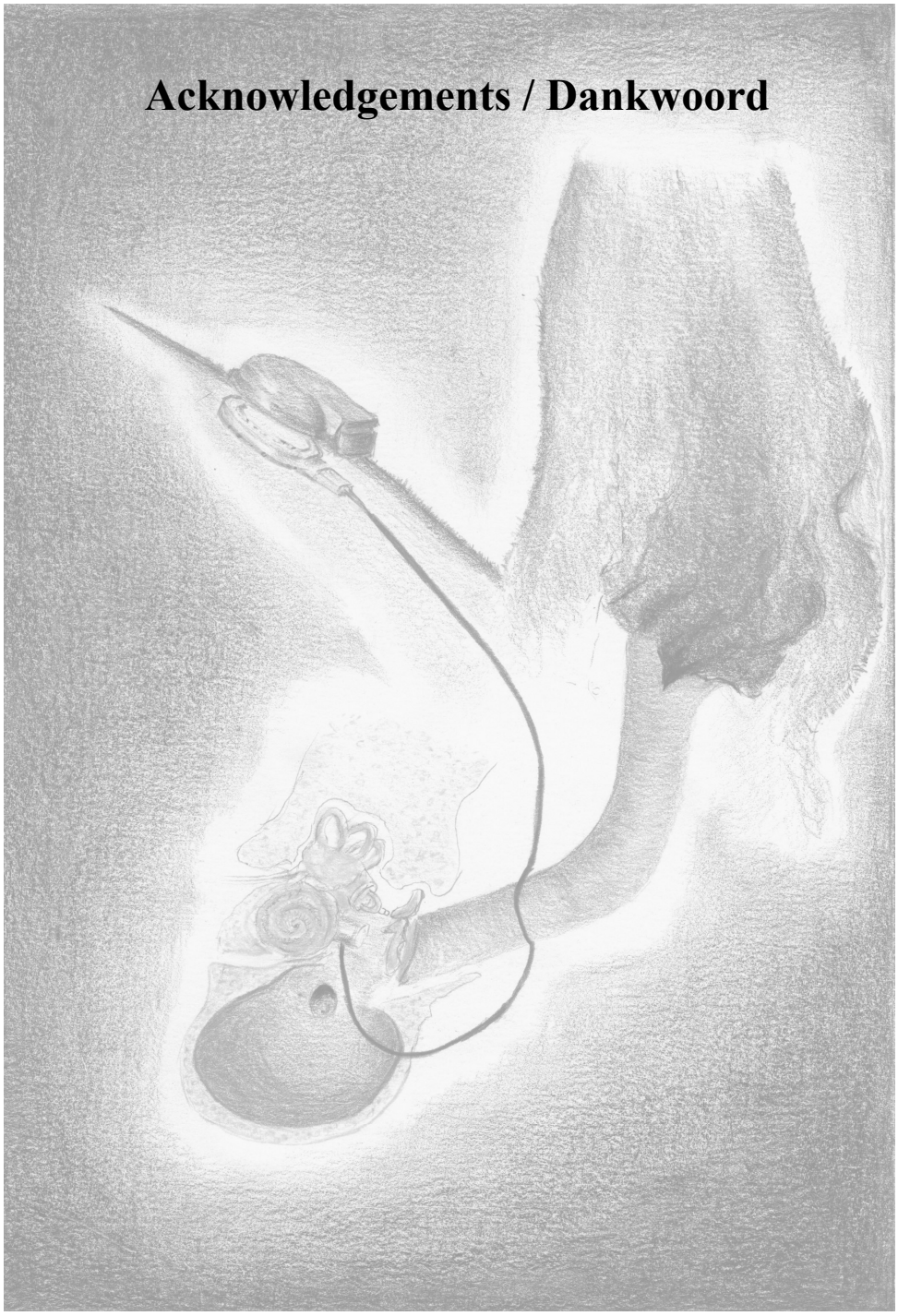
De enorme vooruitgang van de hoortoestellentechnologie in de audiologische geneeskunde van de laatste 20 jaar zal ook meer en meer zijn afspiegeling vinden in de veterinaire keel-, neus-, en oor-heelkunde. Spannende nieuwe ontwikkelingen zijn te verwachten in de geneeskunde van het gezelschapsdier in de nabije toekomst, nu de toepassing van implantatie van de VSB al op het punt staat om in klinische trials geëvalueerd te worden. Niet alleen zal de klinische toepasbaarheid van dit implantaat in de diergeneeskunde moeten worden onderzocht. Wanneer de moderne diagnostische mogelijkheden stelselmatig en op correcte wijze worden toegepast zullen er patiënten gevonden worden die baat hebben bij bot-verankerde hoortoestellen, binnenoorimplantaten of zelfs hersenstamimplantaten. De grote overeenkomsten in audiometrische en histologische karakteristieken van LGGV tussen honden en mensen in beschouwing nemend, moet geconcludeerd worden dat de hond een uitstekend model kan zijn voor toekomstig onderzoek naar LGGV en mogelijk andere vormen van PGV bij mensen. Niet alleen zou dergelijk onderzoek een aanzienlijke verbetering van het kennis- en

behandelings-niveau van LGGV bij honden tot gevolg hebben, maar het relatieve gemak waarmee histopathologische evaluatie van behandelingen kan plaatsvinden bij honden, zou ook van groot nut kunnen zijn voor de ontwikkeling van de juiste behandelingsprotocollen bij mensen.

Conclusies

LGGV is de meest voorkomende vorm van verkregen PGV bij honden, maar heeft tot nu maar weinig aandacht gekregen in de diergeneeskundige literatuur. Dit proefschrift beschrijft de diagnostiek en de audiometrische karakteristieken van LGGV, gebruik makend van BERA met frequentiespecifieke toneburst signalen. Tevens worden de leeftijdgerelateerde histologische karakteristieken van de cochlea beschreven en gerelateerd aan de BERA-afgeleide gehoordrempels. De uitvoerbaarheid en toepasbaarheid van behandeling van LGGV met een VSB middenoor implantaat werd onderzocht in een klinisch experiment.

Acknowledgements / Dankwoord



Velen hebben een bijdrage geleverd aan niet alleen het tot stand komen van dit proefschrift, maar ook aan mijn persoonlijke vorming en ontwikkeling tot specialist KNO. Wat heb ik veel geleerd gedurende dit proces! En wat valt er nog veel te ontdekken… Ik wil iedereen hartelijk bedanken die getracht heeft om me wijzer te maken, me inzicht bij te brengen of mijn onderzoek op welke wijze dan ook heeft ondersteund, ook diegenen die niet specifiek in dit dankwoord worden genoemd.

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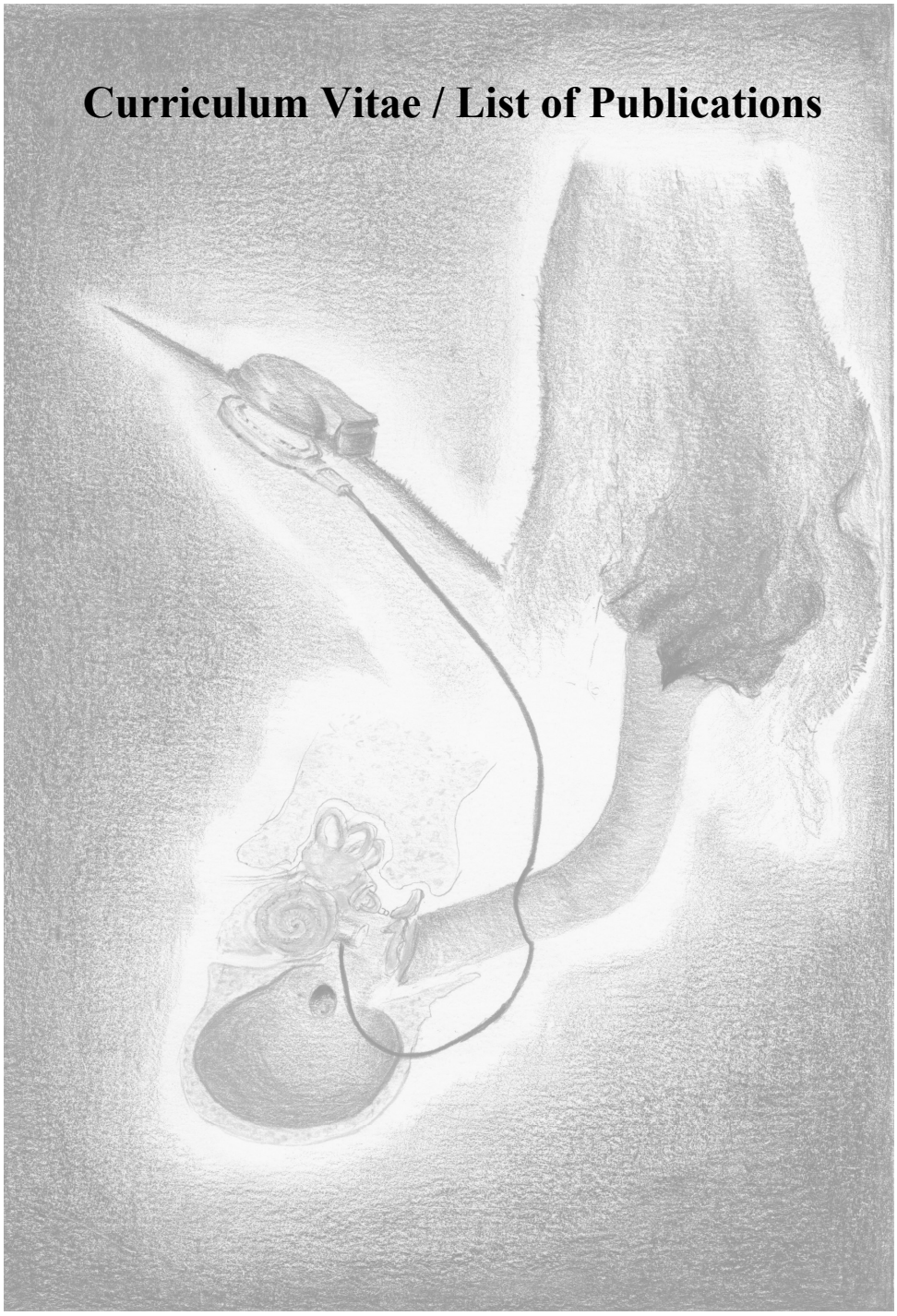
Curriculum Vitae / List of Publications



Curriculum Vitae

Gert ter Haar was born in Eck en Wiel, the Netherlands on the 3rd of February 1972. After following a classical secondary education with Latin and Greek at the Revius Lyceum in Doorn, he studied veterinary medicine at the Faculty of Veterinary Medicine, Utrecht University, The Netherlands and graduated in February 1997 with a differentiation in Small Animal Medicine and Surgery. After having worked for a few months in private practice and being involved in the State Swine Fever Control program, he gratefully accepted a Clinical Rotating Internship position at the Department of Clinical Sciences of Companion Animals from June 1997 – August 1998, at this university, followed by a Residency in Small Animal Surgery from September 1998 – September 2001 at the same Department. Since September 2001, he is a member of the staff at this Department as Assistant Professor in Ear-, Nose-, and Throat- (ENT) diseases and diseases of the upper airways. In July 2002 he passed the surgical specialist certifying examination held in Vienna and became a Diplomate of the European College of Veterinary Surgeons. He became Head of the Department of ENT in February 2003 and secretary of the International Veterinary Ear-, Nose- and Throat- Association (IVENTA) in July 2003. The Basic Qualification for Teaching (BKO) at universities was obtained in July 2004. While teaching, and doing clinical work in the field of ENT-diseases, he gave over 100 national and international lectures on ENT medicine, surgery and hearing research in dogs.

Gert ter Haar werd geboren in Eck en Wiel, Nederland, op 3 februari 1972. Na het doorlopen van het gymnasium aan het Revius Lyceum in Doorn, studeerde hij diergeneeskunde aan de Faculteit Diergeneeskunde van de Universiteit Utrecht, Nederland en studeerde af in februari 1997 met de differentiatie gezelschapsdieren. Na een paar maanden waarnemingen te hebben gedaan in de gezelschapsdierenpraktijk en betrokken te zijn geweest bij de varkenspest-bestrijding nam hij dankbaar de kans waar om een roulatie te volgen bij het Departement Geneeskunde van Gezelschapsdieren van deze universiteit van juni 1997 tot en met augustus 1998. Dit werd gevolgd door een opleiding tot specialist chirurgie gezelschapsdieren bij hetzelfde departement van september 1998 tot september 2001. Sinds september 2001 is hij stafmedewerker bij het departement en werkzaam als junior dierenarts – onderzoeker – docent in het vakgebied van de Keel-, Neus- en Oor-Heelkunde (KNO). In juli 2002 slaagde hij voor het Europese specialisten examen dat gehouden werd in Wenen en werd daarmee Diplomate of the European College of Veterinary Surgeons. Hij werd hoofd van de afdeling KNO in februari 2003 en secretaris van de International Veterinary Ear-, Nose-, and Throat-Association (IVENTA) in juli 2003. De basis kwalificatie onderwijs werd

behaald in juli 2004. Terwijl hij klinisch werkzaam was in de KNO en doceerde in dit vakgebied, gaf hij meer dan 100 nationale en internationale lezingen over de KNO-geneeskunde en heelkunde en gehooronderzoek bij honden.

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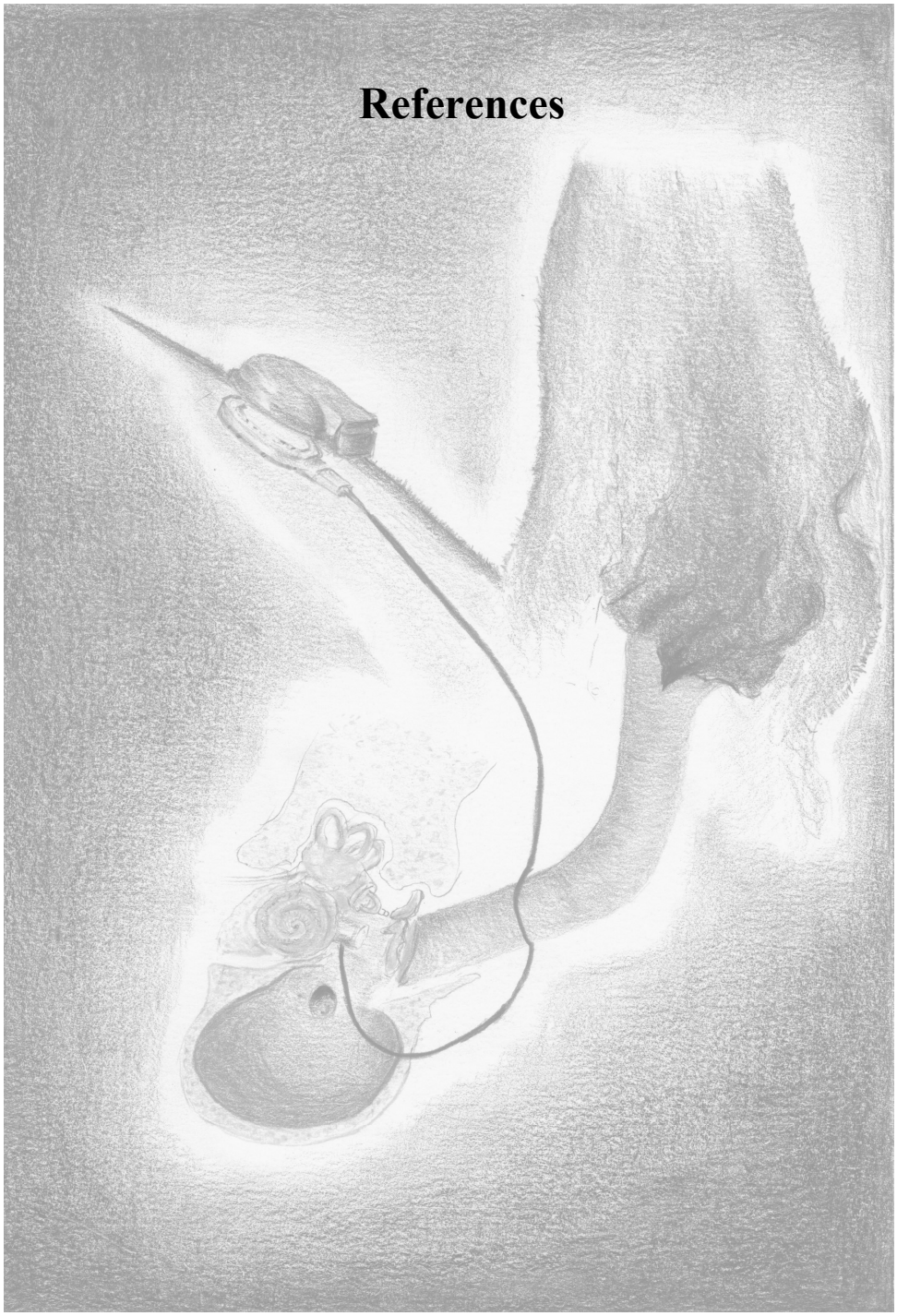

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