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Congenital sensorineural deafness in client-owned pure-breed white cats

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... to my family…

LIST OF ABBREVIATIONS

ABR	auditory brainstem response
AEP	acoustically evoked potentials
B	bel
BAEP	brain stem auditory evoked potential
BAER	brain stem auditory evoked response
c	pink-eyed albino pigment gene
c^a	blue-eyed albino pigment gene
c^b	Burmese dilution albino pigment gene
c^s	Siamese dilution albino pigment gene
c-Kit	tyrosine kinase receptor gene
C	albino gene
CI	confidence interval
CN	cranial nerve
CNS	central nervous system
CSD	congenital sensorineural deafness
Cz	BAER recording electrode placed at vertex
dB	decibel
dBA	decibel, A-weighted
Dipl ACVIM	Diplomate of American College of Veterinary Internal Medicine
Dipl ACVR	Diplomate of American College of Veterinary Radiology
Dipl ECVN	Diplomate of European College of Veterinary Neurology
DFN	x-linked deafness locus
DFNA	autosomal dominant deafness locus
DFNB	autosomal recessive deafness locus
DNA	deoxyribonucleic acid
Dr. med. vet.	Doctor of veterinary medicine
DVM	Doctor of veterinary medicine
et al.	*et alii* - and others
EAEP	early acoustic evoked potential
EDN3	endothelin 3
EDNRB	endothelin receptor type B
GJB2	gap junction protein, beta 2, 26k (connexin 26)

habil.	*habilitatus*
HL	hearing level
Hz	hertz
IHC	inner hair cells
IV	intravenously
kg	kilogramme
kHz	kilohertz
kOhm	kiloohm
KIT	tyrosine kinase receptor gene
KITLG	KIT ligand protein coding gene
LLAEP	long-latency auditory evoked potentials
LMU	Ludwig-Maximilian University
mg	milligramme
ml	millilitre
mm	millimetre
msec	microsecond
mV	microvolt
µPa	micropascal
M	merle gene
MAS	Master of Arts in American Studies
MITF	microphthalmia-associated transcription factor
MLAEP	middle-latency auditory evoked potentials
n	number
nHL	normal hearing level
nm	nanometre
NSAIDs	non-steroidal anti-inflammatory drugs
OHC	outer hair cells
pSPL	peak sound pressure level
P, p	probability value
Pa	Pascal
PAX3	paired box gene 3 (Waardenburg syndrome 1)
S	piebald gene (white spotting gene)
SOX10	SRY (sex determining region Y)-box 10
SC	subcutaneously
SL	sensation level

SPL	sound pressure level
SLUG	zinc finger protein gene
TIP	tubal insert phones
w	self allele of white gene
w^m	much spotted allele of white gene
w^l	little spotted allele of white gene
W	white gene
W^h	high degree of piebald allele of white gene
WS	Waardenburg syndrome

I Introduction

Hereditary deafness has been studied in different animal species in which characteristic features of pigment abnormalities are associated with hearing loss.
The congenitally deaf white cat is one of several animals that exhibits a syndrome of sensorineural hearing loss associated with pigmentary disorders. The first report describing the relationship between white pigmentation and deafness in cats was presented in the first half of the nineteenth century (BREE, 1829). Since then, the interest in this relationship has been increasing steadily, resulting in numerous studies (BOSHER and HALLPIKE, 1965; BERGSMA and BROWN, 1971; MAIR, 1973; PUJOL et al., 1977; REBILLARD et al., 1981a, 1981b; DELACK, 1984; HEID et al., 1998), especially because the congenitally deaf, mixed-breed white cat has been used as an animal model of human deafness. The molecular mechanisms for deafness in white cats are not known; many studies have suggested that the animals are a feline homologue of the human Waardenburg syndrome (BERGSMA and BROWN, 1971; MAIR, 1973; SCHWARTZ and HIGA, 1982; DELACK, 1984).
Congenital sensorineural deafness (CSD) has been described in cat breeds in which the dominant autosomal white gene (W) is segregating. Progressive cochleo-saccular degeneration, resembling Scheibe deformity in humans, is commonly associated with the W gene and causes complete congenital sensorineural deafness in white cats (BOSHER and HALLPIKE, 1965; MAIR, 1973). Interestingly, partial deafness and various types of inner ear degeneration have been reported in some experimental studies of white cats in a setting that is difficult to reconcile with the W gene (REBILLARD et al., 1981a; HEID et al., 1998; RYUGO et al., 1998, 2003). These studies were based on observations in experimental cats in which inbreeding may be assumed to be much greater than in pure-breed cats. Moreover, not all pure-breed white cats necessarily carry the W gene because there are several ways for cats to exhibit a white coat (PEDERSEN, 1991). The dominant white gene (W) currently is present in 17 registered cat breeds, but to the author’s knowledge, up to now, there has been no study that describes the prevalence of deafness among pure-breed client-owned cats. The objective of this study was to provide data on the hearing status and occurrence of unilateral and bilateral CSD in client-owned pure-breed white cats presented for hearing assessment.

II Literature review

1. Auditory pathway- anatomy and physiology

1.1 Anatomy of the ear

The ear is a highly complex sensory organ responsible for the sense of hearing and for vestibular control of posture and eye movements. The ear consists of three compartments: the outer ear, including the pinna (auricula) and the external ear canal down to the tympanic membrane; the middle ear, including the three ossicles (malleus, incus, and stapes) and the connection to the pharynx (the auditory canal), and the inner ear, which includes the vestibule, three semicircular canals, and the cochlea (Figure 1). The cochlea is the sensory organ for hearing and is encased within the bony labyrinth of the petrous temporal bone. It includes the scala vestibuli and scala tympani, which are canals filled with perilymph, a fluid that communicates directly with the cerebrospinal fluid of the subarachnoid space. In cats, the cochlea is rolled-up in a snail form with two and a half turns around the modiolus (KOCH and BERG, 1997).

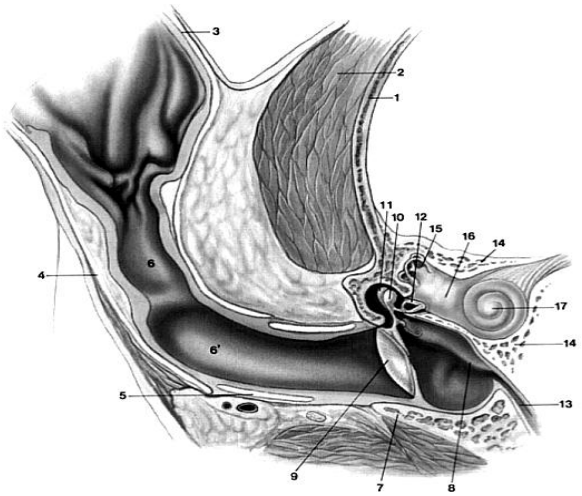

Figure 1 Anatomy of the ear (cat) – transversal cut, frontal view.

1 *Skullcap* ***2*** *M. Temporalis* ***3-6`****: Auris externa* ***3, 4****: Cartilago auriculae* ***3*** *Scapha* ***4*** *Concha* ***5*** *Cartilago meatus acustici externi* ***6*** *Meatus acusticus externus - vertical part* ***6`*** *Meatus acusticus externus - horizontal part* ***7 - 12****: Auris media* ***7*** *Bulla tympanica* ***8*** *Septum bullae* ***9*** *Membrana tympani* ***10 - 12*** *Ossicula auditus* ***10*** *Malleus* ***11*** *Incus* ***12*** *Stapes* ***13*** *Tuba auditiva* ***14*** *Pars petrosa ossis temporalis* ***15 – 17*** *Labyrinthus osseus* ***15*** *Canales semicirculares ossei* ***16*** *Vestibulum* ***17*** *Cochlea* (from HUDSON and HAMILTON, 1993)

The cochlear duct is a coiled portion of the membranous labyrinth that lies within the cochlea and is filled with endolymph. The scala media is a part of the

membranous labyrinth and is filled with endolymph. The basilar membrane separates the scala vestibuli and scala media.

The organ of Corti, the sensory receptor of hearing, lies on the basilar membrane. On the surface of the organ of Corti lie inner hair cells (IHC) and outer hair cells (OHC) that synapse with the spiral ganglion for transmission to the cochlear nerve. The hair bundle is located at the apical surface of the IHC and OHC. It is made up of 20-300 actin-filled stiff microvilli, the stereocilia, which contain the mechano-electrical machinery, and a single cilium, the kinocilium, which is not present in mature cochlear hair cells. Actin filaments are uniformly polarized, the fast growing ends being located at stereocilia tips. Stereocilia in mature hair bundles can, in some species, contain up to 2000 actin filaments (REVENU et al., 2004). The hair bundle is an exquisitely sensitive oscillation detector, as movements as small as 1 nm result in hair potential changes (ROBLES and RUGGERO, 2001). Auditory hair cells project into the gelatinous tectorial membrane of the scala media. The tectorial membrane is necessary for focusing the mechanical oscillations of the sound stimulus onto the sensory hair bundle. Protein-protein interactions appear to anchor the tectorial membrane to sensory and supporting cells of the organ of Corti. There is a vascular bundle on the outer wall of the scala media called the stria vascularis, which is responsible for the production of endolymph (Figure 2).

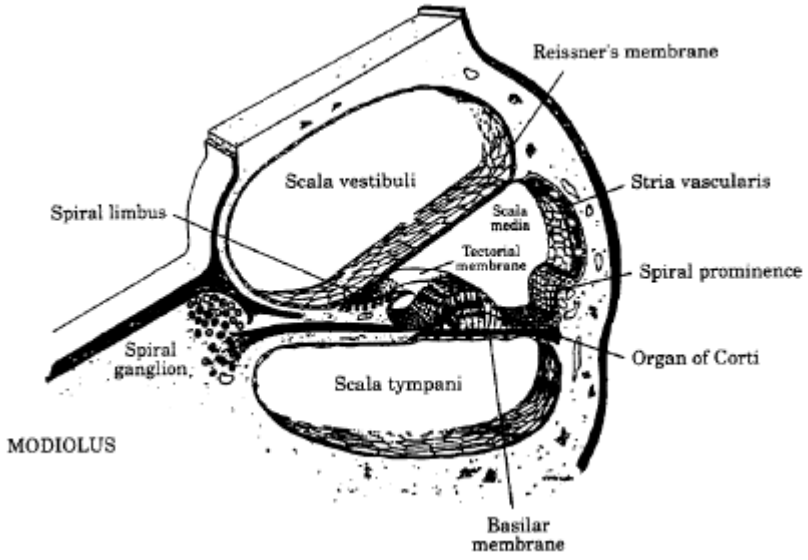

Figure 2 Cross-section of the cochlea. (from BLOOM and FAWCETT, 1975).

Endolymph contains a high concentration of potassium and a low concentration of sodium, which is exactly the opposite of perilymph. As a result of the differences

in ionic composition between the compartments, the potential difference between endolymph and perilymph is about + 80 mV. This positive potential is the largest found in the body. Since the intracellular resting potential of hair cell receptors is around – 70 mV, the potential difference across the hair cell apex is a remarkable 150 mV. This large potential difference represents a tremendous ionic force and serves as the engine driving the mechanoelectrical transduction process of the hair cell (DE LAHUNTA, 1983; KING, 1987; GUYTON, 1991; BAGLEY, 1996; COOK, 2004; EISEN and RYUGO, 2007).

1.2 Physiology of normal hearing

Normal mammalian auditory function relies on two broad categories of function: mechanical and electrochemical. In a normal functioning auditory system, air vibrations are presented to the inner ear via the auditory canals and, subsequently, are interpreted by the nervous system as sound. Several cellular and extracellular specializations within the cochlea function to decompose the mechanical stimulus of sound into its frequency components and to convert these stimuli into an electrochemical signal conveyed by discharges along auditory nerve fibers.

The pinna gathers in sound vibrations to the external ear canal, which serves to direct the vibrations toward the air-filled middle ear.

The middle ear consists of the tympanic membrane and bony ossicles along with ligaments and muscles that coordinate their function. The sound vibrations from the external ear canal are then transmitted through the tympanic membrane to the ossicles, which articulate to amplify sound and transmit vibrations to the fluid-filled inner ear. Thus the major function of the middle ear is to match relatively low impedance airborne sounds to the higher impedance fluid of the inner ear. The foot plate of the stapes connects through the oval window with the cochlear ducts. As sound vibrations pass through the middle ear and the stapes pushes the oval window inward, the perilymph within the scala vestibule is compressed and the basilar membrane is deflected.

The stiffness gradient of the basilar membrane along the length of the cochlea functions like a bank of frequency band-pass filters aligned from the highest frequency at the cochlear base to the lowest frequency at its apex. Deflection of the basilar membrane means that the stereocilia within hair bundles are displaced relative to each other. This displacement puts the tip links under tension and pulls open cation channels. Driven by the endocochlear potential, the flow of cation

depolarizes the hair cells membrane potential. The intracellular processes in response to these changes in membrane potentials differ considerably between the two types of auditory hair cells. IHC contain afferent synapses that relay membrane voltage changes into auditory nerve fiber action potentials; OHC contain electromotile elements and generally serve as mechanical amplifiers of the sound stimuli (DALLOS, 1992).

In IHC, changes in membrane potential produce afferent synaptic activity localized to the hair cell's basolateral surface and the pre-synaptic machinery is geared to generate a graded release of the neurotransmitter molecules. Voltage-dependent calcium channels co-localized with neurotransmitter release sites open in response to membrane depolarization, which in turn results in the release of the neurotransmitter molecules. The amount of transmitter release is modulated by the magnitude of the membrane voltage changes.

The neurotransmitter diffuses across the synaptic cleft and binds to post-synaptic receptors on afferent auditory nerve fiber dendrites. This process begins the generation and propagation of action potentials along the afferent fibers. The peripheral processes (or dendrites) of primary neurons extend from the hair cells to their respective cell bodies that reside in Rosenthal's canal. This canal is carved within the internal bone of the cochlea and spirals medially and in parallel to the organ of Corti. The central axons of the spiral ganglion cells then coalesce within the central core of the cochlea to course through the internal auditory canal towards the brainstem as the cochlear (auditory) nerve. Hair cell stereocilia are activated according to the particular frequency of the vibrations presented to them, causing depolarization of their associated spiral ganglion neurons (KING, 1987; GUYTON, 1991; BAGLEY, 1996; COOK, 2004; EISEN and RYUGO, 2007).

Impulses from the cochlear nerve are transmitted to the cochlear nuclei within the medulla oblongata. Tracts from the cochlear nuclei cross to the opposite side and pass through the lateral lemniscus of the brain stem to the geniculate nucleus of the thalamus (NIEUWENUYS et al., 1991). From the thalamus, there are relays to the cerebral cortex for conscious recognition of sound (DE LAHUNTA, 1983; KING, 1987). There are also tracts connecting the cochlear nuclei with descending motor tracts to the neck and limbs that coordinate reflex movements (that is, rapid turning of the head in response to sudden noises) (KING, 1987; COOK, 2004).

1.3 Sound stimulation

Sound stimuli is the term given to pressure waves generated by vibrating air molecules. Sound waves propagate in three dimensions, creating spherical shells of alternating compression and rarefaction (TER HAAR, 2006). Like all wave phenomena, sound waves have four major features: waveform, phase, amplitude and frequency. These four features determine the perception of sound, especially the frequency and amplitude of the waves. However, in nature, sounds composed of single sine waves are extremely rare; most sounds consist of acoustically complex waveforms.

The frequency of a sound, expressed in cycles per second or Hertz (Hz), roughly corresponds to the pitch of the sound, whereas the amplitude, usually expressed in decibels (dB), determines the loudness of the sound. By changing the frequency and/or amplitude of a sound, a different stimulation of the ear, and thus a different perception, occurs. The receptors, the hair cells, act like miniature amplifiers, each tuned mechanically by shape and function to provide a maximum electrical response when vibrated at a particular frequency by the fluid waves of the inner ear. Along the cochlea, all small groups of hair cells have their own specific frequency by which they are stimulated maximally. The hair cells are thus a set of frequency filters, ordered spatially within the cochlea; those with high-pass frequencies occupy the base and those with low-pass frequencies occupy the apex of the cochlea.

Therefore, a sound with a high frequency causes maximal displacement of a portion of the basilar membrane at the base of the cochlea: the greater the displacement of the basilar membrane, the greater the number of sensory receptor and neurons that are stimulated, leading to increased sound intensity. A sound wave with a higher amplitude leads to a greater basilar membrane displacement. A sound with a low frequency causes displacement of a more apically situated portion of the cochlea (TER HAAR, 2006).

2. **Pathophysiology of auditory pathway**

2.1 Deafness: classification and causes

Classification of hearing impairment has been frequently done according to the localization of the defective anatomical structures involved. The causes of hearing

impairment can be subdivided into two major categories: conductive and sensorineural deafness.

Conductive deafness occurs when there is a failure in the proper transmission of sound vibrations to the inner ear and auditory pathway, without there being any damage to the cochlea. Conductive deafness typically results from middle ear pathology, tympanic membrane perforation, ossicular discontinuity or fixation, or middle ear infection, which are often amenable with surgical procedures. Alternatively, it may result from external ear pathology, such as severe otitis externa and occlusion of the external ear canal through excess cerumen production (STRAIN, 1999).

Sensorineural deafness results from abnormalities of the inner ear structures, the cochlear (auditory) nerve, and/or the central auditory pathways in the brainstem, thalamus and cerebrum (LUTTGEN, 1994). Causes of sensorineural deafness can be sorted into two complimentary categories, each with two types: inherited or acquired and congenital or later-onset.

Acquired sensorineural deafness may result from otitis interna, meningitis, noise trauma, mechanical trauma, anoxia, anaesthesia, or aging (presbyacusis) (STRAIN, 1999). Furthermore, acquired sensorineural deafness has also been associated with ototoxicity from drugs or chemicals (that is, aminoglycoside antibiotics, platinum-based chemotherapeutic agents, some NSAIDs and antimalarial medications) that damage hair cells, the stria vascularis, the organ of Corti, or the cochlear neurons (PICKRELL et al., 1993; MERCHANT, 1994; YORGASON et al., 2006; SELIMOGLU, 2007). Presbyacusis is hearing impairment that accompanies aging in dogs and cats. Affected dogs show a loss of spiral ganglion cells, atrophy of the organ of Corti, atrophy of the stria vascularis, thickening of the basilar membrane, lipofuscin accumulation within cochlear hair cells, and nerve cell loss and gliosis within the cochlear nuclei. These degenerative changes are hypothesised to be the result of aging changes as well as exposure to ototoxic agents (SHIMADA et al., 1998). Most congenital deafness is hereditary, and most later-onset deafness is acquired, although there are human forms of inherited later-onset deafness.

Finally, sensorineural deafness can be primary or secondary. Primary deafness results from the degeneration of hair cells in the cochlea without antecedent events. This occurs in hereditary deafness in Doberman Pinchers, and in some forms of ototoxicity, and presbyacusis. Secondary deafness occurs when hair cells

die as a consequence of other damage in the cochlea, most commonly to the stria vascularis. This occurs in pigment-associated hereditary deafness and some forms of ototoxicity (STRAIN, 2004).

In human audiology, deafness is classified as an isolated form of deafness (nonsyndromic) where impaired auditory function is the only clinical manifestation, or as a syndromic form of deafness where deafness is associated with other symptoms or anomalies. With the exception of embryopathies induced by rubella, toxoplasmosis or cytomegalovirus infection, which might result in different malformations including hearing impairment, other forms of syndromic hearing impairment are of a genetic origin. To date, the genes underlying more then 100 different syndromes that include hearing impairment have been identified (PETIT, 2001; TORIELLO et al., 2004). The non-syndromic (isolated) forms of deafness can be due to either genetic causes or non-genetic causes such as sound trauma, infections, xenobiotics and tumours. The isolated forms of hereditary hearing impairment are categorised according to their mode of transmission: X chromosome-linked (DFN), Y chromosome-linked; autosomal dominant (DFNA), autosomal recessive (DFNB), and maternal inheritance linked to the mitochondrial genome (PETIT, 2006).

The most commonly seen forms of deafness in companion animals are hereditary congenital sensorineural deafness, acquired later-onset sensorineural deafness, and acquired later-onset conductive deafness (STRAIN, 1999).

2.2 Congenital sensorineural deafness (CSD) in dogs and cats

Hereditary CSD is common in many breeds of dogs and cats with a predilection for white coat colours. In the small number of canine breeds where CSD is not associated with white pigmentation (Doberman Pinschers and other breeds not carrying piebald or merle genes), deafness resulting from the loss of the auditory hair cells is a primary event, with an unknown cause (WILKES and PALMER, 1992; STRAIN, 1999). In dogs and cats with white-producing genes, deafness appears to result from a strong expression of the gene.

When the piebald, merle, or white gene is strongly expressed, it suppresses melanocytes not only in the skin, but also in the iris and the stria. Melanocytes, which produce pigment granules in the skin, hair, and elsewhere, originate embryologically in the neural crest, the source of all neural cells, which explains the linkage between pigment and a neurologic disorder. Melanocytes produce

pigment granules, either eumelanin (black or brown) or phaeomelanin (yellow or red) - from the amino acid tyrosine. White colour results from an absence of melanin, usually from an absence of melanocytes.

Furthermore, melanocytes situated in the stria vascularis are of great importance for maintaining the ionic environment that is necessary for the normal function of the cochlear hair cells (STEEL and BARKWAY, 1998; TACHIBANA, 1999, 2001). The stria is responsible for the secretion of endocochlear fluid (endolymph) and maintenance of its high K^+ concentration, which is essential to sound transduction by the sensory hair cells. In pigment-associated hereditary deafness, when the strial melanocytes are absent, the stria degenerates, resulting in secondary loss of hair cells, and therefore, deafness. Whether hair cell death is from primary or secondary mechanisms, the loss is permanent, as mammals are unable to regenerate cochlear neuronal tissue. In pigment-associated hereditary deafness, the strial degeneration and hair cell death usually occur 2 - 4 weeks after birth (STRAIN, 1999, 2003).

3. Congenital sensorineural deafness in white cats

Scientific interest in the association between white coat colour, blue iris colour and deafness in cats can be traced to the first half of the nineteenth century (BREE, 1829; SICHEL, 1847; DARWIN, 1859). BREE (1829) gave the first known report regarding deafness in white cats and observed that white cats with blue eyes were invariably deaf. After 20 years of observation, SICHEL (1847) reported in his study that white cats without blue eyes were never deaf. However, TAIT (1883) disagreed with previous findings and reported that deafness could occur in cats, with either blue or yellow eye colour, but believed that only white coat males were affected. A series of reports published afterwards disagreed with those findings and have been the source of many contradictory statements and anecdotal observation regarding congenital deafness in white cats (STEVENS, 1884; PRZIBRAM, 1907; BEAUMONT, 1911).

First, systematic breeding experiments were carried out by WHITING (1918), after which he concluded that solid white (W) is a simple inherited gene, with complete dominance over other colours (w) and is unrelated to albinism. He also identified a white-spotting factor that might account for the observed irregularities in the inheritance of iris colour. WHITING (1919) proposed a single quadruple

allelic series: W- solid white; w^m - much spotted; w^l - little spotted and w- self (no-white), with the dominance in the direction of decreasing pigmentation. BAMBER (1929) showed conclusively that not all blue-eyed white cats were deaf, nor were all white male cats deaf, as had been stated by several authors.

More recently, WILSON and KANE (1959) suggested that a single gene (rather than three closely linked genes) was responsible for determining the characters of white coat, blue eyes and deafness, with high penetrance for the suppression of pigmentation and lower penetrance with respect to eye pigmentation and abnormal ear development.

An intensive study involving several hundred cats showed that, in many backcross matings of white to solid-pigmented cats, no segregation in any litter of white offspring occurred, but two distinct classes of piebald spotting were produced (BERGSMA and BROWN, 1971). The latter report suggested that, because piebald genes could not be contributed by the solid-pigmented parent, only one gene could vary at the piebald locus in the white parent and, thus dominant white was an allele at that locus. Furthermore, BERGSMA and BROWN (1971) in their study made a differentiation between piebald with pleiotropic effects (W^h - high degree of piebald) and white spotting without pleiotropic effects (that is, S), although the distinction between the two phenotypes might depend on the background genotype rather than on the major gene involved. Therefore, it has been stated that for analytic purposes, it was useful and convenient to treat W and S as representing distinct loci (ROBINSON, 1959, 1970).

Unlike dogs, which are homozygous with the dominant merle pigmentation gene, homozygous white cats do not typically have visual or reproductive defects, but they are prone to the occurrence of blue irises (one or both) and deafness (either unilateral or bilateral), and likelihood of deafness increases with the number of blue eyes (DELACK, 1984).

In the recently published study by GEIGY et al. (2007), a complex segregation analysis, using maximum likelihood procedures, was performed in experimental colonies of mixed-breed white cats to determine the most probable mode of inheritance of deafness and blue eyes. Their results suggested the best model is a pleiotropic gene segregating for deafness and blue irises, with additional polygenic effects. This recognition that deafness in blue-eyed white cats does not follow simple Mendelian genetics was not surprising, as a simpler mode of

inheritance would probably have been recognized long ago and would currently be used to reduce deafness prevalence (STRAIN, 2007).

3.1 Pathophysiology and histopathology of CSD

The congenitally deaf white cat has long been of interest to numerous investigators because of the similarities of inner ear pathology between cats and humans (BAMBER, 1933; WOLFF, 1942; WILSON and KANE, 1959; BOSHER and HALLPIKE, 1965, 1967; SUGA and HATTLER, 1970; BERGSMA and BROWN, 1971; MAIR, 1973). Typically, congenitally deaf white cats exhibit cochleo-saccular degeneration; this is also found in humans where it was first described in congenitally deaf patients (SCHEIBE, 1892, 1895). Because of that, the congenitally deaf white cat has been promoted as a model for the Scheibe deformity. This particular pattern of deafness in humans is characterised by the collapse of Reissner`s membrane onto the undifferentiated organ of Corti, thinning of the stria vascularis, and malformation of the tectorial membrane.

First, thorough anatomical investigations of congenitally deaf white cats were published by BOSHER and HALLPIKE (1965). According to this study, the first degenerative changes in the organ of Corti can be seen from postnatal day five and are virtually complete by postnatal day 21; therefore, the organ of Corti degenerates during the period in which the normal cochlea matures. The changes in the inner ear included a collapse of the inner tunnel; a degeneration and subsequent disappearance of the sensory hair cells; change in shape and structure of the tectorial membrane which then clings to the inner sulcus; a progressive atrophy of the stria vascularis; and the collapse of Reissner`s membrane and its folding and covering of the basilar membrane. These researchers also found simultaneous hair cell degeneration in all coils of the cochlea.

In contrast, MAIR (1973) concluded that deafness begins with the progressive loss of hair cells initiated during the first postnatal week of life and continues throughout the first and second years of life. He claimed that the degenerative process follows a regular pattern, whereby degeneration of the organ of Corti starts in the upper half of the basal turn spreading from there in both basal and apical directions. Moreover, he found that secondary to hair cell loss was the loss of primary neurons, and the general inference was that older animals suffered more severe deafness than did young animals.

Subsequently, published reports agreed that hereditary degeneration in the cochlea of white cats was not necessarily a regular process, and that, in a few cases, ganglion cell loss preceded cochlear degeneration, which is opposite to the previously published reports (PUJOL et al., 1977; REBILLARD et al., 1981a, 1981b). Recently, a distinct type of cochlear pathology associated with CSD in cats has been detected consisting of the hypertrophy of Reissner`s membrane resulting in an irregular and folded structure, eventually filling the scala media, and the tissue exhibits an overall "spongiform" appearance (RYUGO et al., 2003). Only some cats investigated in the afore-mentioned study showed the well-known Scheibe degeneration while others showed both epithelial overgrowth and Scheibe degeneration (Figure 3).

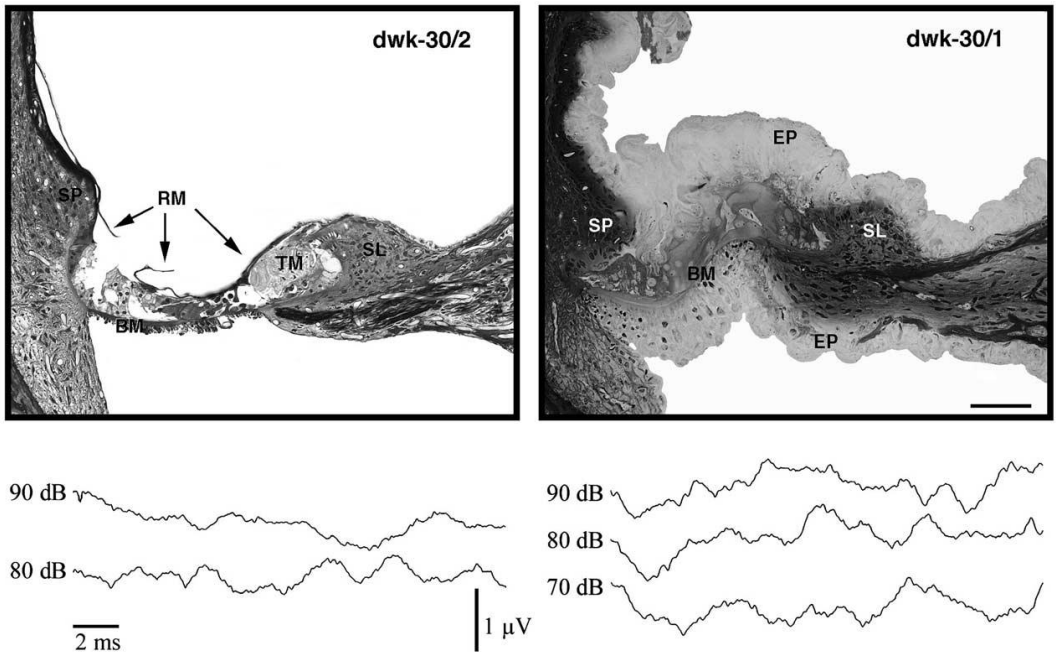

Figure 3 Representative morphology and ABRs of two forms of cochlear pathology. *Neither ear of these deaf white kittens (dwk) exhibited a click-evoked ABR at PN30. Each cochlea exhibits distinct structural differences. One form of pathology (dwk-30/2) resembles the Scheibe deformity with Reissner`s membrane collapsing and obliterating the scala media. The other form (dwk-30/1) reveals a kind of exuberant growth of epithelial cells that smothers the organ of Corti and stria vascularis. Abbreviations: BM, basilar membrane; EP, epithelial cell layer; RM, Reissner`s membrane; SL, spiral limbus; SP, spiral prominence; TM, tectorial membrane*.
(from RYUGO et al., 2003)

3.2 Hearing status in white cats with CSD

It should be emphasised that the early publications on the congenitally deaf white cats (for example, BOSHER and HALLPIKE, 1965; BERGSMA and BROWN,

1971; MAIR, 1973) addressed deafness as an all-or-none phenomenon, occurring either unilaterally or bilaterally.

Interestingly, the notion of a progressive development of complete deafness is in sharp contrast to reports of partially hearing cats (REBILLARD et al., 1976; REBILLARD et al., 1981a, 1981b; RYUGO et al., 1998, 2003). Some mixed-breed white cats from these studies showed partial deafness (or hearing) with elevated BAER thresholds that did not change over time (that is, BAER thresholds did not get progressively worse). According to the afore-mentioned studies, progressive cochlear dysplasia resembling the Scheibe deformity may be the final result for some cats, but there are clearly other forms of congenital deafness that do not proceed to complete hearing loss. Finally, the variations in histopathology and hearing status across the experimental populations of mixed-breed congenitally deaf white cats suggest that the general class of deaf white cats is not homogenous and that causes other than hereditary sensorineural degeneration must be responsible for the hearing disorder. Unfortunately, to date, no data about histopathology and hearing status in pure-breed white cats with CSD have been published.

3.3 Prevalence of deafness

The prevalence of deafness is high in the cat population in which the dominant white gene (W) is segregating, especially in cats with blue eyes. The W gene has been studied exclusively in domestic longhair and shorthair mixed-breed cats and to date, there have been no studies in any pure-breed cats. The W gene is present in 17 registered cat breeds, but the true prevalence of congenital deafness among those breeds has never been published (GEBHARDT et al., 1979).

Deafness prevalence in mixed-breed white cats has been studied by several investigators, but many of these investigations involved short investigative series and were primarily concerned with physiological and histological findings (WOLFF, 1942; WILSON and KANE, 1959; SUGA and HATTLER, 1970). Combined, the afore-mentioned three studies describe ten deaf cats in a total of 16 white cats. With respect to iris colour, inner ear degeneration was observed in 60 % of cats with both irises that were blue, 30 % of cats with one blue iris, and 10 % of cats with irises of a colour other than blue. Unilateral inner ear degeneration was observed in a cat with both irises of a colour other than blue (WILSON and

KANE, 1959). Of the ten deaf cats, degeneration was present in 17 of 20 ears, which is an incidence of 85 %.

The most complete data on prevalence in white cats come from the breeding studies of BERGSMA and BROWN (1971) and MAIR (1973) who examined crosses between white and non-white parents and between hearing and deaf parents. Prevalence rates in these two studies among white kittens for combined - unilateral and bilateral deafness, were 42.6 % (n = 162) and 51.5 % (n = 66), respectively. When kittens were homozygous for white (WW), the rates were 52.0 % and 96.0 % in the two studies; the rates for heterozygotes kittens were 24.3 % and 27.4 %.

In the study by MAIR (1973), at least one parent was always bilaterally deaf. While BERGSMA and BROWN (1971) included all possible hearing combinations in the parents, these differences hindered comparison between the results of the studies. From these complex studies, it is difficult to cite a single prevalence rate, but clearly the prevalence rates are high. The authors also found a clear association between blue eyes and deafness. The prevalence of deafness (combined - unilateral and bilateral) in cats with two blue irises was 85 % and 65 %, respectively. In cats with one blue eye it was 40 % and 39 %, respectively, and in cats with no blue irises it was 17 % and 22 %, respectively. It has been suggested that long-haired cats have a higher prevalence of blue eyes and deafness than have short-haired cats (MAIR, 1973), but this has not been confirmed.

DELACK (1984) analysed three studies of deafness in mixed-breed white cats that included a total of 256 cats (BOSHER and HALLPIKE, 1965; BERGSMA and BROWN, 1971; MAIR, 1973); 12.1 % were unilaterally deaf and 37.9 % were bilaterally deaf, or a total of 50 % were affected. When cats that were the offspring of two white parents were examined, the prevalence of deafness (unilateral or bilateral) ranged from 52 % to 95 % (Table 1). Therefore, not all white cats are deaf and not all blue-eyed white cats are deaf, but a great many of them are so affected. DELACK (1984) also presented proportions of cats with the white phenotype in urban and rural regions, which ranged from 0 % - 11.1 %.

Table 1 Association between inner ear degeneration and eye color in white cats older than 14 days. (from DELACK, 1984)

		No. of Blue Eyes				
Source of Research	***Inner Ear Degenaration***	***2***	***1***	***0***	***Total***	***Incidence (%)***
Mair (1973)	Bilateral	13	5	2	20	77.5
	Unilateral	4	3	1	8	68.8
	Absent	3	12	15	30	30.0
	Total	20	20	18	58	51.7
	Deafness (%)	85.0	40.0	16.70	48.3	
Bergsma and Brown (1971)	Bilateral	36	9	8	53	76.4
	Unilateral	14	9	5	28	66.1
	Absent	27	28	46	101	40.6
	Total	77	46	59	182	54.9
	Deafness (%)	64.9	39.1	22.0	44.5	

GEIGY et al. (2007) described the prevalence rate in an experimental colony of 104 mixed-breed white cats. The prevalence of completely deaf individuals was 67 % and that of partial hearing ones was 29 % and thus was similar to prevalence rates from studies by BOSHER and HALLPIKE (1965) and MAIR (1973) for comparable mating schemes. It has been stated that the evaluation of hearing in cats is additionally complicated because, unlike with pigment-associated deafness in dogs, deafness in white cats may be partial or complete in a given ear (REBILLARD et al., 1981a), and more than one type of underlying cochlear pathology may exist (RYUGO et al., 2003). Because there is no internationally standardized classification, evaluation of partial hearing in cats is not well defined.

3.4 Genetics of deafness

Domestic cat populations are heterogeneous for coat colour and hair quality, but the numbers of mutant genes responsible for these traits are few and their mode of inheritance and phenotype interactions have been largely elucidated, but not yet clarified on a molecular genetic basis (ROBINSON, 1959; STRAIN, 2007).

CSD in cats is linked to the so-called white gene (W), which is dominant and epistatic over all colour loci (LITTLE, 1957; SEARLE, 1968). Although the white gene is dominant, not all carriers are deaf, thus deafness is not simply inherited.

Cats carrying the W gene are not always solid white, often having coloured spots on their heads that may fade or disappear with age.

The W gene interferes with the migration of the cells from the neural crest of the early embryo, reducing in that way the number of melanocytes distributed in the skin, hair, iris and inner ear (SEARLE, 1968). Whether the cat is heterozygous or homozygous for W, the blue eyes and deafness have incomplete penetrance. GEIGY et al. (2007) support the hypothesis of a pleiotropic major gene segregating for deafness and blue eyes, and claim that the high heritability coefficient for both traits indicates that besides the major gene there was an important influence of polygenic effects.

Another gene responsible for hypopigmentation in cats is the dominant piebald gene (S), also known as the white-spotting gene. White spotting in cats varies greatly according to the degree to which the gene S is expressed. Coat colours under the genetic influence of the dominant piebald gene S can range from all black to all white, with any gradation between, but there has been no report of deafness associated with its presence (SEARLE, 1968; PEDERSEN, 1991).

The albino gene (C) with its four mutant alleles can also result in a solid white coat colour and blue irises in cats, but deafness does not seem to be associated with albinism (LITTLE, 1957; PEDERSEN, 1991). Albinism, in which regular numbers of melanocytes are present but one of the enzymes responsible for melanin production (tyrosinase) is absent or diminished, does not have an association with deafness. Recently, 12 different genes have been identified that, when mutated, result in an albino coat colour in different species, but none of these was associated with deafness (OETTING et al., 2003).

Furthermore, cats carrying the underlying c^s Siamese dilution pigment gene can have blue eyes without deafness, and it has been suggested that the presence of this gene explains why pure-breed white cats are less often deaf than mixed-breed white cats (PEDERSEN, 1991), but no studies have documented this assertion about prevalence.

Despite the long-standing recognition of deafness in white cats and the many descriptive studies, the molecular basis for hearing problems in cats with white coat colour is not known. It has often been suggested that the disorder is a feline homologue of the human Waardenburg syndrome (BERGSMA and BROWN, 1971; MAIR, 1973; WEST and HARRISON, 1973; REBILLARD et al., 1976; REBILLARD et al., 1981a, 1981b; SCHWARTZ and HIGA, 1982; DELACK,

1984). In humans, mutations in the PAX3 gene were identified as causal for this disorder, and mutations in this gene are also candidates for deafness in mice (STEEL and BROWN, 1996; DESTEFANO et al., 1998). In any case, by comparing the DNA sequences of the canine PAX3 gene of healthy and deaf Dalmatian dogs, no causative mutations in the analysed coding regions were found (BRENIG et al., 2003). To date, there is no published report of whether the mutation in the PAX3 gene might be the cause of CSD in cats.

The main candidate gene with an effect on hearing and eye colour in cats is the W gene. In mice, this gene encodes for a growth factor receptor known as the c-kit, which is involved in the formation, migration, proliferation and/or differentiation of germ cells, haemopoietic tissues and melanoblasts (CHABOT et al., 1988; GEISSLER et al., 1988). A study of melanoblast development suggests that it is primarily the survival of melanoblasts that is affected by defects in the W gene (CHABOT et al., 1988). Progress in reducing deafness will most likely require identification of the genomic identity of the W gene, followed by identification of mutations in that gene that are causative for deafness.

4. **Clinical evaluation of auditory function in dogs and cats**

Deafness in an animal is usually not a life-threatening disorder and it is not a painful condition, but it does put an animal at risk from undetected dangers, such as motor vehicles or predators, and deaf animals create their own liabilities and present great training challenges to their owners. Because large numbers of deaf puppies and kittens are euthanized, it is important to identify those animals affected by the hereditary forms of deafness - both unilaterally and bilaterally deaf - and remove them from the potential breeding pool to reduce the number of future deaf animals. For this reason, a complete clinical examination of the auditory system should be thoroughly done based on electrodiagnostic assessments.

4.1 Behavioural evaluation of hearing

Hearing in dogs and cats can be evaluated by observing an animal’s behaviour in response to sounds that are part of its natural environment, or sounds that are produced under artificial conditions in the laboratory. These sounds should be produced outside of the visual fields, avoiding visual clues, vibratory cues, touch

and air movements. Under ideal circumstances, a sort of psychophysical audiogram can be constructed from an animal's reaction to sounds of varying intensities and frequencies (ROSE, 1977). However, behavioural evaluation has a limited value - animals in a clinical setting are usually so apprehensive that their attentiveness to the examiner is minimal and animal responses rapidly adapt even when hearing is present. Moreover, in unilaterally deaf animals, the only behavioural sign of deafness is difficulty in localizing the source of a sound, and many animals adapt to that also, so that unilaterally deaf animals cannot be detected with any reliability. As a consequence, the behavioural hearing assessment of the dogs and cats in the clinic or at home is of limited reliability, and electrodiagnostic tests are used for objective assessment (SIMS, 1988; STRAIN, 1999).

4.2 Electrodiagnostic evaluation of hearing

Hearing in animals can be evaluated using electrodiagnostic procedures that selectively assess the integrity of peripheral and central auditory components. These procedures include tympanometry, acoustic reflex testing and auditory evoked responses. All of these procedures are noninvasive and evaluate components of the external ear canal, middle and inner ear cavities, cranial nerves and selected areas of the brain stem and cortex. Electrodiagnostic testing procedures do not require conscious cooperation and are particularly useful in testing very young animals.

4.3 Auditory-evoked responses

Following a transient acoustic stimulus, such as a click or a brief tone pip, the ear and parts of the nervous system generate a series of electrical signals with latencies ranging from milliseconds (msec) to hundreds of msec. These auditory evoked potentials (AEP) are conducted through body tissues and can be recorded from electrodes placed on the skin to evaluate noninvasively the function of the ear and portions of the nervous system activated by the acoustic stimulation. The short-latency or brainstem auditory evoked potentials (BAEP) have proved to be valuable tools for hearing assessment and for the diagnosis of neurologic, otologic or audiologic dysfunction in animals (BENNET et al., 1977; BODENHAMER et al., 1985; CONLEE et al., 1984; KAY et al., 1984; HOLLIDAY et al., 1992;

STRAIN et al., 1992, 1999; UZUKA et al., 1998; SHIU et al., 2000; PONCELET et al., 2000, 2002; MURRELL et al., 2004).

AEP have been divided into a sequence of three different wave forms with increasing latencies:

a) short-latency potentials (BAEP), with latencies of under ten msec,
b) middle-latency auditory evoked potentials (MLAEP), with latencies between 10 and 50 msec,
c) long-latency auditory evoked potentials (LLAEP), with latencies exceeding 50 msec.

Generally, components of successive types of AEP represent the activity of neural generators at progressively higher levels in the neuroaxis.

Short-latency AEPs have achieved the greatest clinical utility because they are relatively easy to record and their waveforms and latencies are highly consistent across normal subjects. The earliest components derive from electrical processes within the inner ear and action potentials in the auditory nerve. For the early-latency components, generators are thought to be located mostly within the brainstem, so that this series of AEP is commonly called brainstem auditory evoked potentials (BAEP). However, this term is not completely accurate because the roster of generators clearly includes the distal (with respect to the brainstem) cochlear nerve and may also include thalamocortical auditory radiations, neither of which is within the brainstem. Other synonyms or related designations include brainstem auditory evoked response (BAER), auditory brainstem response (ABR), early acoustic evoked potentials (EAEP), far-field electrocochleography, and brainstem audiometry.

AEP components generated within the brainstem may reflect both action potentials and postsynaptic potentials. Auditory-evoked neural activity becomes increasingly affected by temporal dispersion as the poststimulus latency increases and the contribution of short-duration electrical phenomena, such as action potentials, is eliminated. Thus, AEP components that are longer in latency are also wider, and the middle- and long-latency AEP are predominantly generated by postsynaptic potentials within areas of the cerebral cortex that are activated by the acoustic stimulation.

Middle-latency AEPs are small, subject to contamination by myogenic signals, highly sensitive to most anaesthetics and rather variable from subject to subject, which limits their clinical application in both human and veterinary medicine.

Long-latency AEPs, mostly used in human medicine, are profoundly affected by the degree to which the subject is attending to the stimuli and analysing stimulus features. They have, therefore, been used in humans as probes of cognitive processes, but their variability as well as uncertainty about the precise identity of their cortical generators limits their utility for neurological diagnosis (SIMS, 1988; LITSCHER, 1995).

5. **Brainstem auditory evoked potentials (BAEP)**

The recording of brainstem auditory evoked potentials is probably the most widely used electrophysiological test in veterinary medicine. The brainstem auditory evoked response (BAER) test was first used in veterinary research applications in the 1970s and in clinical veterinary applications in the early 1980s. The BAER test detects electrical activity in the cochlea and auditory pathways in the brain in much the same way that an electrocardiogram detects electrical activity in the heart (STRAIN, 1999). Sounds are used to stimulate the auditory system and the resultant electrical activity is recorded by electrodes placed at strategic sites on the skull. Because auditory stimuli are used in this test, the functional integrity of the structures of the outer, middle and inner ear is evaluated in addition to the nervous system.

5.1 Stimulation

BAEPs are most commonly elicited by brief acoustic click stimuli produced by delivering monophasic square pulses of 0.1 msec durations to headphones or other electromechanical transducers (tubal inserts) at a rate of about 10 Hz. They are generated predominantly by the region of the cochlea responding to 2000- to 4000-Hz sounds, although wave V may also receive contributions from lower-frequency regions of the cochlea (GORGA et al., 1985).

A rate of exactly 10 Hz or other submultiples of the power line frequency should be avoided; otherwise, the inevitable line frequency artefact will be time-locked to the stimuli and will not be removed by the averaging process. Audiometric headphones that have a relatively flat frequency response are desirable so that “broad-band” clicks, whose energy is spread over a wide frequency range, will be produced. The headphone transducer reacts to the electrical stimulus by generating a short-duration damped sine pressure wave. If the electrical square

pulse causes the diaphragm of the acoustic transducer to move toward the patient's ear, a propagating wave of increased air pressure, termed a compression click or a condensation click is produced. Reversing the polarity of the electrical square pulse that activates the transducer produces a rarefaction click. Most investigators work with alternating polarity click stimuli, as this cancels the electromagnetic artefact from the transducer. Unfortunately, this also cancels the diagnostically relevant cochlear microphonic potentials resulting from cochlear hair cell electrical activity (SCHWARTZ et al., 1990).

Stimuli are delivered monaurally so that a normal BAEP to stimulation of one ear does not obscure the presence of an abnormal response to stimulation of the other ear. An acoustic stimulus delivered to one ear can reach the other ear via air and bone conduction. To prevent contralateral stimulation from occurring and possibly being misinterpreted as a BAEP arising from stimulation of the ipsilateral ear, the contralateral ear is masked with continuous wide band masking noise at an intensity of 30 to 40 dB below that of the BAEP stimulus.

The stimulus intensity is probably the most important stimulus parameter because it has the greatest effect on the latency and amplitude of the component waves. The stimulus intensity should be loud enough to elicit a clear BAEP waveform without causing discomfort or ear damage. Several terms are used to describe the intensity of a stimulus. Sound pressure level (dB SPL) is an absolute physical measure of the sound intensity for sound in air and other gases, relative to 20 micropascals (μPa) = 2×10^{-5} Pa, the quietest sound a human can hear. The measurement unit for dB SPL is Bel (B) or 1/10 Bel - Dezibel (dB). The decibel (dB) is a logarithmic unit of measurement that expresses the magnitude of a physical quantity (usually power or intensity) relative to a specified or implied reference level. A-weighted decibel (dBA) is a unit representing the sound level measured with the A-weighting network on a sound level meter. Most measurements of occupational, industrial and environmental noise are taken using A-weighting. A-weighting is necessary to reduce the effects of the low and high frequencies with respect to the medium frequencies. Hearing levels (dB HL) are referenced to the threshold of hearing of the normal population, and normal hearing level (dB nHL) is referenced to the threshold of the specific control population used to establish a laboratory's normative database. Another useful unit of measure is the sensation level (dB SL), which is relative to the threshold of the ear being tested.

Even though the different investigators used variable standards for the detection of the hearing thresholds, threshold of the BAER is usually determined by decreasing the stimulus intensity in 5 to 10 dB steps to a level that evokes no response (that is, the disappearance of the wave V). The threshold value is then fixed midway between intensities for which a signal is still detected and the one for which no signal is present. A latency-intensity curve can be produced for each of the waves by plotting the latency of the component waves as a function of stimulus intensity (SIMS, 1988; PONCELET et al., 2000).

The brief acoustic click stimulus, the one normally used, contains a small range of audible frequencies of the dogs and cats (HEFFNER and HEFFNER, 1985). Accordingly, the click evoked BAER is a frequency non-specific test that is more useful for detecting the presence or total absence of auditory function without quantifying hearing loss in decibels and can also be used as a reliable diagnostic tool to differentiate between conductive and sensorineural deafness.

Current available information about tone-evoked auditory potentials in dogs (used to assess hearing at different frequencies) has been obtained under widely different technical conditions and cannot be effectively compared (UZUKA et al., 1998; PONCELET et al., 2000, 2002).

5.2 Recording

BAEPs are usually obtained in dogs and cats under sedation or anaesthesia due to their poor tolerance of the skull electrodes and of the tubal inserts that are placed in the ears. Recording electrodes are typically placed at the vertex (location Cz) and at both earlobes or mastoids. The ground electrode is usually placed on the forehead, but its precise location is not critical. Recording electrodes are typically stainless steel partially Teflon-insulated needles that are placed subcutaneously. Optimally, the same type of electrode should be used at all recording positions, and electrode impedance (ideally, less than 5 kOhm) should be as consistent as possible across all recording electrodes, since mismatched electrode impedance can increase the amount of noise in the BAEP data (CAMPBELL and BARTOLI, 1986). BAEPs should be recorded between the vertex electrode and the ipsilateral mastoid or earlobe electrode. A minimum of a two recording-channel systems, with the vertex electrode and the contralateral mastoid or earlobe electrode in the second channel, has been recommended, because this channel may aid the

identification of waves IV and V, which may be fused in the one-channel waveform (LEGATT, 1995).

The raw analogue data are amplified by high-input impedance differential amplifiers. A typical analogue filter bandpass is 100 Hz or 150 Hz to 3000 Hz (-3 dB points). The analogue gain depends on the input window of the analogue-to-digital converter; a value of approximately 100,000 is used normally. Data are usually digitized over an epoch duration or analysis time of approximately 10 msec (the analysis time in some recording systems is actually 10.24 msec). The analogue-to-digital conversion should use at least 256 points per epoch; sampling of a 10.24 msec epoch at 256 time points corresponds to a sampling interval of 0.4 msec and a sampling rate of 25,000 Hz. The display calibration usually ranges from 0.5 to 2.5 microvolt per division (cm) for the BAER in small animals.

Far-field BAEPs are too small to be visible in unaveraged raw data, so signal averaging is required. The improvement in the signal-to-noise ratio is proportional to the square root of the number of data epochs included in the average. Automatic artefact rejection is used to exclude from the average sweeps with high-amplitude noise. The number of epochs per trial is typically between 250 and 1000, although a larger number may be required if the signal-to-noise ratio is poor. At least two separate averages should be recorded and superimposed to assess reproducibility of the BAEP waveforms. Latency replication to within one percent of the sweep time and amplitude replication to within 15 percent of the peak-to-peak amplitude have been recommended as standards for adequate reproducibility (LEGATT, 1995).

5.3 Wave identification and analysis

A typical BAEP tracing in small animals consists of five to seven successive positive/negative deflections numbered with Roman numerals and experimental studies in the cat have shown that they correspond to anatomical relay stations of the auditory pathway (JEWETT, 1970; LEV and SOHMER, 1972; BUCHWALD and HUANG, 1975; ACHOR and STARR, 1980).

There is general agreement that the origin of wave I is in the most distal portion of the auditory nerve. There is less agreement concerning the origin of wave II. However, it appears to be generated by the ipsilateral cochlear nucleus and the unmyelinated central terminals of the cochlear nerve. Evidence of the generator of wave III, points to the dorsal nucleus of the trapezoid body of the ipsilateral

and/or contralateral brainstem. Studies of wave V of the BAER have indicated the ipsilateral and/or contralateral caudal colliculus as the generator with the central nucleus as the primary source. Generators of waves IV, VI, and VII have not been clearly defined.

Waves I and V are easily identified in most BAER, wave I because it is first to appear, and wave V because of its large amplitude and characteristic negative trough following the positive peak. The amplitude of a wave is measured from the peak of the wave to the nadir of the following negative trough. Latency is measured from the beginning of the stimulus to the positive peak of the wave. Interpeak latency may be measured for any wave pair, but particularly for wave pairs I and III, and I and V. Interpeak latency for wave pairs I and III represents the approximate time required for the activity created in the auditory nerve to arrive at the level of the pons, and the interpeak latency for wave pairs I and V is an approximate measure of the time from the action potential in the cochlear nerve to the level of the mesencephalon. The interpeak latency for wave peaks I and V is referred to as the central conduction time.

The overall morphologic features of the canine and feline BAER are similar to those in other animals and humans. Wave amplitude ranges from less than one microvolt to approximately six microvolts. For most stimulus intensities and rates, waves I, II, and V in dogs have large amplitudes, and waves III, IV, and VII have smaller amplitudes. This same basic pattern, or a slight variation, occurs in cats, except that wave II may be larger than wave I (SIMS and HOROHOV, 1986). Wave VI is present in most responses, and wave VII occurs infrequently in dogs and cats. In the click-evoked BAER test, the greatest variation occurs in waves III and V. In dogs and cats, waves III and IV may combine to form a single wave, with wave III predominating. The separation of waves III and IV seems to be a combination of individual variation, stimulus characteristics, and age.

5.4 Clinical use of the BAEP

The BAER test has been widely used in the general assessment of neurological diseases, of hearing impairment in human adults and children, as a measure of CNS maturation, and in monitoring the treatment of CNS disease. Because the auditory pathways traverse the brainstem extensively from side to side and back to front, consequently, the BAER test provides a fairly good evaluation of the brainstem integrity in general and can be used in cases of head trauma,

inflammatory disease, and other conditions when a patient is comatose and cranial nerve reflexes cannot be evaluated. Since a typical surgical level of anaesthesia produces only minor alterations in the BAER test, they can be used for the intraoperative monitoring of the ears and the auditory pathways, particularly in patients undergoing cochlear implantation surgery. Some reports showed changes in waves II to V of the BAER test in association with various brainstem lesions (FISCHER and OBERMAIER, 1994; STEISS et al., 1994). These investigations proved that BAEPs are useful as a screening method for brainstem lesion; they would not assist in finding their precise localization, but could potentially be useful in anticipating life-threatening conditions, such as intracranial pressure elevations, cerebellar herniation and brainstem compression. BAEPs may be of special interest in vestibular syndrome because recognition of simultaneous auditory impairment helps in the differentiation between central and peripheral localization of what is of paramount prognostic significance. In animals, the main clinical use of the BAER test has been in the evaluation of deafness, particularly inherited and senile deafness.

5.5 BAEP in CSD

The BAEP recording is widely used as a screening test to identify complete deafness in individuals of cat and dog breeds prone to hereditary hearing loss. It has prove to be an invaluable tool in the investigation and control of congenital deafness in numerous breeds of dogs and cats (PONCELET et al., 2000; STRAIN, 2004; PLATT et al., 2006; FAMULA et al., 2007). Regardless of the methodological differences among investigators, BAEP to high intensity click stimuli (60 – 90 dB nHL) has proved extremely efficient with a very low occurrence of equivocal results in puppies (HOLLIDAY et al., 1992; STRAIN et al., 1992).

The BAER test demonstrates maturational changes. Puppies and kittens are not born with mature auditory system. Maturational studies in puppies and kittens have revealed that waves I and II are the first to appear after birth, and are particularly well developed during maturation. During maturation, the general pattern is an increase in wave amplitude, a decrease in wave latency, and a decrease in the interpeak latency for wave pairs I and IV (KAY et al., 1984; MARSHALL and REDDING, 1985). BAER latencies and amplitudes have been shown to approach adult values as early as two weeks of age, but more frequently,

they mature between six and eight weeks of age (SIMS, 1988). For this reason, it is advisable to wait until at least 6 weeks of age to perform screening BAER tests for inherited deafness to avoid erroneous conclusions. Moreover, if the results are unclear, a second test a few weeks later should be performed.

The diagnostic potential of BAER recordings is enhanced considerably when the whole range of stimulus intensities is used. This approach makes it possible to define the wave V threshold and to build a wave V latency-intensity curve and, therefore, to use the BAER test as a diagnostic tool for the detection of partially hearing cats (PONCELET et al., 2000).

The interpretation of the findings considers the presence of waveforms, their latency and their amplitude, although identification of congenitally deaf animals is simply based on the presence or absence of waveforms.

III Chapter I:

Unilateral and bilateral congenital sensorineural deafness in client-owned pure-breed white cats

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Unilateral and Bilateral Congenital Sensorineural Deafness in Client-Owned Pure-Breed White Cats

Short title: Deafness in White Cats

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Abstract

Background - Congenital sensorineural deafness has been reported frequently in experimental mixed-breed white cats but there is a paucity of data on occurrence of deafness in client-owned pure-breed white cats.

Objective - To describe hearing status in client-owned pure-breed white cats.

Animals - Eighty-four pure-breed client-owned cats with white coat color of 10 registered breeds presented for routine hearing evaluation before breeding (1995 – 2008).

Methods - Hearing was assessed by click-evoked brainstem auditory evoked response (BAER).

Results - Overall deafness prevalence was 20.2%; 9 cats (10.7%) were bilaterally deaf and 8 cats (9.5%) were unilaterally deaf. There was no association between sex and deafness status (P=0.85). Deafness status was associated with iris color (P=0.04).

Conclusions and clinical importance - Congenital sensorineural deafness frequently occurs in pure-breed cats with white coat color. Unilateral sensorineural deafness was as common as bilateral deafness.

Keywords:

feline, hereditary, hearing disorder

Congenital sensorineural deafness in white cats is a well-known phenomenon. The interest in this relationship has been steadily increasing, especially because the congenitally deaf mixed-breed white cat has been used as an animal model of human deafness.[1-5]

Congenital sensorineural deafness has been described in cat breeds in which the dominant autosomal white gene (W) is segregating. Progressive cochleo-saccular degeneration, resembling Scheibe deformity in humans, is commonly associated with the W gene and causes complete congenital sensorineural deafness in white cats.[1,2] Interestingly, partial deafness and various types of inner ear degeneration were reported in some experimental studies of white cats in a setting that is difficult to reconcile with the W gene.[6-9] These studies were based on observations in experimental cats in which inbreeding may be assumed to be much greater than in pure-breed cats. Moreover, not all of pure-breed white cats necessarily carry W gene because there are several ways for cats to exhibit a white fur.[10] The dominant white gene (W) currently is present in 17 registered cat breeds,[11] but to the authors' knowledge, there is no study up to now that describes the prevalence of deafness among pure-breed cats.

The objective of this study was to provide data on the hearing status and occurrence of unilateral and bilateral congenital sensorineural deafness in client-owned pure-breed white cats presented for hearing assessment.

Materials and methods

Animals

Medical records and electrodiagnostic files were searched for cats with white coat color presented for routine hearing evaluation with BAER before breeding (1995 - 2008) as required by their respective breed associations. Inclusion criteria were: ≥ 8 weeks old; normal physical, neurological and otoscopic examination; no history of either ear disease or topical or systemic administration of drugs with potential ototoxicity. Information about breed, age, sex and eye color was retrieved from the medical records. The cats belonged to 10 different registered breeds (31 British Shorthair, 14 Maine Coon, 11 Turkish Angora, 9 Persian, 6 Foreign White, 6 Norwegian Forest, 4 Highlander, 1 Balinese, 1 Devon Rex, 1 Oriental Shorthair). Fifty-five cats were female and 29 were male. Median age of the cats was 4 months (range, 2 - 108 months). Iris color was documented in 55 cats: in 18 cats both eyes were blue, in 10 cats 1 eye was blue (odd-eyed) and in 27 cats both eyes were of other colors than blue.

Recording

Hearing testing was performed by click-evoked brainstem auditory evoked response (BAER). Cats were anesthetized with diazepam[a] (0.5 mg/kg) and propofol[b] (4mg/kg) IV or sedated with medetomidine hydrochloride[c] (80µg/kg) IM and placed in sternal recumbency. Rectal temperature was monitored and care was taken to avoid a decrease in body temperature. All recordings were done in a dark, quiet, but not soundproof room.

Viking IV/Quest[d] was used as the electroacoustic devices for all BAER measurements. Recordings (10 ms) were obtained ipsilateral to the stimulated ear with needle electrodes (disposable 12mm platinum/iridium EEG electrodes) placed SC at the vertex (Cz) and at the base of the stimulated ear. Ground was a needle electrode of the same type positioned SC in the neck. In accordance with the current international convention, positive activity recorded from Cz was displayed as an upward deflection. The signal was amplified 120,000 times and then filtered using a bandpass filter with 150 Hz and 3 kHz cut off frequencies (-3dB point, roll-off: 12 dB/octave). Recordings were averaged using 200 - 500

sweeps. All recordings were repeated once and superimposed on each other on the screen.

Stimuli

The acoustic stimuli were clicks, rectangular square waves of 100-microseconds` duration generated by the Viking IV/Quest Software[e]. The click stimuli were delivered to insert earphones[f] , which were placed in the external ear canal. The repetition rate of the stimulation was 11.1 Hz and click polarity was alternating.
Cats were routinely stimulated at 70 dB or 80 dB normal hearing level (nHL) with clicks delivered monoaurally, and in case of absent BAER the stimulation and recording procedure was repeated at 90 dB nHL. The nHL at 0 dB was equivalent to the peak sound pressure level (pSPL) at 30 dB (Viking IV/Quest product specification). In order to eliminate crossover recordings, white masking noise, at 30 dB below the level of the stimulation intensity, was delivered to the contralateral (non-stimulated) ear.

Data Collection

Bilateral sensorineural deafness was diagnosed if BAER were absent on repeated recordings from either ear even at stimulation with maximum stimulation intensities. Unilateral sensorineural deafness was diagnosed if BAER were absent from 1 ear and a normal BAER was recorded from the contralateral ear.
For estimation of the hearing threshold, the intensity of the click stimulus was decreased from 90 dB nHL in 15 dB steps to the lowest end of the amplifier range (30 dB nHL). Hearing threshold was defined as < 30 dB nHL if there was a recordable signal of wave V at 30 dB nHL.

Statistical Analysis

Deafness prevalence rates were calculated for bilateral deafness, unilateral deafness and combined bilateral and unilateral deafness. Chi-square test was used to test for associations between iris color or sex and deafness status, and odds ratios were calculated. Hearing thresholds of non-affected ears from unilaterally

deaf cats were compared with those of bilaterally hearing cats. The significance level for all statistical tests was set as $p < 0.05$. Statistical calculations were run using a commercially available statistical program[g].

Results

A total of 84 cats with white coat color presented for routine hearing evaluation before breeding (1995 – 2008) were identified.

Overall, 20.2% (17/84) of the cats were deaf in 1 or both ears (Table 1). Nine cats were bilaterally deaf (10.7%) and 8 cats (9.5%) were unilaterally deaf (Figure 1). Deaf cats belonged to 6 different breeds (Turkish Angora, British Shorthair, Maine Coon, Norwegian Forest, Persian, Foreign White). There was no association between sex and deafness (p=0.85).

Blue-eyed cats were more likely to be deaf than cats with other eye colors (p=0.04). The combined prevalence of uni- and bilateral deafness was 44.4% in cats with 2 blue eyes (n=18), 20.0% in odd-eyed cats (n=10), and 18.9% in cats with other eye colors (n=27). The odds ratio for a cat with at least 1 blue eye being deaf was 3.72 (95% CI 0.9-18.4) compared to cats with other eye colors. The odds ratio for cats with 2 blue eyes being deaf was 5.75 (95% CI 1.2-31.2) compared to cats with other eye colors than blue. The hearing threshold was < 30 dB nHL (lowest end of the amplifier range) in the non-affected ear of unilateral deaf cats and in both ears of bilaterally hearing cats.

Discussion

The present study shows frequent occurrence of congenital sensorineural deafness in pure-breed cats with white coat color even after many years of recommendations from breed associations to exclude deaf animals from breeding programs. Furthermore, breeding of white cats has been strictly discouraged by the German Animal Rights Law since 1998.[12]

Few studies have investigated the occurrence of deafness in client-owned cats of registered breeds. On the other hand, congenital sensorineural deafness has been

widely described in experimental mixed-breed cat colonies serving as an animal model for congenital deafness in humans. Specifically, cochlear pathology, cochlear and higher neuroanatomic structure function and more recently cochlear implants have been investigated in the congenital deaf white cat.[6,7,9,13]

The overall prevalence rates of unilateral and bilateral congenital sensorineural deafness (20.2%) in the present study were much lower than in experimental studies of mixed-breed cats with white coat color that reported 89.3%, 42.6%, 51.5%, and 67.0% occurrence of deaf cats, respectively.[1-3,5] Differences among deafness rates could result from a different genetic basis of deafness in experimental and client-owned white cats. Many experimental studies of mixed-breed cats included subjects homozygous for the dominant W allele which may not be the case in the present study.[1-3,5] Deafness occurrence rates of pure–breed white cats reported here also could support the hypothesis that some pure-breed white cats may carry the cs-Siamese dilution pigment gene and thus be less prone to deafness.[10] Furthermore, mating policies within the registered breeds aim at avoiding matings that could lead to deaf offspring whereas the purpose of mating in experimental studies is to promote deafness, resulting in high inbreeding coefficients.

To date, neither the mechanism of inheritance nor the molecular genetic basis of congenital sensorineural deafness in cats with white coat color has been elucidated completely. White color-associated congenital sensorineural deafness in the cat commonly has been linked to the W gene, which is dominant over other colors and is unrelated to albinism. The absence of melanocytes produces white coat color which is a consistent feature of the W gene. The cats evaluated in our study belonged to the 10 registered pure-breed cats described as carrying the dominant white gene (W). White albino cats with Siamese *cs*, blue-eyed *ca* or pink-eyed *c* recessive alleles from gene locus C, which determines the quantity of pigment granules in melanocytes, usually are not affected because they have a normal distribution of melanocytes.[10] Attempts to identify the genomic identity and changes in the W gene as a cause for deafness in white cats are in progress.[14] Another way for cats to exhibit white fur is the white spotting gene (S), sometimes called piebald, but to our knowledge there is no report of deafness associated with its presence in cats. Also, it is not clear that it is the same gene as in dogs.[15]

Considerable progress has been made over the past decade in identifying genetic loci and genes associated with mammalian deafness. To date, the genes underlying more than 100 different syndromes that include hearing impairment have been identified.[16] Mutation in the genes PAX3, MITF, C-KIT and SOX10 which are closely involved in cochlear melanocyte development cause hearing loss in humans and in other mammals.[17,18] Congenital sensorineural deafness in white cats also is often interpreted as the feline homologue of the human Waardenburg syndrome.[1,3,4,8,19] Disruption of melanocyte differentiation is the etiology of Waardenburg syndrome, which includes sensorineural hearing loss and pigmentary abnormalities of the skin, hair, and eyes.[20]

Recent reports suggested that different types of underlying cochlear pathology may exist in congenital sensorineural deafness in white cats, and that some cats may only be partially deaf.[6-9] Contrary to these findings, none of the cats of the present study had evidence of partial deafness. Furthermore, hearing thresholds determined in the normal ear of unilaterally deaf cats were in the same range as in bilaterally hearing cats (< 30 dB nHL). One limitation of this study is that histopathologic examination was not performed because the cats were client-owned pets.

Unilateral deafness was diagnosed in 9.5% of the pure-breed white cats presented in this paper and thus the rate roughly equals bilateral deafness (10.7%). Although not emphasized in publications, unilateral deafness also was a frequent finding in experimental investigations. Yet, unilateral deafness appeared less frequently (12%) than did bilateral deafness (49%) in 256 experimental mixed breed white cats [1-4] Different from cats, unilateral deafness is diagnosed more frequently than bilateral deafness in dogs.[21] Interestingly, unilateral deafness in humans is rarely recognized as a manifestation of congenital deafness.[22]

The results clearly show that congenital unilateral sensorineural deafness exists at least in white color-associated deafness syndromes in cats. The pathophysiology and genetics of unilateral deafness have yet to be completely elucidated. One possibility is that there are different stages of degeneration in the 2 ears, even though most studies failed to show progressive cochlear degeneration.[2,6] Another hypothesis is that there are different forms of pathology in the 2 ears, 1 milder and 1 more severe. In all of the 8 unilaterally deaf white cats in our study, non-affected ears showed normal BAER waveforms and hearing thresholds. In agreement with this finding, some studies failed to identify pathological changes

after histology examination in the normal hearing ears of unilaterally deaf white cats.[1,19] Experimentally, in a small number of cats increased hearing threshold and mild cochlear pathology were demonstrated in the normal hearing ear of unilaterally deaf animals.[1,6] Although click-evoked BAER testing suggests that the normal ear in unilateral deafness is not impaired, this remains to be proven by more sophisticated, frequency-specific testing of hearing function.

To our knowledge the present study is the first to provide data on deafness prevalence in client-owned pure-breed white cats using BAER for hearing assessment. Additional studies with larger numbers of pure-breed white cats are needed to establish prevalence rates for each cat breed separately. Furthermore, more detailed information is needed on genotypes in future reports about deafness prevalence in client-owned cats.

Footnotes

[a]Diazepam-ratiopharm 2, Ratiopharm GmbH, Ulm, Germany

[b]Narcofol, CP Pharma, Burgdorf, Germany

[c]Domitor, Orion Corporation, Espoo, Finland

[d]Nicolet Biomedical, Madison, Wisconsin,USA

[e]Neurodiagnostic Master Software, Nicolet Biomedical, Madison, Wisconsin, USA

[f]TIP 300 Insert Phones; Nicolet Biomedical, Madison, Wisconsin, USA

[g]Stata, Version 9.0, StataCorp LP, College Station, Texas, USA

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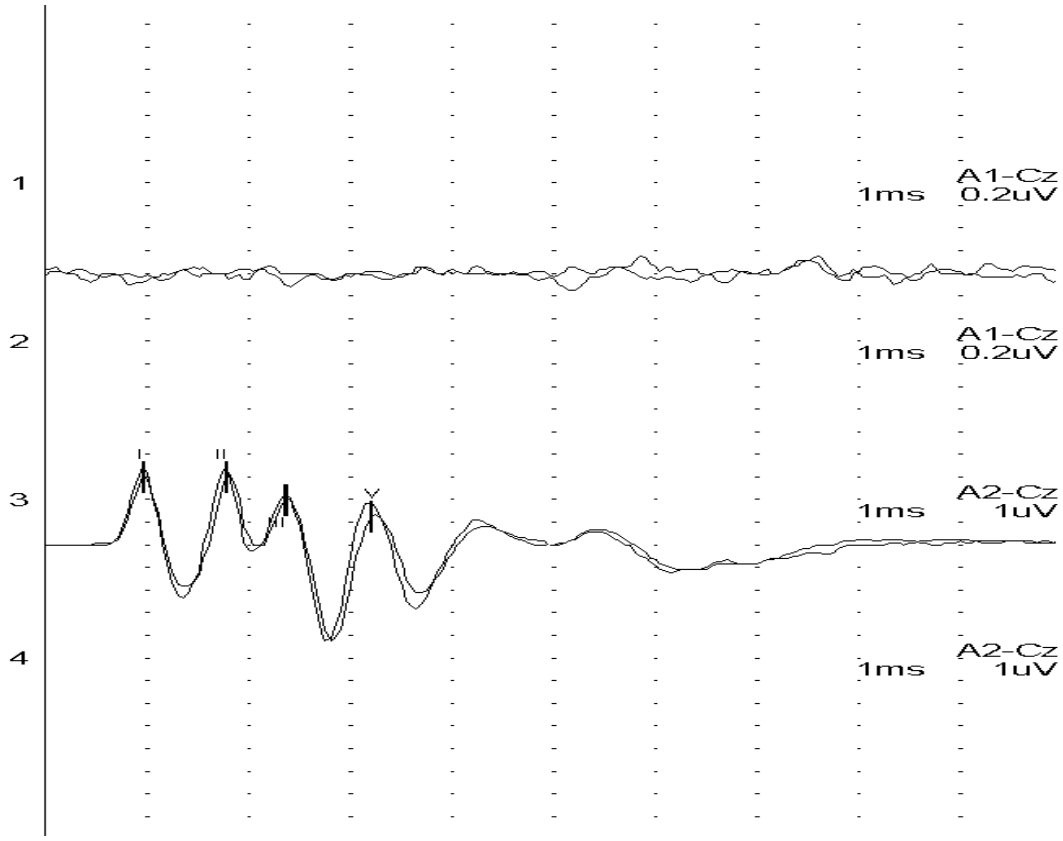
Table 1.

Breed and hearing status in 84 pure-breed white cats

Breed	Number of cats	Normal hearing	Unilateral deafness	Bilateral deafness
British Shorthair	31	26	4	1
Maine Coon	14	9	1	4
Turkish Angora	11	10	1	0
Persian	9	8	0	1
Foreign White	6	5	1	0
Norwegian Forest	6	2	1	3
Highlander	4	4	0	0
Balinese	1	1	0	0
Devon Rex	1	1	0	0
Oriental Shorthair	1	1	0	0

Figure 1.

BAER derived from unilateral deaf, 3 months old, female white Main Coon cat; stimulation intensity 80 dB nHL (ipsilateral ear), white masking noise 50 dB nHL (contralateral ear)



IV Discussion

1. General aspects and limitations of the study

The present study was designed so that one part of the study was retrospective and the other part was prospective. This was inevitable because of the long period of time (that is, 1995 - 2008) needed before data concerning the required number of client-owned pure-breed white cats were collected. Unfortunately, as a consequence of this, for some white cats (29/84), no data about eye colour were available for the calculation of the association between eye colour and deafness.
Nevertheless, the cats examined for the purpose of this study had all undergone the same click-evoked BAER procedure, or at least, there were only slight differences in the technical conditions of the electrodiagnostic equipment, which had no significant influence on the results of the examination. The measurement and results that were not done by the author of this study were re-checked by the author himself and additionally and independently checked by two senior neurologists working at the Clinic for Small Animal Medicine, LMU Munich.
The main limitations of the present study and all other studies that have been published to date and that have used the click-evoked BAER test as a method for assessment of hearing impairment in cats is that the hearing frequency range in cats goes up to 80 kHz and the highest frequency value used for BAER measurement in the present study was 8 kHz (HEFFNER and HEFFNER, 1985). Because of the limited technical features of the auditory stimulator used for hearing assessments (Viking IV/Quest, frequency range 250 Hz - 8 kHz) it is not possible to define true hearing status in cats, particularly in the high frequency hearing range. Future investigations should focus on providing technical possibilities to survey the whole frequency range in cats and thus to distinguish clearly partial from normal hearing cats.

2. Occurrence of CSD in pure-breed white cats

Even though it is accepted that cats with white coat colour are more likely to be deaf, to date there has been no study to describe the real prevalence rates of deafness in pure-breed white cats.

The present study is the first one to give reliable results on the occurrence rates among client-owned pure-breed white cats using the BAER test as an objective method for hearing assessment.

On the other hand, many reports have been published describing the association between white fur, blue eyes and deafness in experimental mixed-breed white cats (BOSHER and HALLPIKE, 1965; BERGSMA and BROWN, 1971; MAIR, 1973; DELACK, 1984; HEID et al., 1998; GEIGY et al., 2007). Most of these studies were done because mixed-breed white cats in experimental colonies were being used as an animal model for congenital deafness in humans. In detail, cochlear pathology, cochlear and higher neuroanatomic structure function and more recently cochlear implants have been investigated in the congenitally deaf white cat (MAIR, 1973; REBILLARD et al., 1976; PUJOL et al., 1977; REBILLARD et al., 1981a, 1981b; SCHWARTZ and HIGA, 1982; RYUGO et al., 1998, 2003; REBSCHER et al., 2007; FALLON et al., 2009).

The overall occurrence rates of unilateral and bilateral CSD (20.2 %) in the present study show that deafness occurs frequently in pure-breed cats with white coat colour. This relatively high percentage of deaf pure-breed client-owned white cats among the examined population (84 cats) gains a greater significance considering the year-long explicit recommendation from the breeding association to exclude deaf animals from breeding programs. Moreover, the breeding of cats with white coat colour has been strictly discouraged by the German Animal Rights Law since 1998 (SCHMITZ, 2004).

In the study by GEIGY et al. (2007), hearing prevalence data for three specific cat breeds namely, Norwegian Forest, Maine Coon, and Turkish Angora, with deafness prevalence rates of 18 %, 17 %, and 11 %, respectively, based on 329, 134, and 474 subjects were reported. Analyses of iris and coat colour were not reported; the subjects included both white and pigmented variants and the applied method for testing hearing was not uniform (some cats were BAER-tested, others were diagnosed based on behavioural assessments instead of the more reliable BAER). All this limited the possibility of comparing prevalence rates from this report to the overall deafness occurrence rate (20.2 %) from the present study. Although the data from the study by GEIGY et al. (2007) are limited (underestimates), they still represent the only other published data on deafness prevalence in client-owned pure-breed cats.

The occurrence rates of unilateral and bilateral CSD (20.2 %) in the present study were much lower than in experimental studies in mixed-breed cats with white coat colour by BERGSMA and BROWN (1971), MAIR (1973), DELACK (1984) and GEIGY et al. (2007) which resulted in 42.6 %, 89.3 %, 51.5 %, and 67.0 % of deaf cats, respectively. Differences among deafness rates could result from a different genetic basis of deafness in experimental and client-owned white cats. Many experimental studies of mixed-breed cats included subjects that were homozygous for the dominant W allele, which may not be the case in the present study (BOSHER and HALLPIKE, 1965; BERGSMA and BROWN, 1971; MAIR, 1973; DELACK, 1984; GEIGY et al., 2007). Deafness occurrence rates of client-owned pure-breed white cats reported here could also support the hypothesis that some pure-breed cats may carry the c^s-Siamese dilution pigment gene and thus be less prone to deafness than are mixed-breed cats (PEDERSEN, 1991).
Furthermore, different prevalence rates could result from different mating policies. Mating policies within the registered breeds aim to avoid matings that could lead to deaf offspring whereas the purpose of mating is always the reverse in experimental studies (that is, promoting deafness), resulting in high inbreeding coefficients.

3. Unilateral CSD in pure-breed white cats

The results from the present study clearly show that unilateral CSD exists at least in white colour-associated deafness syndromes in cats. Unilateral CSD was diagnosed in 9.5 % of the examined pure-breed white cats. Although not emphasized in publications, unilateral deafness also was a frequent finding in experimental investigations. The prevalence of unilateral deafness was 12 % compared to 49 % of bilateral deafness in 256 mixed-breed white cats from four experimental investigations (BOSHER and HALLPIKE, 1965; BERGSMA and BROWN, 1971; MAIR, 1973; DELACK, 1984). Yet, unilateral deafness appeared less frequently (12 %) than did bilateral deafness (49 %) in mixed-breed white cats, whereas in the present study, the rate of unilateral deaf cats (9.5 %) was roughly equal to the rate of bilateral deaf pure-breed white cats (10.7 %).
Similarly, unilateral deafness is frequently observed in dogs affected with CSD. For the studied canine breeds - Dalmatians, Bullterriers, English Setters, English Cocker Spaniels, Australian Cattle Dogs and Border Collies - the prevalence rates

of unilateral CSD were 21.9 %, 9.9 %, 6.5 %, 5.9 %, 12.2 %, 2.3 %, respectively (HOLLIDAY et al., 1992; STRAIN, 2004; PLATT et al., 2006). In contrast to cats, the frequency of unilaterally affected dogs is generally higher than that of bilaterally deaf animals. Indeed, most of the studies show that about two to three times more Dalmatians are unilaterally rather than bilaterally deaf (HOLLIDAY et al., 1992; FAMULA et al., 1996; WOOD and LAKHANI, 1997; MUHLE et al., 2002; JURASCHKO et al., 2003b; STRAIN, 2004).

The pathophysiology and genetics of unilateral deafness have yet to be completely elucidated. One possibility is that there are different stages of degeneration in the two ears, even though most studies failed to show progressive cochlear degeneration (BOSHER and HALLPIKE, 1965; RYUGO et al., 2003). Another hypothesis could be that there are different forms of pathology in the two ears, one being milder and the other being more severe. One further limitation of the present study is that histopathologic examinations were not performed because the cats were client-owned pets.

In all of the eight unilaterally deaf white cats in this study, non-affected ears showed normal BAER waveforms and hearing thresholds. Furthermore, hearing thresholds determined in the normal ear of unilaterally deaf cats were in the same range as in bilaterally hearing cats (< 30 dB nHL). In agreement with these results, some studies have shown that no pathological changes after histological examination can be noticed in normal hearing ears in unilateral deaf white cats (WILSON and KANE, 1959; MAIR, 1973; REBILLARD et al., 1981b). Experimentally, an increased hearing threshold and mild cochlear pathology was demonstrated in the normal hearing ear of a few unilaterally deaf animals (MAIR, 1973; RYUGO et al., 2003). Although the preliminary click-evoked BAER testing suggests that the normal ear in unilateral deafness is not impaired and has a normal hearing threshold, this remains to be proven by more sophisticated, frequency-specific testing of hearing function. In addition, it should be noticed that precise measurement of the hearing threshold in the present study was limited because of the technical characteristics of the auditory stimulator that was used (that is, the lowest end of the stimulator was 30 dB nHL, which is relatively high).

The high occurrence of unilateral deafness needs to be taken into account if the cat is used as a model in human hearing research. Interestingly, unilateral deafness in humans is rarely recognized as a manifestation of congenital deafness. In studies by LINA-GRANADE et al. (1995) and DIKKERS et al. (2005), it has

been stated that possible causes for the unilateral appearance of CSD in humans can be the variable expression of bilateral isolated hereditary deafness, incomplete Klein-Waardenburg syndrome with stria vascularis anomalies and highly variable gene expression, or unilateral cochlear aplasia. The prevalence rates for hereditary unilateral congenital deafness in humans are unknown. Finally, as the CSD in white cats is mostly associated with the presence of the W gene, which is responsible for the early embrional migration of the melanocyte precursor cells to the inner ear, it may be that a specific mutation in the W gene affects the migration process, resulting in the unilateral appearance of the hearing disorder.
The fact that CSD in cats and dogs can be unilaterally expressed proves the great importance of using the BAER test as an objective method for hearing assessment before breeding. The behavioural method for diagnosing hearing impairment should not be used because unilateral deafness cannot be detected with any reliability. In unilaterally deaf animals, the only behavioural sign of deafness is a difficulty in localizing the source of a sound, and many animals adapt to that also. Since the unilaterally deaf cats and dogs are carrying a genetic defect, which is probably just not completely expressed like in bilaterally deaf subjects, unilaterally deaf animals should be excluded from further breeding programs.

4. Molecular genetic basis of CSD in white cats

Up to now, neither the mechanism of inheritance nor the molecular genetic basis of CSD in white cats has been annotated completely.
White colour-associated CSD in cats has been generally linked to the W gene, which is dominant over other colours and is unrelated to albinism. The absence of melanocytes produces white coat colour which is a consistent feature of the W gene. Melanocytes have been suggested as being of great importance for normal stria vascularis development and function, although their actual function has not been fully described. The intermediary cells of the stria vascularis are melanocyte-derived cells that migrate to the developing inner ear from the neural crest and are thought to play an important role in the generation of the endocochlear potential by the stria vascularis (WESTON, 1970; STEEL and BARKWAY, 1998; TACHIBANA, 1999). Defective and insufficient numbers of melanocytes lead to stria vascularis malformation and dysfunction in the inner ear of the German waltzing guinea pig (JIN et al., 2007). In mice, mutations of the c-kit receptor

tyrosine kinase encoded at the W locus do not alter early migration or differentiation of melanoblasts, but severely affect melanoblasts` survival (CABLE et al., 1995). Mutation in the genes PAX3, MITF, C-KIT and SOX10 which are closely involved in cochlear melanocyte development, cause hearing loss in humans and in other mammals (PRICE and FISCHER, 2001; TACHIBANA, 2001).

The cats evaluated in this study belonged to the ten registered pure-breed cats described as carrying the dominant white gene (W). White albino cats with Siamese *cs*, blue-eyed *ca* or pink-eyed *c* recessive alleles from gene locus C, which determines the quantity of pigment granules in melanocytes, usually are not affected because they have a normal distribution of melanocytes (PEDERSEN, 1991). Attempts to identify the genomic identity and changes in the W gene as a cause for deafness in white cats are in progress (STRAIN, 2007). Another way for cats to exhibit white fur is the white spotting gene (S), sometimes called piebald, but to our knowledge there is no report of deafness associated with its presence in cats. In addition, it is not clear that it is the same gene as in dogs (KARLSSON et al., 2007). Furthermore, the effects of white-producing genes can be modified by currently undefined genes resulting in either strong or weak gene expression. A pleiotropic gene segregating for deafness and blue irises, with additional polygenic effects has been suggested (GEIGY et al., 2007).

Considerable progress has been made over the past decade in identifying the genetic loci and genes associated with mammalian deafness. To date, the genes underlying more than a hundred different syndromes that include hearing impairment have been identified (VAN CAMP and SMITH, 2009).

Deafness is the most common human sensory disorder world-wide, with approximately one in every thousand children born with a serious permanent hearing impairment, and about 60 % of people over 70 suffering from progressive hearing loss (BITNER-GLINDLZICZ, 2002). In humans, congenital sensorineural deafness can be the only clinical manifestation (nonsyndromic forms of deafness) or be associated with other symptoms or anomalies (syndromic forms of deafness). Some of these syndromes are genetically heterogeneous (that is, they are the result of mutations in different genes), and other syndromic forms of deafness resulting from mutations in a single gene. The nonsyndromic forms of congenital deafness are categorized according to their mode of inheritance i.e. autosomal dominant (DFNA), autosomal recessive (DFNB), X chromosome-

linked (DFN), Y chromosome-linked, or mitochondrial genome linked. The autosomal recessive form (DFNB) accounts for approximately 80 % of the cases of early-onset congenital deafness in humans (PETIT, 2006). Frequent involvement of the connexin-26 gene (GJB2), which encodes a gap junction protein in the inner ear and whose biallelic mutations causes the DFNB1 form of deafness, is of great medical significance in early-onset congenital deafness (KENNESON et al., 2002; DEL CASTILLO et al., 2003).

Moreover, the CSD in white cats is often interpreted as the feline homologue of the human Waardenburg syndrome (BERGSMA and BROWN, 1971; MAIR, 1973; REBILLARD et al., 1976; REBILLARD et al., 1981a, 1981b; DELACK, 1984). Disruption of melanocyte differentiation is the etiology of Waardenburg syndrome, which includes sensorineural hearing loss and pigmentary abnormalities of the skin, hair, and eyes (READ and NEWTON, 1997). The Waardenburg syndrome is classified into four types, depending on the presence or absence of additional symptoms, which are caused by mutations in the six genes EDN3, EDNRB, MITF, PAX3, SLUG and SOX10. These genes are known to be expressed in the neural crest (EDN3, EDNRB, PAX3, SLUG, SOX10) or directly in the melanocytes (MITF) and, are involved in the migration, differentiation or survival of melanocytes (BONDURAND et al., 2000; SANCHEZ-MARTIN et al., 2002). DESTEFANO et al. (1998) and WATANABE et al. (1998) showed that the PAX3 gene transactivates the MITF promoter, and that failure of this regulation, due to casual mutation in the PAX3 gene, causes the auditory-pigmentary symptoms in humans with Waardenburg syndrome. The genes causing human Waardenburg syndrome are only examples of a few suitable candidate genes for deafness in cats, and many more genes with mutations known to result in human cochleo-saccular degeneration could possibly be involved in feline congenital sensorineural deafness. The fact that different types of underlying cochlear pathology may exist in CSD in white cats, makes analysis of the mode of inheritance in cats even more complex (REBILLARD et al., 1981b; SAADA et al., 1996; RYUGO et al., 2003).

Recent molecular genetic studies in pigment-associated deafness in dogs identified the genomic identities of the piebald gene S and the merle gene M. For the piebald gene, it is shown to be the MITF pigmentation gene and the merle gene is a retrotransposon insertion in the SILV pigmentation gene (CLARK et al., 2006; KARLSSON et al., 2007). Both of these genes have been identified in other

animal species, and mutation in MITF has been identified as causative for deafness in humans and mice (TASSABEHJI et al., 2004). However, it has also been shown that some of the pigment-associated genes are not causative for either white pigmentation or for deafness in several dog breeds. ZEMKE et al. (1999) detected no differences in the sequences of the coding regions of EDNRB, MITF and PAX3 between hearing and deaf dogs from different breeds. PAX3 has also been excluded as a candidate for deafness in a predominantly Swiss Dalmatian population (BRENIG et al., 2003). This finding does not rule out point mutation; therefore, the PAX3 gene cannot be definitively excluded as a cause of congenital sensorineural deafness in cats and dogs. Moreover, the genes EDNRB and KIT have been shown not to be responsible for white spotting in Border Collies (METALLINOS and RINE, 2000), whereas KITLG has been excluded as candidate gene for merle in Australian Shepherds (SCHMUTZ et al., 2003). Nonetheless, these excluded genes could still remain as candidates for pigment-associated deafness in dogs, since different founder effects may occur in different dog breeds or in geographically isolated lines of one breed leading to the existence of different mutations in one gene or in different genes (RAK et al., 2003). Unsurprisingly, a wide variety of molecules has now been implicated in the causation of deafness in humans and mice, including transcription factors, motor molecules (for example, unconventional myosins), extracellular matrix components, gap and tight junctions, ion channels and ion channel activators, and many more. The situation is likely to be similar in cats.

Although the BAER test is a reliable method for identifying unilaterally and bilaterally deaf cats, the high occurrence of deafness presented in this study (20.2 %) showed that the BAER test does not seem to be an effective way of reducing the occurrence of deafness in affected breeds, particularly in a recessive mode of inheritance, so that hearing cats can still be genetic carriers. There is no doubt that the future challenge in the study of human and feline deafness will be to identify and analyse the function of (additional) deafness-causing genes using high-density genome screens or genome-wide association screening. The use of comparative genomics can be a powerful and very effective approach towards unravelling the genetic basis of feline and human deafness. If causal mutations for CSD in cats are identified, breeding strategies can be developed to reduce the prevalence in affected cat breeds while gaining new insights into the molecular mechanism of

auditory function, and possibly translating these basic findings into therapeutic strategies.

5. Association between CSD and blue eyes in pure-breed white cats

An association between deafness and blue-eyed white cats was noted as early as 1828, and DARWIN commented on it in his famous publication *The Origin of Species* (BREE, 1828; DARWIN, 1859). Of all mammals, cats apparently present with the greatest variation in iris colour. The eyes of blue-eyed white cats are partially depigmented. The iris and retina epithelia are normally pigmented, but pigment is absent, wholly or in part, from the iris stroma, choroid and tapetum. The partial depletion of pigment is comparable to the macroscopically observable heterochromia of the iris (THIBOS et al., 1980). The absence of the tapetum usually results in marginal or obvious dilation of the pupil (BERGSMA and BROWN, 1971). Tissues affected by the W gene originate from the neural crest, while those derived from the embryonic optic cup (iris and retinal epithelium) are not affected (THIBOS et al., 1980).

With respect to iris colour, inner ear degeneration was observed in 60 % of cats with blue-blue irises, 30 % of cats with blue-pigmented ones, and 10 % of cats with bilateral pigmented irises; therefore blue eyes were associated with deafness 90 % of the time in three experimental studies using a small number of white mixed-breed cats (WOLFF, 1942; WILSON and KANE, 1952; SUGA and HATTLER, 1970). BERGSMA and BROWN (1971) and MAIR (1973) also found clear association between blue eye colour and deafness. The prevalence of deafness (unilateral and bilateral combined) in mixed-breed white cats with two blue eyes was 65 % and 85 %, respectively. In cats with one blue eye it was 39 % and 40 %, respectively, and in cats with no blue eyes it was 22 % and 17 %, respectively. In general, deafness was found three to five times more often in white cats with two blue eyes than in white cats without blue eyes and about two times more often in white cats with one blue eye. Examination of inner ear degeneration and ipsilateral eye colour indicates that the blue eye color is much more frequently associated with inner ear defects than is the pigmented eye colour (approximately 4:1). BERGSMA and BROWN, (1971) found that, of nine cats with one blue iris and inner ear degeneration, no one-to- one correlation could be established; that is, the observed frequency of unilateral expression did not differ

from the product of expectation of unilaterality alone, thus suggesting that these two aspects of the syndrome are expressed independently of each other (DELACK, 1984). In unilaterally deaf individuals, however, it can be stated that if the ipsilateral eye colour is not blue, then neither is the contralateral blue (MAIR, 1973).

Pure-breed blue-eyed cats examined for the purpose of the present study were more likely to be deaf than were cats with other eye colours. The combined occurrence of unilateral and bilateral deafness was 44.4 % in cats with two blue eyes, 20.0 % in odd-eyed cats, and 18.9 % in cats with other eye colours. The odds ratio for a cat with one or two blue eyes being deaf was 3.72 (95 % confidence interval, 0.9 - 18.4) compared to cats with other eye colours. The odds ratio for cats with two blue eyes being deaf was 5.75 (95 % confidence interval 1.2 - 31.2) compared to cats with eye colours other than blue. These results are in agreement with results from other investigations mentioned previously, confirming the fact that white cats with blue eyes are more likely to be deaf than are white cats with eye colours other than blue. Nevertheless, findings from the presented study are the first ones describing the association between eye colours and deafness in pure-breed white cats up to now.

The fact that the predisposition to deafness and eye colour is genetically controlled has been known for many decades (DARWIN, 1859; BOSHER and HALLPIKE, 1965; BERGSMA and BROWN, 1971; GEBHARDT et al., 1979; DELACK, 1984). The inheritance of eye colour appears to be polygenic, although it is equally apparent that genes from dominant white (W), Burmese dilution (c^b), Siamese dilution (c^s), and blue-eyed albino (c^a) indicate a certain degree of monogenic control (WAARDENBURG, 1951; ROBINSON, 1977). A recently published report by GEIGY et al. (2007) supports the hypothesis of a pleiotropic major gene segregating for deafness and eye colour and indicates also that besides the major gene, there was an important influence of polygenic effects. The model for a joint segregation analysis of hearing status and blue eyes included, in the afore-mentioned study, the independent and additive contribution of a single gene with a pleiotropic effect on hearing and eye colour, additive genetic effects for a number of independent genes affecting both traits and a genetic correlation between these genes as well as individual-specific environmental effects on both traits with the corresponding environmental correlation. Unfortunately, for the examined pure-breed white cats in the study presented here no information on the

genetic base was available, so it was not possible to look for plausible modes of inheritance for eye colour and hearing status. As long as the responsible genes and modes of inheritance are definitively not known and the marker tests are not available, mating and selection programs using BAER recordings remain the only alternative to reduce the occurrence of CSD in pure-breed white cats. However, reliable and complete records on genotypes for cat's families in the whole breeding population are necessary.

V Summary

Congenital sensorineural deafness in client-owned pure-breed white cats

Dejan Cvejić

The objective of this study was to provide data on the occurrence of congenital sensorineural deafness in client-owned pure-breed white cats presented to the Clinic for Small Animal Medicine (1995 - 2008). For this purpose, 84 pure-breed white cats that were presented for a routine hearing test before breeding were evaluated. The cats belonged to ten different registered cat breeds. Hearing status was assessed using the click-evoked brainstem auditory evoked response (BAER), as an objective electrodiagnostic method for hearing assessment in animals; 20.2 % of the examined pure-breed white cats were deaf in one or both ears; 10.7 % were bilaterally deaf and 9.5 % of the cats were unilaterally deaf. The deaf cats belonged to six different registered cat breeds (Turkish Angora, British Shorthair, Maine Coon, Norwegian Forest, Persian, Foreign White). The deafness occurrence rate was 20.0 % in female cats and 20.6 % in male cats. There was no association between gender and deafness ($p = 0.851$).

Among 55 pure-breed white cats in which the eye colour was documented, the combined occurrence of unilateral and bilateral deafness was 44.4 % in cats with two blue eyes, 20.0 % in odd-eyed cats, and 18.9 % in cats with other eye colours. Blue-eyed cats were more likely to be deaf than were cats with other eye colours ($p = 0.040$). The odds ratio for cats with two blue eyes being deaf was 5.75 compared to the cats with eye colours other than blue.

The presented study has shown that unilateral and bilateral deafness continues to occur frequently in pure-breed white cats in Germany despite the recommendation from breeding organizations to avoid the breeding of white cats and the fact that the breeding of white cats is discouraged by the German Animal Rights Law.

VI Zusammenfassung

Untersuchungen zum aktuellen Vorkommen angeborener Taubheit bei weißen Rassekatzen

Dejan Cvejić

Ziel dieser Arbeit war es, objektive Daten zum Vorkommen angeborener Taubheit bei weißen Rassekatzen zu erheben. Hierfür wurden die Gehörtests - Click-Evoked Brainstem Auditory Evoked Response (BAER) - von 84 reinrassigen weißen Katzen ausgewertet, die zur Beurteilung des Hörvermögens im Rahmen der Zuchtzulassung in der Medizinischen Kleintierklinik vorgestellt wurden. Die Katzen gehörten zehn verschiedenen registrierten Katzenrassen an. Das Hörvermögen wurde mit einer objektiven elektrodiagnostischen Methode, den click-evozierten Hirnstammpotentialen (BAER) für jedes Ohr untersucht. Insgesamt wurden 20,2 % der untersuchten Katzen entweder als ein- oder beidseitig taub beurteilt. 10,7 % der Katzen waren beidseitig und 9,5 % einseitig taub. Die tauben Katzen gehörten sechs verschiedenen Rassen an: Türkisch Angora, Britisch Kurzhaar, Maine Coon, Norwegische Waldkatze, Perser, Foreign White. Männliche und weibliche Katzen waren gleichermaßen betroffen ($p = 0,851$). Eine Taubheit wurde bei 44,4 % der Katzen mit zwei blauen Augen, bei 20,0 % der Katzen mit einem blauen Auge und bei 18,9 % der Katzen mit einer anderen Augenfarbe beobachtet. Bei Katzen mit blauen Augen würde häufiger eine Taubheit festgestellt ($p = 0,040$). Katzen mit zwei blauen Augen hatten ein größeres Risiko für eine ein- oder beidseitige Taubheit (Odds ratio = 5,75) als Katzen anderer Augenfarben.

Die hier vorliegende Studie zeigt, dass einseitige und beidseitige Taubheit immer noch häufig bei reinrassigen weißen Katzen in Deutschland vorkommt; trotz der Empfehlungen des Zuchtverbandes, das Züchten weißer Katzen zu vermeiden und der Tatsache, dass das deutsche Tierzuchtgesetz eine Zucht, die zu erblich bedingten Defekten führt, nicht erlaubt.

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X Appendix

Figure 4 Normal BAER, 3 months old, male Turkish Angora cat. Stimulation intensity 80 dB nHL (ipsilateral ear); white masking noise 50 dB nHL (contralateral ear)

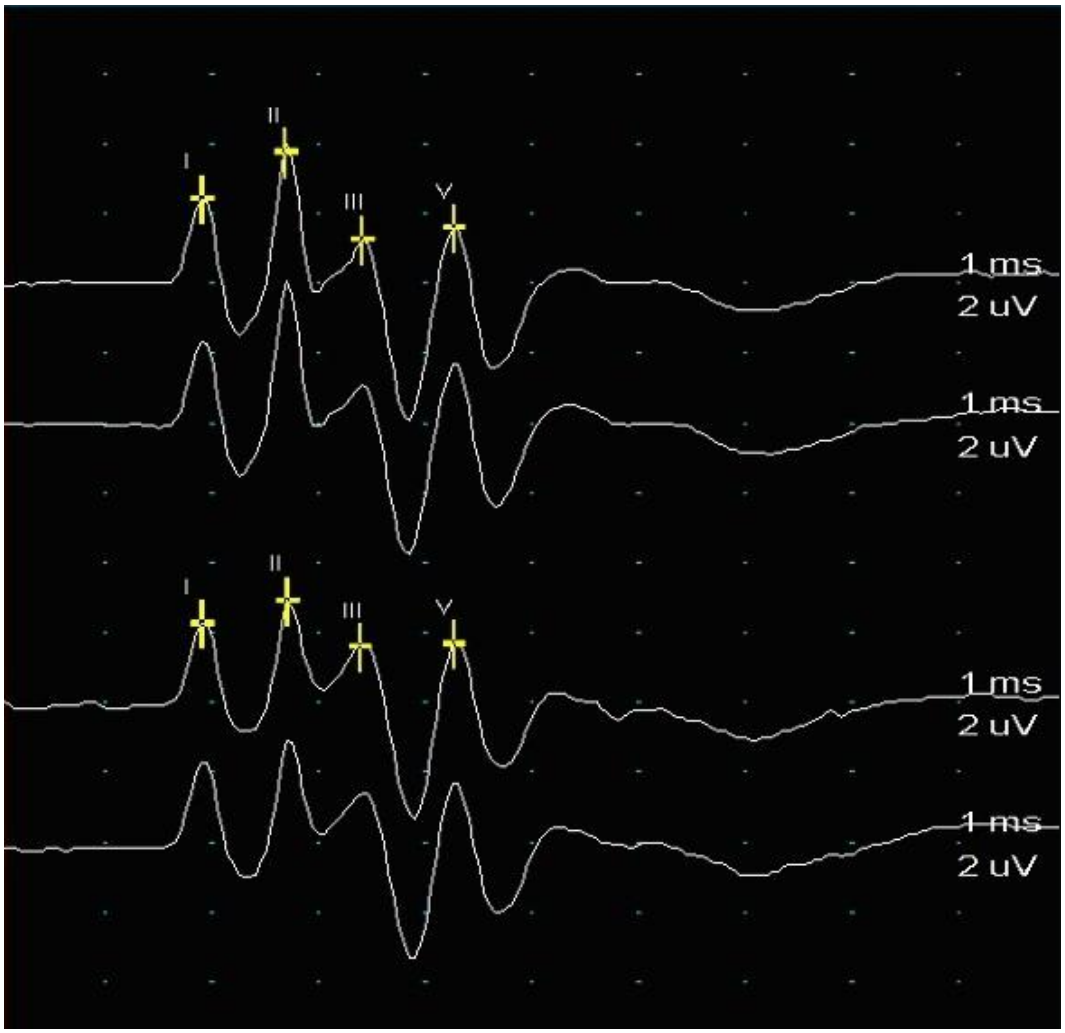


Figure 5 BAER from bilateral deaf, 4 months old, male Norwegian Forest cat. Stimulation intensity 90 dB nHL (ipsilateral ear), white masking noise 50 dB nHL (contralateral ear)

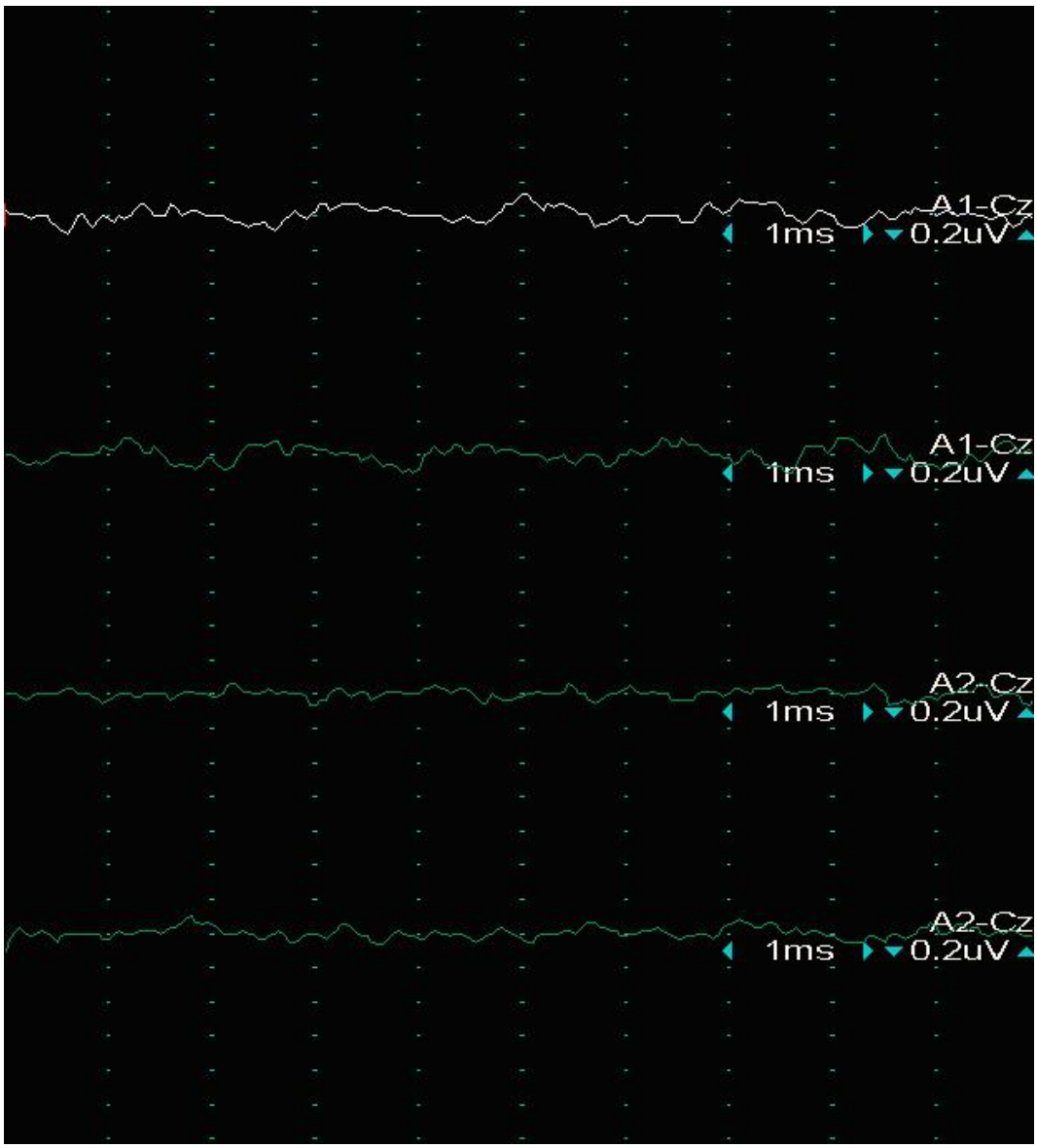


Figure 6 BAER from unilateral deaf, 6 months old, female British Shorthair cat. Normal BAER waveform recorded from right ear (1st and 2nd trace) after stimulation with 90 dB nHL; BAER response from left ear (3rd and 4th trace) after stimulation with 90 db nHL was a flat line that is, cat was deaf in left ear. Contralateral ear has been masked with white noise at the intensity from 50 dB nHL.

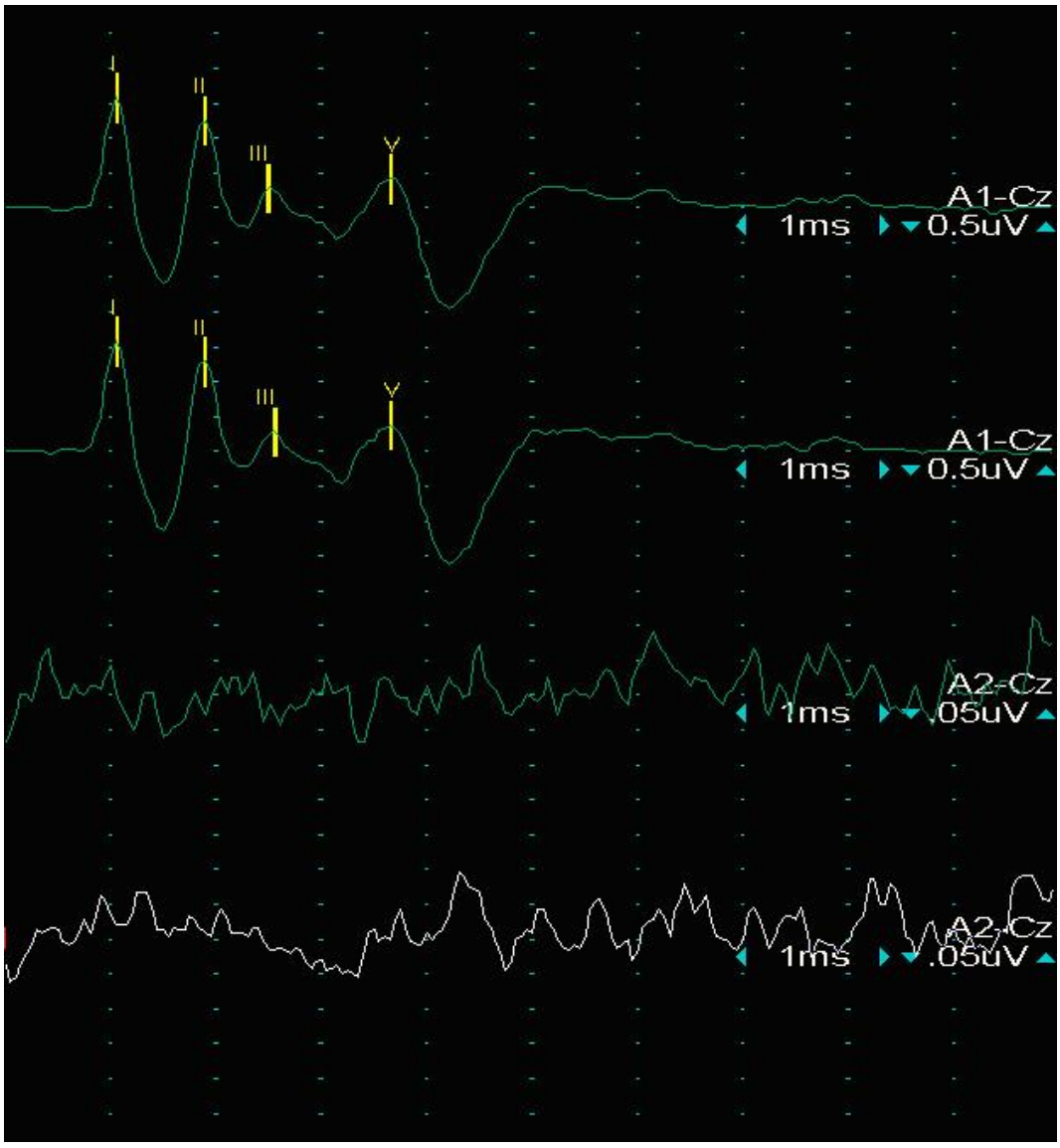


Figure 7 Electrodiagnostic device Viking Quest* used for BAER measurement in this study.

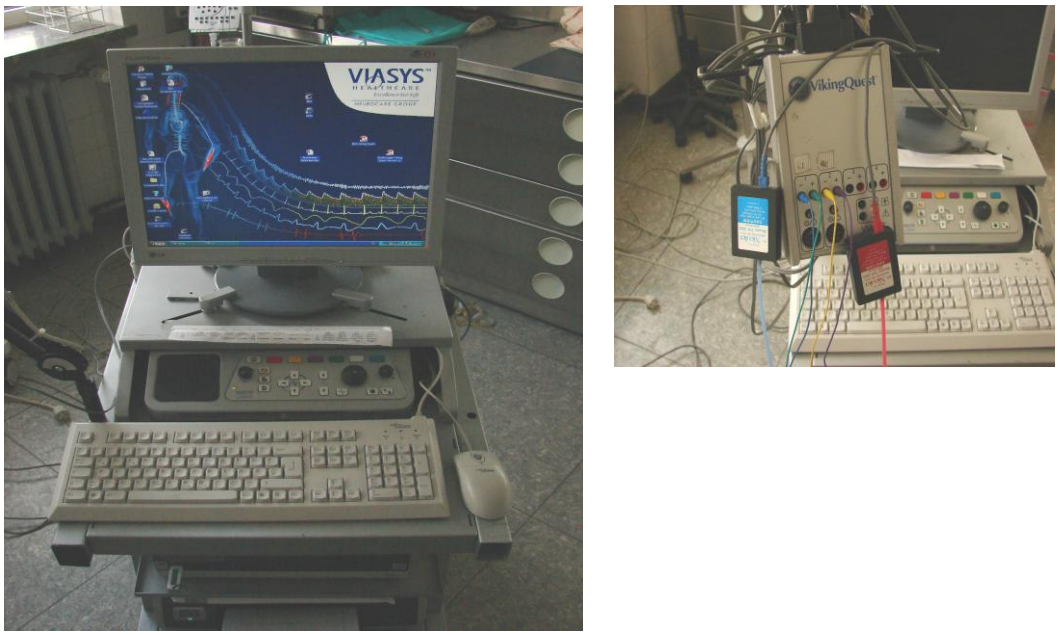


Figure 8 13 months old, male Persian cat during the BAER recording set.

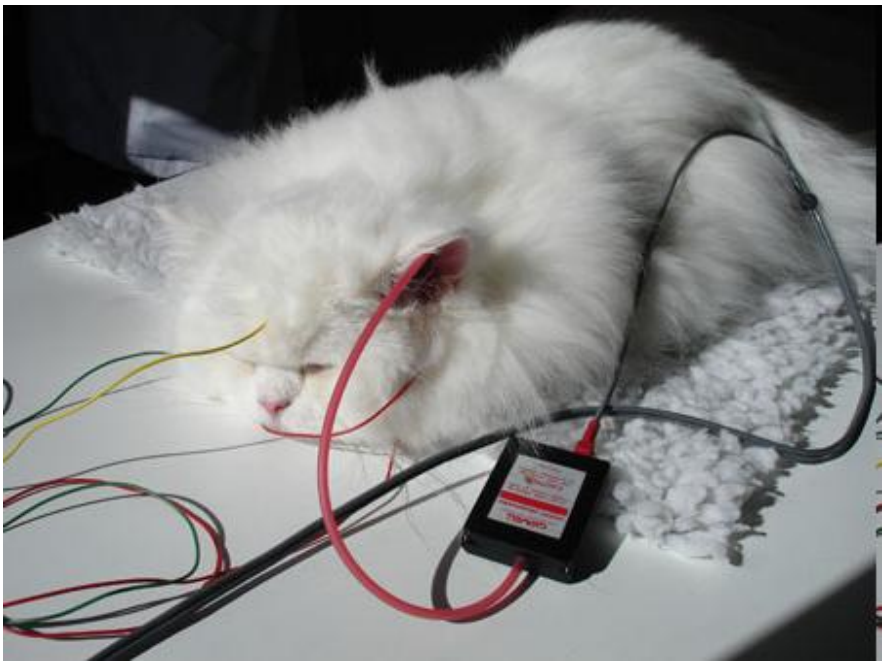


Figure 9 Disposable 12mm platinum/iridium EEG electrode* used as needle electrode for BAER recordings.

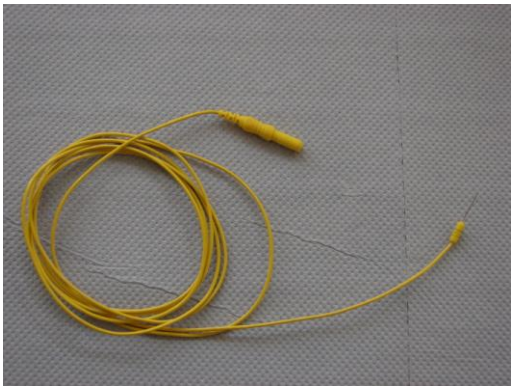


Figure 10 Insert phones TIP 300*; used to deliver the click stimuli to the external ear canal of examined cats.

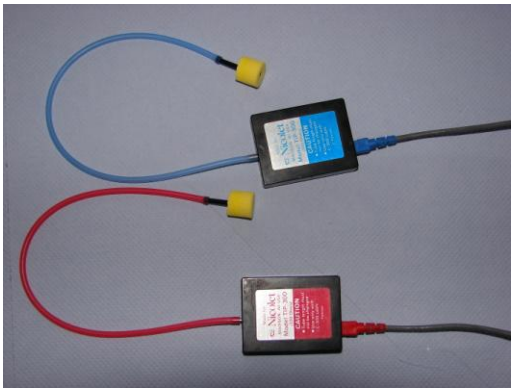
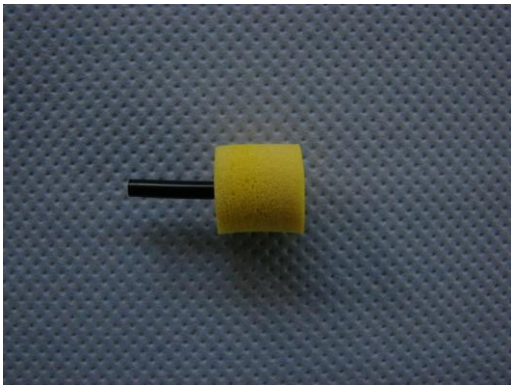


Figure 11 Foamed eartip (13 mm)*; end part of the insert phones TIP 300, placed directly in the external ear canal of examined cats.



* Nicolet Biomedical, Madison, Wisconsin, USA