NTNU
Innovation and Creativity

Diffusion-Based Model for Noise-Induced Hearing Loss

Sverre Aas
Tron Vedul Tronstad

Master of Science in Electronics
Submission date: June 2007
Supervisor: Odd Kr. Pettersen, IET

Norwegian University of Science and Technology
Department of Electronics and Telecommunications

Problem Description

Damage to the hearing arises either as a direct mechanical destruction at high sound pressure levels, or as exhaustion of cells and tissue over time at lower levels. A model of this exhaustion can be found through studies of the hearing organ with regard to blood flow and other transportation of energy and nutritions. Such model should probably contain one or more diffusion elements. Parameters for the model could be found through studies of earlier experiments. If the model is correct it can give a better estimate of noise-induced hearing loss than the prevailing models of hearing damage today.

Assignment given: 15. January 2007
Supervisor: Odd Kr. Pettersen, IET

Preface

This thesis is the result of the work done during the 10th and final semester of the Master's program at the Department of Electronics and Telecommunications, NTNU. The work has been carried out between January and June 2007, with professor II Odd Kr. Ø. Pettersen as head supervisor and research scientist Olav Kvaløy at SINTEF IKT as teaching supervisor. This thesis is written after a thesis proposal from SINTEF IKT Akustikk, and is part of their research into noise-exposure and its effect on hearing.

Being involved in this project has given us further insight into the interesting and important subject of noise-induced hearing loss.

The thesis is submitted to the Department of Electronics and Telecommunications at the Faculty of Information Technology, Mathematics and Electrical Engineering, NTNU, as a final fulfillment of our M.Sc. degrees.

Acknowledgments
The authors thank Olav Kvaløy and Odd Kr. Ø. Pettersen at SINTEF IKT for their support and advise, and for their genuine interest in our work.

Trondheim, June 13, 2007

Sverre Aas

Tron Vedul Tronstad

Abstract

Among several different damaging mechanisms, oxidative stress is found to play an important role in noise-induced hearing loss (NIHL). This is supported by both findings of oxidative damage after noise exposure, and the fact that upregulation of antioxidant defenses seem to reduce the ears susceptibility to noise. Oxidative stress mechanisms could help explain several of the characteristics of NIHL, and we therefore believe that it would be advantageous to estimate noise-induced hearing impairment on the basis of these, rather than the prevailing energy based methods.

In this thesis we have tried to model progress of NIHL using diffusion principles, under the assumption that accumulation of reactive oxygen species (ROS) is the cause of hearing impairment. Production, and the subsequent accumulation, of ROS in a group of outer hair cells (OHCs) is assessed by different implementations of sound pressure as in-parameter, and the ROS concentration is used in estimation of noise-induced threshold shift. The amount of stress experienced by the ear is implemented as a summation of ROS concentration with different exponents of power.

Measured asymptotic threshold shift (ATS) values are used as a calibrator for the development of threshold shifts. Additionally the results are evaluated in comparison to the standards developed by the International Organization for Standardization (ISO) and the American Occupational Safety and Health Administration (OSHA).

Results indicate that ROS production is not directly proportional to the sound pressure, rather an accelerated formation and accumulation for increasing sound pressure levels (SPLs). Indications are also that the correlation between concentration of ROS and either temporary threshold shift (TTS) and/or permanent threshold shift (PTS) is more complex than our assumption.

Because our model is based on diffusion principles we get the same tendency of noise-induced hearing loss development as experimentally measured TTS development. It also takes into account the potentially damaging mechanisms which occur during recovery after exposure, and has the ability to use TTS data for calibration. We therefore suggest that modeling of ROS accumulation in the hair cells could be used advantageously to estimate noise-induced hearing loss.

Contents

List of Figures

List of Tables

Abbreviations

ABR	Auditory Brainstem Responses
AO	Antioxidant
ARN	Acquired Resistance to Noise
ATS	Asymptotic Threshold Shift
BM	Basilar Membrane
CAP	Compound Action Potential
CAT	Catalase
CBF	Cochlear Blood Flow
CNS	Central Nervous System
DC	Deiters' cell
DNA	Deoxyribonucleic Acid
EEH	Equal Energy Hypothesis
EQ	Effective Quiet
GSH	Glutathione
GSSG	Glutathione disulphide
HeC	Hensen's cell
IHC	Inner Hair Cell
ISO	International Standard Organization
LMS	Least Mean Square
NIHL	Noise-Induced Hearing Loss
NIPTS	Noise-Induced Permanent Threshold Shift
MET	Mechano-Electrical Transduction
OC	Organ of Corti
OHC	Outer Hair Cell
OSHA	Occupational Safety and Health Administration
PTS	Permanent Threshold Shift
RM	Reissner's Membrane
RMS	Root Mean Square
RNA	Ribonucleic Acid
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SL	Spiral Ligament
SOD	Superoxide Dismutase
SPL	Sound Pressure Level
SV	Stria Vascularis
TM	Tectorial Membrane
TTS	Temporary Threshold Shift

Chapter 1

Introduction

Noise-induced hearing loss (NIHL) has been know since the beginning of written history, and deafness is found used as a term already among those who lived near the Nile falls in the first century A.D.. However, deafness was first used as a standard term of diagnosis for boiler workers in 1850, where noise was found to be the main cause of hearing impairment [45]. Since the beginning of industrialization NIHL has been, and still is, an ever increasing problem especially among workers in the industry. It has been estimated that at least 600 million people, in developed and developing countries, are working in environments with potentially dangerous levels of noise today (50-60 millions of these in USA and Europe), and that a substantial percentage of these have, or will develop, hearing impairment [48].

Earlier mechanical stress was thought to be the main reason for NIHL, with perhaps some influence of intense noise on inner ear blood flow, but recent studies have found metabolic fatigue to play an essential role in such impairment [53]. In 1971, Lim and Melnick [56] was the first to propose that intense metabolic activity could contribute to noise-induced inner ear pathology, but it did not gain any significant credibility or supporters before Yamane et al. [107], in 1995, demonstrated formation of free radicals in the inner ear tissues, and a subsequent loss of functionality after noise exposure.

Indications are that, when exposed to loud noise, the ears, i.e. the cells and tissues inside, will experience lack of oxygen and nutritions causing elevated production, and a subsequent accumulation, of waste products. This accumulation is found to be unhealthy for the sensory cells of the cochlea, and can result in permanent damage. We want to look at the mechanisms involved in accumulation of waste products and try to use these in estimation of hearing impairment as a result of noise exposure.

1.1 Determination of noise exposure and estimation of NIHL

Today, the prevailing way to evaluate noise exposure is by its total energy. ISO 1999 [42] makes the basis for this method, and uses an energy equivalent over eight hours in estimation of noise-induced permanent threshold shift (NIPTS). There is, however, reason to believe that energy considerations do not give a correct picture as to assess hearing impairment as a result of noise. Estimation of NIPTS from continuous noise would perhaps be quite accurate, but when introducing impulsive or intermittent noise, with shorter or longer uphold in noise exposure, the energy based method's validity has been found to decrease [5, 31, 76].

It is believed that, as long as daily sound exposure is below the 85 dBA[1] equivalent level limit, ears are fully recovered and ready to endure new eight hours of noise exposure every day. It would therefore be reasonable to believe that the ears regain some resistance to noise if the exposure stops for a period of time, and therefore have a somewhat larger tolerance when noise exposure returns, also during one single day (i.e. eight hours).

Through this study we want to explore the use of diffusion principles in a model for NIHL, and exploit the knowledge of noise-induced oxidative stress mechanisms in the cochlea, to estimate damage risk. The study aims to estimate and predict the progress of NIHL based on diffusion theory, and oxidative stress theory. The hypothesis is that accumulation of oxidative species will eventually lead to destruction of delicate tissues in the inner ear, and that the accumulation process is advantageously modeled with the use of diffusion principles.

1.2 Thesis overview

Our hearing is one of the most delicate mechanisms in the whole body. Although its functionality is not yet fully understood, our understanding is constantly being expanded and the complexity by which it works is becoming more and more evident. In the next chapter (Ch. 2) we will give a review of the hearing, and the most important mechanisms and processes necessary for hearing functionality.

In Chapter 3 we will give an overview of the most important mechanisms of noise-induced hearing loss, and try to demonstrate the complex effects of noise on our hearing organ. Many different damaging pathways could lead to impairment of hearing, and it is likely that several contribute to the total, experienced hearing impairment, but indications are that the oxidative stress pathways are more important than the others, at least for low and medium level exposures.

In Chapter 4 we will present oxidative stress literature, and try to demonstrate the medical and biochemical basis for our modeling approach. As mentioned above; studies through the last couple of decades have demonstrated oxidative stress to be one very important factor in NIHL.

The model is, as mentioned above, based on diffusion principles, and in Chapter 5 we will

[1] Many countries use a 85 dBA equivalent level over 8 hours as an upper limit for occupational noise exposure per day.

explain our way of thinking. We will use the repeatedly mentioned principles to build a model, and try to show why and how it is possible to use such model in predicting TTS progress. We will *not* try to estimate PTS progress, but rather try to estimate the time at which permanent damage could start to occur when exposed to noise. It is assumed known that exposure to noise not necessarily cause irreversible, or permanent, damage, but rather a temporarily increased hearing threshold. It should therefore be possible to use TTS-data (and PTS-data, if it exists) as a kind of calibration of our model.

During the development of our model we have tested different hypotheses on how the generation of waste products elapses as a function of sound pressure. In Chapter 6 we give a presentation of the different alternatives, and try to point out advantages and disadvantages found.

We will, in Chapter 7, give a general discussion of the model. This includes the fundamental assumptions made, model parameters, the different alternatives, and try to give a clue on what the main modeling challenges are. We will also try to demonstrate what we believe to be the main focus in potential further studies.

Finally, we will, in Chapter 8, give an overall conclusion of the work done.

Chapter 2

Anatomy and Physiology of the Ear

The ear is often thought of as just the cartilaginous part visible outside our head, but there is much more to it than that. In this chapter we will try to demonstrate which, more or less, complex mechanisms are involved in hearing, and what makes us able to perceive pressure fluctuations as sound.

2.1 The outer ear

The outer ear consists of the pinna and the ear canal. Pinna is the latin name for the cartilaginous part of the ear that sticks out from our head. Its purpose is to catch and collect sounds, and guide it into the ear canal. The pinna is also important for our directional hearing, and thus help us to locate the source of the sound. The pinna can vary both in shape and size from person to person.

The ear canal is an approximately 25 mm long canal, with a diameter of approximately 6 mm [65]. The most medial (innermost) part of the ear canal is a nearly circular opening in the skull (temporal) bone, and the lateral (outermost) part is cartilage. The purpose of the ear canal is simply to guide sounds to the tympanic membrane (eardrum).

2.2 The middle ear

The middle ear consists of the tympanic membrane, and three small bones (ossicles); hammer (malleus), anvil (incus) and stirrup (stapes), see Fig. 2.1. The purpose of these bones is to act as an impedance matching transformer from the low impedance of the air to the higher impedance of the fluid filled cochlea (see Sec. 2.3).

There are also two small muscles located in the middle ear; the tensor tympani muscle and the stapedius muscle. The tensor tympani muscle is attached to the hammer near the tympanic membrane, and when activated the tensor pulls the tympanic membrane inwards, and thereby

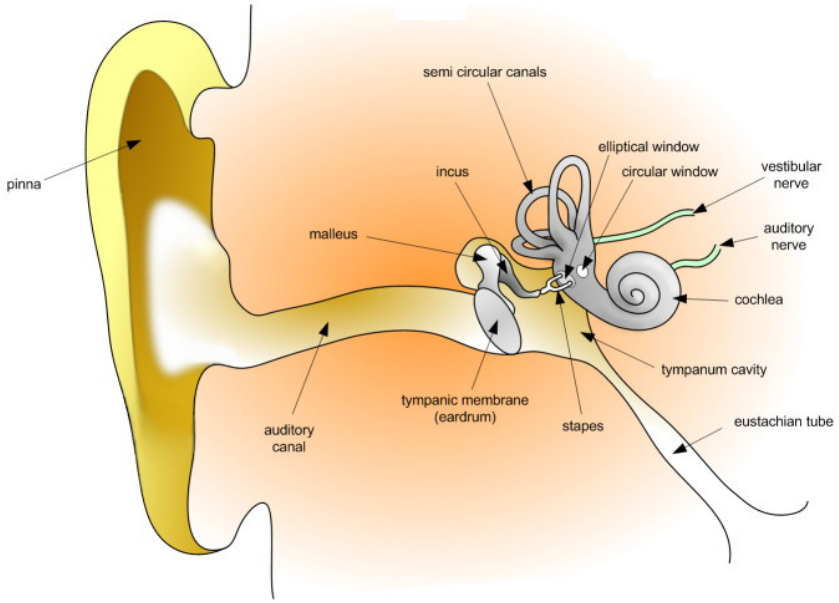


Figure 2.1: Schematic drawing of the ear. Adapted from [74].

stiffening the tympanic membrane. The stapedius muscle is attached to the stirrup at the end that is coupled to the anvil, and when activated it pulls the head of the stirrup perpendicular to the direction of movement for sound conduction. Both muscles are connected to the middle ear cavity wall at the other end. When tensed these muscles act together to reduce transmission of sound to the inner ear by increasing the stiffness of the middle ear system.

The eustachian tube is a connection between the pharynx and the middle ear. The tube's purpose is to equalize pressure between the middle ear and the atmosphere, and is usually closed, but opens when swallowing or yawning. The eustachian tube is also used as a drain for mucus that is produced in the middle ear.

2.3 The inner ear

The inner ear has two functional parts; the cochlea (organ of hearing) and the vestibular apparatus (organ of balance). Here we will only describe the cochlea and the parts that are important for the hearing mechanism.

2.3.1 The cochlea

The cochlea is a snail shell shaped bone in the inner ear, in which sound is converted into neural code, which in turn can be interpreted by the brain. The cochlea has a little more than $2\ ^1/_2$

turns, and if uncoiled it would have a length of approximately 31-33 mm [65]. Coiled, it has a hight of 5 mm in humans.

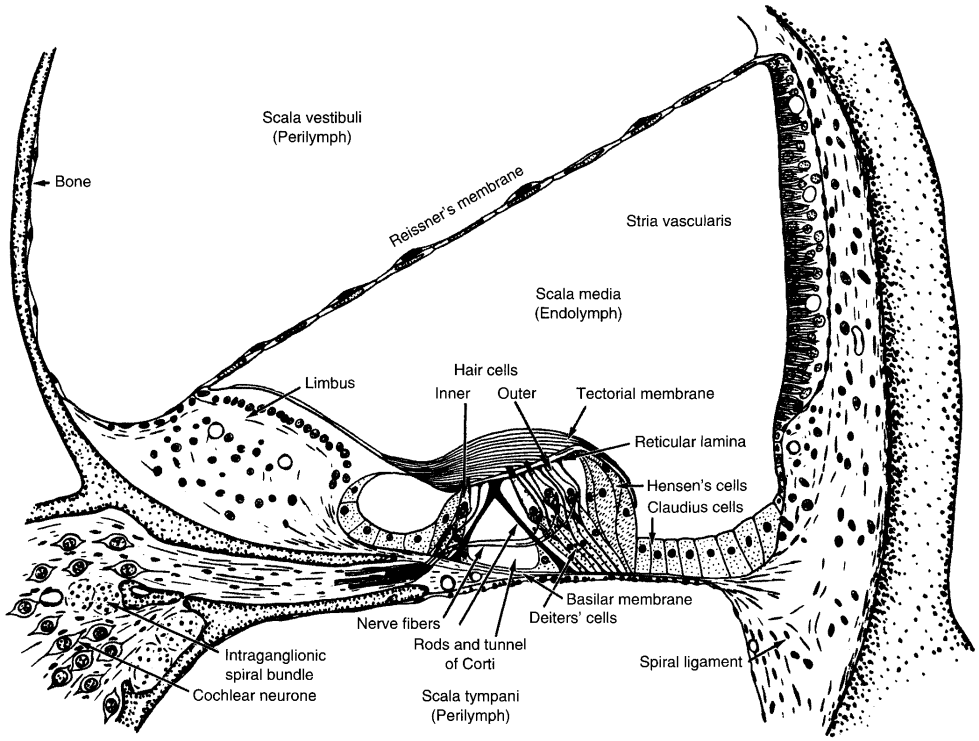


Figure 2.2: Schematic drawing of the cross-section of the cochlea. Figure adapted from Møller [65].

The cochlea is divided into three compartments (see Fig. 2.2) which are filled with fluids. The upper (Scala vestibuli) is separated from the middle (Scala media) by the Reissner's membrane, and the Scala media is separated from the lower (Scala tympani) by the basilar membrane. Both the Scala vestibuli and the Scala tympani compartment are filled with perilymph-fluid, while the Scala media is filled with endolymph-fluid. These two fluids are different in ionic composition (see Table 2.1), which is of great importance for our hearing. The perilymph is similar to extracellular fluids such as cerebrospinal fluid (i.e. rich in sodium ions (Na^+)), while the endolymph is similar to intracellular fluids (i.e. rich in potassium ions (K^+)). The difference in chemical composition of these two fluids make their potentials different, whereof endolymph has a potential of +80 mV relative perilymph [19].

Transmission of vibrations, from the bones of the middle ear to the fluid of the cochlea, appears through the oval window, in which the stapes is anchored. The oval window is an "opening", or boneless area, of the cochlea. The "opening" is covered by a membrane, and a similar membrane covers the round window, which is another hole in the bone structure. The oval window leads into Scala vestibuli, and the round window to the Scala tympani. The round window is displaced in opposite phase of the oval window, and the energy is eventually dissipated through here.

Table 2.1: Composition of cochlear fluids. This table is adapted from Wangemann [105].

Component	**Unit**	**Endolymph** (Scala media)	**Intrastrial fluid**	**Perilymph** (Scala vestibuli)	**Perilymph** (Scala tympani)	**Plasma**
Na^+	(mM)[a]	1.3	85	141	148	145
K^+	(mM)	157	2	6.0	4.2	5.0
Ca^{2+}	(mM)	0.023	0.8	0.6	1.3	2.6
Cl^-	(mM)	132	55	121	119	106
HCO_3^-	(mM)	31	n.a.	18	21	18
Glucose	(mM)	0.6	n.a.	3.8	3.6	8.3
pH	(pH units)	7.4	n.a.	7.3	7.3	7.3
Protein	(mg dl^{-1})	38	n.a.	242	178	4238

[a]Concentration of a solution measured as the number of moles of solute per liter of solution. For example, a 6 M HCl solution contains 6 moles of HCl per liter of solution. 1 mol = $6.022 \cdot 10^{23}$ (Avogadros number).

2.3.2 Basilar membrane

The basilar membrane (BM) is responsible for the frequency tuning of our hearing. Its mechanical properties is decisive for this tuning. The BM is narrow (approximately 150 μm) at the base, i.e. closest to the oval window, and widest (approximately 450 μm) at the apex, i.e. top of the cochlea. And, as Fig. 2.3 suggests, the stiffness decreases along the membrane, being stiffer at the base than at the apex. This indicate that the location of sensory transduction for high frequencies is at the base, and that of lower frequencies is towards the apex. The vibrations travel along the BM until they reach the point of resonance, at which point BM deflections are the greatest, and stops here. This means that high frequencies travel a short distance, while lower frequencies travel further. Frequencies below appr. 20 Hz on the other hand, reaches the top of the cochlea. These low frequency vibrations pass through the helicotrema, a small opening (approximately 0.05 mm^2) in the BM at the apex, which allows very low frequencies to be transmitted without setting any sensory mechanisms in motion. That is; such frequencies do set the BM into vibration, but does not hit any point of resonance.

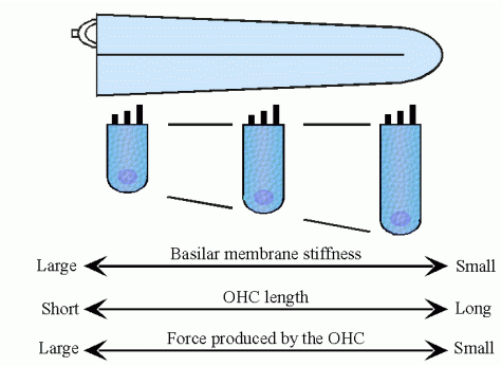


Figure 2.3: Properties of the BM and the OHCs. Figure adapted from [102].

2.3.3 Stria vascularis

Stria vascularis (SV) is a multi-layered[1] epithelium on the lateral wall of the Scala media that is responsible for maintaining the potential of the endolymph. Its functional purpose is therefore to supply the endolymph with potassium ions (K^+) [104]. The process of transporting the ions from the SV to the endolymphatic space is energy consuming, thus the SV is rich on blood vessels and mitochondria (see Tutorial box 1). The SV pushes ions against a positive chemical gradient trying to uphold the 80 mV potential difference, and is therefore required to work extra hard if the efflux of K^+-ions is big and the potential drops.

Stria vascularis is wider at the base than at the apex, and strial width appear to be linearly related to the number of marginal cells. The radial area of the SV also increases towards the base of the cochlea. It is also found that the volume density of the cells and capillaries of the SV is constant along the length of the Scala media [83].

Tutorial box 1.
Mitochondrion.
Mitochondria are the organelles in cells that are responsible for providing energy for the cell through the respiration of glucose using oxygen. The process occurs in three phases. In each phase, molecules of adenosine diphosphate (ADP) have a third phosphate group added to them to create adenosine triphosphate (ATP). Each addition of a phosphate group is called phosphorylation. The process stores a great deal of energy in each molecule of ATP, making ATP the true "battery" of the cell. Conversion of ATP to ADP, by removing one of the phosphate groups, releases a great deal of stored energy that then is used to power the cell.
(Adapted from Henderson et al. [36].)

K^+-cycling

Recycling of K^+-ions is necessary because potassium resources are limited, and an obstruction of these recycling pathways would deprive SV of K^+ and result in loss of endolymphatic potential. According to Kikuchi et al. [46] two independent recycling systems of cells exist (see Fig. 2.4), defined by interconnecting gap junctions:

1. The first system, the epithelial cell gap junction system, is mainly composed of all cochlear supporting cells, and also includes interdental cells in the spiral limbus and root cells within the spiral ligament (SL).

2. The second system, the connective tissue cell gap junction system, consists of strial intermediate cells, strial basal cells, fibrocytes in the spiral ligament, mesenchymal cells lining the bony otic capsule facing the Scala vestibuli, mesenchymal dark cells in the supralimbal zone, and fibrocytes in the spiral limbus.

[1] Three layers of cells; marginal cells facing the endolymph compartment, intermediate cells, and basal cells which are in connection with the spiral ligament (see Fig. 2.4).

Here cochlear supporting cells include pillar cells, Deiters cells, Hensen cells, and Claudius cells, and these supporting cells are electrically and metabolically coupled by gap junction channels.

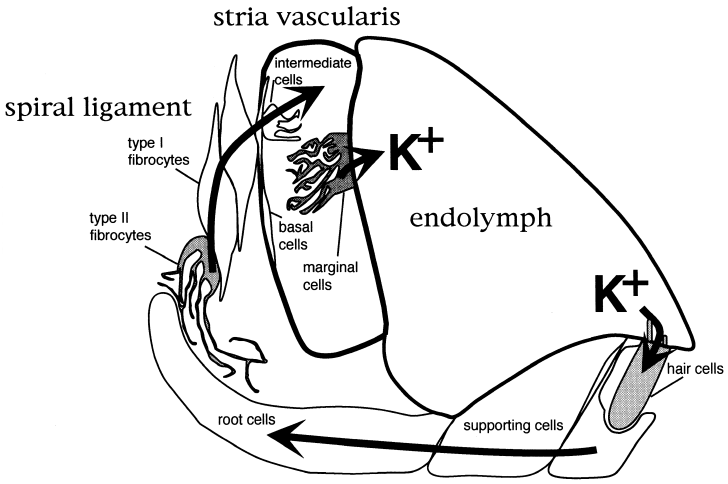


Figure 2.4: Figure indicating a possible recycling pathway for K^+-ions. It shows the endolymphatic compartment with the K^+-flow through the hair cells, supporting cells, root cells of the SL, fibrocytes of the SL and the cells of the SV. Figure adapted from Kikuchi et al. [46].

2.3.4 Organ of Corti

The organ of Corti (OC) is located on the BM, and is a complex structure of different types of cells. The most important cells are the hair cells, which got their name because of the hair like structures on top, called stereocilia (see Fig. 2.7). Hair cells are organized in rows along the BM, whereof three rows of OHCs and one single row of IHCs.

Other cells of the OC, believed to be of crucial importance for the hearing mechanism, are outer and inner pillar cells, Deiters' cells and Hensen's cells. These cells will be described in further detail below. There are also other cells in the organ of Corti, but these are not included in this review, because they are not assumed to take any vital part in hearing pathology.

Tectorial membrane

Another important structure of the OC is the tectorial membrane (TM). This is a non-cellular tissue that covers the cells mentioned above, and plays a key role in the process of hearing. The TM is composed mainly by water (97 %), but the remaining solid fraction (3 % of protein and carbohydrate) forms a matrix that contains ionizable charge groups, attracting mobile counterions from the surronding fluid. The TM is fixed to the OC at two points; the outermost part is attached to the Hensen's cells and a section called the Hensen's stripe attaches the TM to the OC near the IHC [58]. This fixation is made up of fine gelatinous "threads" (trabeculae)(not visible

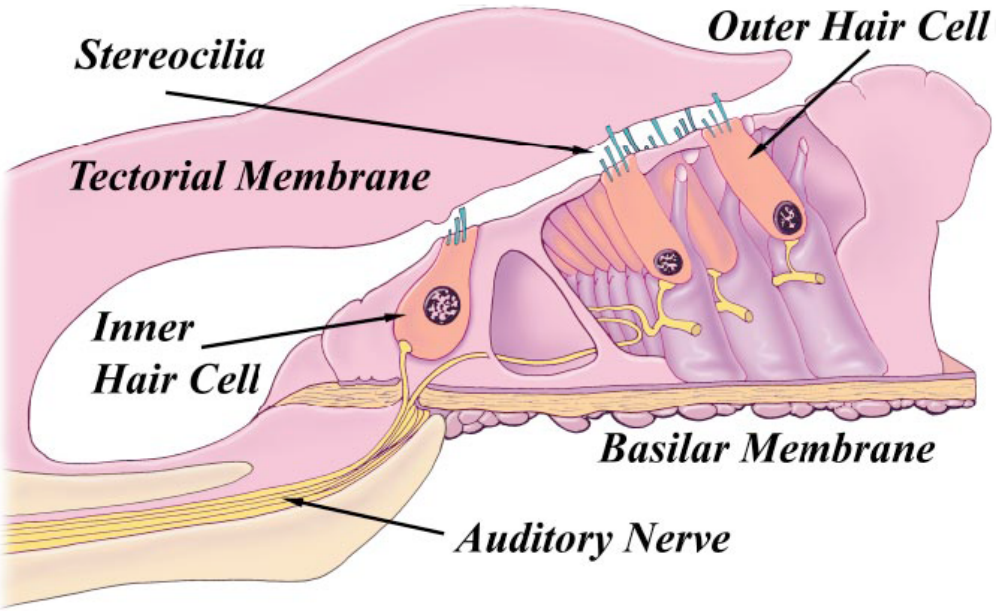


Figure 2.5: The organ of Corti. Figure adapted from Brownell et al. [9].

in Fig. 2.5), which allows the endolymph fluid to move freely, and pass these points without being obstructed in any way.

The longest stereocilia of the outer hair cells (OHCs) are embedded into the TM. This causes a shear force to act upon the stereocilia when the BM is set into motion, thus enhancing the motion of the stereocilia in response to sound.

2.3.5 Inner hair cell

The inner hair cells (IHCs) are sensory cells responsible for conversion of BM vibrations into neural code. These cells are innervated by mostly afferent[1] nerves, thus assumed to be responsible for the actual process of hearing. Approximately 95 % of all afferent nerve endings in the cochlea ends up at the IHCs [65].

IHCs are pear-shaped cells (see Fig. 2.7(a)), closely surrounded by other supporting cells. The IHCs have a resting potential of approximately -40 mV relative to the perilymph fluid, which gives a net potential difference across the apical membrane of about 120 mV relative endolymph [19].

Stereocilia (about 60) on top of the IHC are arranged in the shape of an U, and contain channels for conduction of ions, often referred to as mechano-electrical transduction (MET) channels. These channels are opened when the stereocilia are deflected towards the tallest cilium (see

[1] towards the central nervous system (CNS) (brain)

Fig. 2.6). Opening of the MET channels causes an influx of K^+-ions, and Ca^{2+}-ions, into the IHC, driven by the potential difference. A rise in the intracellular potential triggers voltage controlled channels along the cell membrane to open, causing an efflux of K^+ out of the cell, and an influx of additional Ca^{2+} into the cell. These calcium-ions trigger release of glutamate[1] from the base of the cell, which binds to the afferent nerve terminals surrounding the base of the hair cell, resulting in an action potential being propagated down the afferent nerve fibers [82]. These signals are interpreted by the brain, and we have the sensation of sound.

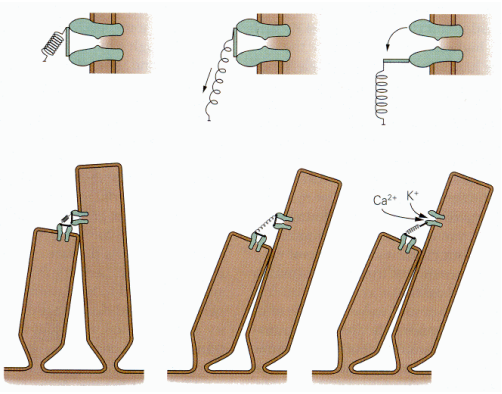


Figure 2.6: Tip-links and MET-channels of the stereocilia. Tightening of the tip-links cause the MET-channels to open, and influx of K^+ and Ca^{2+} occurs. Figure adapted from [60].

2.3.6 Outer hair cell

There are approximately 12 000 outer hair cells (OHCs) in the cochlea, and each OHC has 50-150 stereocilia arranged in 3-4 rows in the shape of a W or V, whereof the longest are embedded into the TM [65]. As seen in Fig. 2.7(b) OHCs have a cylindrical shape, with the cell nucleus at the bottom, mitochondria along the lateral walls and several below the nucleus, and other organelles at different places. The cell membrane consists of lipids, and between the membrane and the subsurface cisternae there is a cortical lattice of proteins.

OHC cell membrane is unique in composition because its ability to lengthen and shorten in response to an increase or decrease in cross-membrane potential. This action is not directly dependent on cellular energy (ATP, see Tutorial box 1), but is rather a voltage to force action, hence called electromotility. The active compound in the membrane is a protein, identified as prestin [20, 55]. According to Liberman et al. [55] a loss of this OHC electromotility would cause a 40-60 dB loss of cochlear sensitivity.

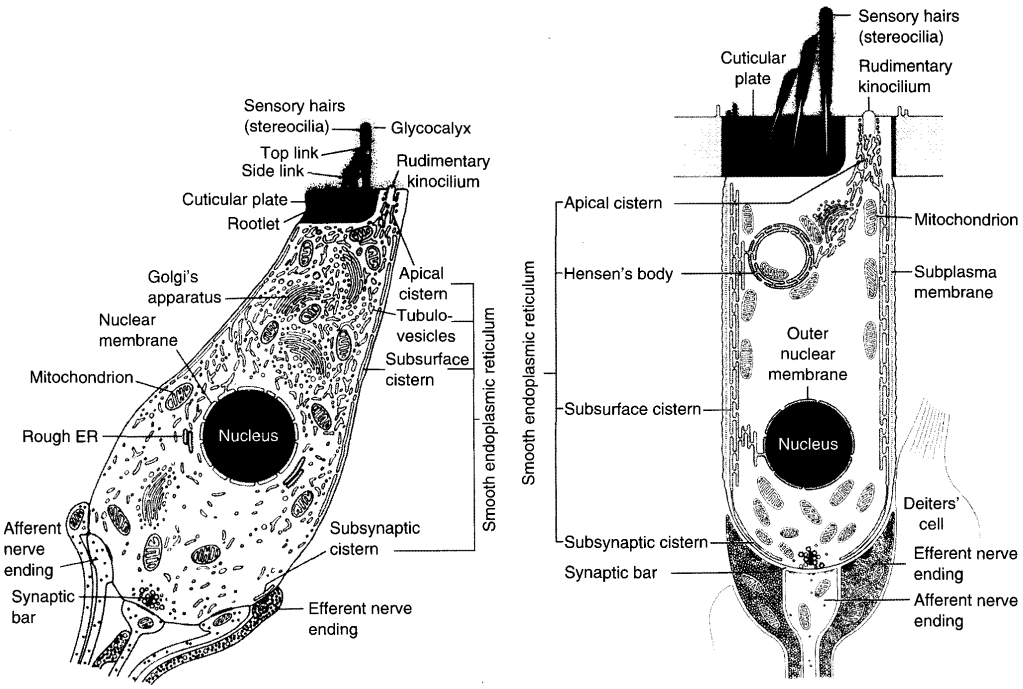
As the IHC's stereocilia, OHC's stereocilia contain MET channels, and when deflected towards the tallest cilium tip-links are tightened and the channels are opened. The influx of K^+- and Ca^{2+}-ions into the cell increase cellular potential, thus decreasing cross membrane potential. This depolarization causes the cell membrane to contract, thus the cell to shorten. When the hair

[1] glutamate (an amino acid) is the main excitatory neurotransmitter used by the IHCs [36]

bundle is deflected the opposite way channels are closed, causing the intracellular potential to rebuild, and even hyper-polarize, which causes the cell to lengthen.

When OHCs' stereocilia are deflected as a cause of vibrations of the BM, OHCs depolarize and contract at their specific frequency. This OHC electromotility is found to be responsible for active tuning in the organ of Corti [99]. OHCs are arranged from the base to the apex according to their length (see Fig. 2.3), and hence their contraction frequency [8, 9]. Force produced by the OHCs adds to the input force, substantially amplifying the vibration of the BM at the cut-off point.

OHCs are innervated by mostly efferent[1] nerve endings, and are therefore assumed not to take any major part in sending information of BM vibrations to the brain. However, the OHCs functions are to act as an amplifier for low intensity sounds, and amplify BM displacement, and to enhance frequency selectivity. There are suggestions that the efferent innervation of the OHCs is controlling the motility [18, 82]. These studies report that efferent nerve connections may decrease the amplification provided by some of the OHCs, and in this way offering the potential to improve the detection of signals in background noise, to selectively attend to particular signals, and to protect the periphery from damage caused by overly loud sounds.



(a) The inner hair cell (IHC) (b) The outer hair cell (OHC)

Figure 2.7: Schematic drawing of the hair cells of the inner ear. Here we see some of the anatomical differences between the IHC and the OHC. Figures are adapted from Møller [65].

[1] from CNS (brain) to a cell in the body

However, studies performed by Scharf et al. [86, 87] indicate no such role of the efferent system, but they find that sectioning of efferent nerve supply cause detection of signals at unexpected frequencies. Scharf and colleagues therefore suggest that efferent innervation plays a role in focusing attention at a specific frequency.

Frolenkov [30] suggests in his study that efferent innervation modulates OHC electromotility, but also suggests other mechanisms that may regulate electromotility.

Zheng et al. [114] suggest that the cochlear efferent system may influence the ear's ability to develop resistance to noise trauma. In their study, Zheng et al. cut the efferent nerve supply to OHCs in chinchillas, and demonstrated a substantially TTS, greater PTS and larger cochlear lesions of OHCs. In another study, Zheng et al. [115] suggest that a malfunction, or obstruction, of efferent nerve supply will cause greater susceptibility to IHC loss induced by noise.

2.3.7 Other cells of the cochlea

Both Hensen's (HeC) and Dieters' cells (DC) are innervated by both afferent and efferent nerve endings. This would probably speak for a role of these cells in hearing, but there is not very much knowledge about their function and participation. HeCs and DCs are however important for keeping the structure of the organ of Corti, and plays in this way an important role in normal functionality.

As suggested above (Sec. 2.3.3) Hensen's, Dieters' and Claudius' cells may play an important role in recycling of potassium ions. Further functional importance is suggested by Flock et al. [28]; supporting cells contribute to control of hearing sensitivity.

Tunnel of Corti

The pillar cells are responsible for holding the structure, which is essential for the function of the organ of Corti. The structure with the pillar cells is called tunnel of Corti, and is a cavity separating the row of IHCs from the rows of OHCs.

Reticular lamina

Deiters' cells form in conjunction with OHC apices the reticular lamina, which is a structure separating OHC body environment from the endolymphatic fluid. This is very important for keeping the cross membrane potential of the OHCs.

2.3.8 Blood supply of the cochlea

The cochlea receives its blood supply from a tight network of extremely slender vessels embedded in the highly compact bone of the otic capsule[1] [66]. The supply is managed through the inner ear artery (labyrinthine artery), which is a branch of the anterior inferior cerebellar artery. The labyrinthine artery branches to form the vestibular-cochlear artery and the spiral modiular artery. The first is used to supply parts of the cochlea, while the latter is thought to serve as a collateral blood supply to the cochlea [65]. The cochlea is richly supplied with blood vessels at the spiral ganglion and along the lateral wall (i.e. SV and spiral ligament). The capillaries of the basilar membrane, which are terminal vessels of the spiral lamina, have been considered to supply oxygen to the organ of Corti [67]

An important feature with the labyrinthine artery is that it is not one single artery, rather a bundle of smaller arterioles. Together with a distal reservoir these small diameter arteries function as a low pass filter attenuating fast changes in blood flow. Without this low pass filter we would probably hear our own pulse.

Regulation of cochlear blood flow

Nitric oxide (NO) has been implicated as a mediator of vasodilation and neurotransmission in the mamalian cochlea, and it is found that a NO donor placed at the round window causes an increase in cochlear blood flow (CBF). And the other way around; a NO inhibitor causes reduction in CBF. These findings suggest an important role for NO in regulating CBF [81].

[1] otic capsule – the bone that surrounds the inner ear

Chapter 3

Noise-Induced Hearing Loss

With the exception of presbyacusis[1] the most common reason of sensorineural hearing impairment is noise [45]. Noise-induced hearing loss (NIHL) is a diagnosis of impaired hearing as a result of exposure to noise, an exposure which can be of both long and short duration, in occupational and leisure time activities. Noise level and exposure time are key determinants for how noise affects hearing, and is also the basics for the two different ways noise can injure the ear; (1) high level, short[2] duration noise exposure can stretch inner ear tissues and structures beyond their elastic limits, thus tearing them apart, or (2) lower level, long duration noise exposure can fatigue the ear's delicate tissue.

Overstimulation by intense sound gives rise to several structural and functional alterations in the organ of Corti. These changes include shrinking of the tectorial membrane [12], disruption of the tip-links of the stereocilia [75], fracture of the actin core and bending of the stereocilia [24, 84], shortening and swelling of OHC bodies [33], contortion, blebbing, and degeneration of the cell body [27, 39], distension of the Deiters' cells (DCs) [28], swelling of afferent nerve endings [26, 54, 77], and degeneration of afferent neurons [114].

3.1 Noise and oxidative stress

There has been suggested for many years that metabolic exhaustion could play an important role in NIHL. Metabolic exhaustion results in an overproduction of waste products in the cochlea, called reactive oxygen species (ROS) and reactive nitrogen species (RNS), which in turn could cause oxidative stress and damage to vital structures and tissue. Oxidative stress is the process in which these reactive species either take ("steal") or give an extra electron to a compound, thus altering its properties. After Yamane et al. (1995) [107] demonstrated oxidative stress to result from noise exposure, metabolic exhaustion hypothesis has regained new faith and been

[1] Presbyacusis – age-related hearing loss.

[2] High level, *long* duration would, of course, also damage the ear. Extensively!

examined thoroughly.

Several recent studies have pointed out metabolic fatigue, and oxidative stress, to be an important factor in NIHL (e.g. [36, 44, 45, 57]). These studies have sought to influence oxidative mechanisms in different ways to emphasize their role in NIHL. The effect of oxidative stress can be enhanced in two different ways; either by enhanced production of oxidizing compounds, or by depletion of antioxidant defenses. There is reason to believe that production of oxidizing compounds could occur transiently, thus overwhelming the body's defense. Indications are that the additive effect of increased ROS/RNS formation and depleted antioxidant capacity can lead to cell injury or death.

We will study this in further detail in the next chapter (Ch. 4).

3.2 Noise and mechanical damage

Very high level noise, and impulsive noise in particular, have been shown to cause direct mechanical damage to our hearing organ. This is assumed to be by far the main reason for sudden hearing loss, and have been found at different locations in the ear. Listed below are the most common mechanical damages.

- Outer Ear
  - rupture of the tympanic membrane
- Middle Ear
  - broken ossicles
- Inner Ear
  - broken, bent or fused stereocilia (e.g. [24, 84]). This would have huge implications on hearing because stereocilia contain the channels that convert vibration and motion into electric currents, which in turn alters the cross-membrane potential, and is fundamental for hearing.
  - broken tip links (e.g. [76]). This would decrease, or even stop electrical currents into the hair cells.
  - OHC stereocilia can be torn loose from their point of insertion in TM [85]. This would significantly reduce movement of the stereocilia, thus causing reduced influx of K^+-ions into the cells.
  - pillar cells can be injured, or destroyed, thus altering the local impedance of vibration [79].
  - Hensen's and Deiters' cells can be displaced [28].
  - OC can be detached from BM, deteriorate, and be replaced by scar tissue [15].

Some of the injuries listed above are to consider as permanent, while others are temporary, i.e. some are irreversible while others are repairable. According to Kopke and Danielson [49] these mechanical types of injury start to occur at levels of 125 - 130 dB SPL and becomes more severe for higher levels. Others state that one would have to be exposed to levels exceeding 140 dB before mechanical damage occurs [15]. Brüel [6] mentions in a acoustical resumé that "someone" have tested high level noise on humans, and found that there is a critical limit somewhere around 150 dB SPL, which separates severe and not so severe mechanical damage to the ear.

3.3 Noise and biochemical processes

As sound, and noise, is being processed by the ear, different systems are set into action, both mechanical and chemical. In the following section we want to look at the effect of noise on some chemical compounds in the cochlea.

3.3.1 Glutamate excitotoxicity

IHCs code vibrations and motions into neural signals which in turn are sent along the afferent nerve fibers to be interpreted by the brain. If the noise level becomes too high, the release of neurotransmitter (glutamate) can be elevated and result in the condition of exitotoxicity, or synaptic exhaustion (for a review see Pujol and Puel [77]). This condition is characterized by swelling (and rupture) of the postsynaptic cellbodies and dendrites. Robertson [80] demonstrated that the affected cells recover over time and regain normal function post exposure, but he also states that this would not always be the case. Robertson suggests that if the glutamate insult is too big it would lead to degeneration of the afferent neurons.

Kopke et al. [48] state that glutamate excitotoxicity can be followed by numerous harmful cellular and molecular consequences. They suggest that it could lead to i.a. reduction in several key mitochondrial molecules, increased ROS production, loss of mitochondrial membrane integrity and reduced energy production, all of which could contribute to elevated hearing threshold.

3.3.2 Lateral wall degeneration

Some studies have found acute swelling of SV in response to high level noise exposure, and loss of intermediate cells as a consequence [103]. Degeneration of intermediate cells is permanent, but the swelling disappears after exposure [39]. As a long term result the capacity of the SV is reduced as a provider of endocochlear potential, but indications are that this does not cause permanent hearing loss. It would, however, lead to lowered tolerance for noise before temporary threshold shift occurs.

3.3.3 Calcium overload

It is known that calcium is one of the key regulators of mitochondrial function, and acts at several levels within the organelle to stimulate ATP synthesis [7]. Sound exposure increases intracellular Ca^{2+} level, which is suggested to be an effective cofactor in the enhancement of the electromotile response of these cells [95]. There is, however, also suggested that acoustic overstimulation could increase OHC Ca^{2+} concentration beyond healthy limits, and that this could cause contraction of the hearing organ, which would, as a consequence, alter hearing properties [29].

Others have studied influence of drug-induced increase, or decrease in endoplasmic Ca^{2+} level on hearing functionality, and found that; (1) mitochondrial matrix Ca^{2+} overload can lead to enhanced generation of ROS [7]; (2) rising Ca^{2+} concentration induces a slow shape change in isolated OHCs [23]; (3) alterations in intracellular Ca^{2+} concentration can lead to cell death through different pathways [53]; and (4) calcium channel blockers protects OHCs during intense noise exposure [35].

3.3.4 Other effects

According to Patuzzi [73] noise can alter MET channel properties, resulting in loss of permeability. Patuzzi states that this probably is caused by some kind of molecular change rather than as a result of mechanical destruction. He stresses the importance of this as a cause of temporary loss of hearing, and that it is just some form of prolonged closure of MET channels.

As Saunders et al. [85] suggest, noise exposure can also lead to swelling of cells and cell organelles. Swelling of mitochondria in response to noise would inhibit its function and lead to lack of energy to the energy consuming processes. Cell body swelling could eventually lead to rupture, and what is called necrosis (see Tutorial box 2.).

According to Henderson et al. [36] there is also reason to believe that cell death through necrosis can induce further damage to surrounding tissue. Spillage of the cell's contents initiates inflammatory responses in the surrounding tissue, which often result in the death of groups of cells.

Tutorial box 2.
Types of cell death.
Cell death occurs through one of two processes, either as necrosis or apoptosis.

- Apoptosis: Apoptosis is a form of active cell death where active processes eliminate a cell, and plays a vital role in normal biological function. It is through this mechanism cells are renewed and replaced in normal development. Initiation of this type of death occurs via both wanted and unwanted stimulus.
- Necrosis: Necrosis is a form of passive cell death, and is often observed after gross physical or chemical insult. Necrosis is associated with cell swelling, which eventually results in rupture of the cell and spillage of the cell's contents.

(Adapted from Henderson et al. [36].)

3.4 Noise and cochlear blood flow

Although the literature is inconsistent, several studies have reported signs of decreased cochlear blood flow (CBF) in response to noise exposure [59, 62, 78, 90, 98, 113]. Any reduction in cochlear blood supply, even if brief, could induce threshold shifts and lead to damage to vital cochlear tissue.

According to Seidman et al. [90] most blood vessels contract in response to noise, also large extremity vessels, and therefore it would be likely that also inner ear vessels do so. Seidman and colleagues review multiple studies which have found reduction in CBF in response to noise, but notice that not all studies have found such decreased CBF. They also state that there are multiple mechanisms which can contribute to reduction of CBF (i.a. high cholesterol, age, stress).

Nakashima et al. [67] give an overview of mechanisms involved in altering CBF, and suggest that noise exposure could be one of them. According to Nakashima and colleagues vasoconstriction of the capillaries of the BM, SL and SV in response to noise has been reported. They also state that it has been demonstrated that noise overstimulation can induce elevation of arterial blood pressure in animals and humans, and that NIHL may be associated with alterations in magnesium metabolism. Nakashima et al. also point out that the origin of the frequency dip[1] could be vasoconstriction of blood vessels at the tonotopic location in the cochlea corresponding to maximum basilar membrane activation. It has also been noted that the tonotopic location of the frequency dip has an anatomical correspondence to the anastomosing region[2] between the main cochlear artery and the cochlear branch of the vestibulo-cochlear artery.

Yamane et al. [107] hypothesize that an initial deposit of ROS that they observed along the marginal cells of the stria vascularis after noise, was the result of overdriving the mitochondria in the absence of available oxygen, and that the oxygen deficiency was due to a noise induced reduction in CBF or ischemia. Henderson et al. [36], however, suggest that there is reason to believe that noise-induced ROS and ROS activity causes CBF reductions, rather than ROS resulting from the CBF changes initially.

3.5 Other effects of noise on hearing

There are also other mechanisms that could be influenced by noise and in turn lead to hearing loss. One mechanism that could be influenced is suggested to be recycling of K^+. As mentioned earlier K^+-ions are responsible for the driving potential in the cochlea (see Sec. 2.3). Wang et al. [103] suggest that noise could destroy the recycling pathway in the region where the OHCs were most heavily damaged by noise exposure.

Chan et al. [13] find in their study that exposure to high level noise causes reversible stiffness changes in auditory cells, which then would indicate a role in TTS. Chan et al. also claim that the

[1] the dip, or notch, seen in the audiogram of noise impaired ears around 4-6kHz.
[2] region with open connections between two blood vessels

deterioration, but also the recovery of the mechanical properties of OHCs may form important underlying factors in all kinds of NIHL.

Bohne and Rabbitt [4] found holes in the reticular lamina as a result of noise in their study. They suggest that this could be caused by degeneration of OHCs, thus being a secondary consequence of OHC death, which may be caused by oxidative stress. If, however, holes should occur in the reticular lamina, for any reason, this would result in leakage of endolymphatic fluids into the OHC body compartment, causing lowered cross membrane potential.

3.6 Summary

As demonstrated above there can be multiple mechanisms causing noise-induced hearing loss, of which many have different origins. It has been known for a long time that very high levels of noise can cause instantaneous mechanical damage, and that long exposure to noise can eventually cause permanent damage to hearing. In the last 10-20 years it has become more and more evident that oxidative stress is one very important factor in NIHL. We will study this in further detail in the following chapter.

Chapter 4

Oxidants and Antioxidants in NIHL

Oxidative stress is common throughout the body, and is found to take part in many diseases and injuries, and is also thought to play an important role in aging [100]. Studies performed in the last couple of decades have, however, found oxidative stress to play a crucial role in noise-induced hearing loss, i.e. degeneration of cochlear tissue and cells.

In healthy cells, a balance exists between the production of oxidants and the antioxidant defense of the cell. A traumatic event, such as exposure to high level noise or prolonged exposure to medium level noise, can shift this balance, resulting in oxidative stress and cell damage.

For a chemical approach on this subject see Appendix A.

4.1 Active compounds

The process of oxidation is caused by compounds of different chemical composition, but they all have one thing in common; they are highly reactive and capable of injuring cells and tissue. Because of their reactivity these compounds are commonly referred to as reactive oxygen species (ROS) or reactive nitrogen species (RNS), of which the most reactive are called free radicals. This latter group has one unpaired electron in their outer shell, which makes them the most dangerous oxidizing compounds.

An unpaired electron represents a higher energy state than a paired electron, and free radicals are therefore highly active as electron “stealers”. The free radicals will, to reduce themselves to a lower energy state, react with almost whatever they come in contact with, and are therefore not wanted in the cells (see App. A.1). ROS/RNS are compounds that are easily converted into such radicals, and it is therefore critical that these are taken care of before being converted. For simplicity both radicals and non-radicals are referred to as ROS or RNS throughout this paper.

There are mainly three different types of ROS associated with NIHL; superoxide ($O_2^- \cdot$) [1], hydroxyl ($OH \cdot$) and hydrogen peroxide (H_2O_2), and two RNS; nitrogen oxide (NO) and peroxynitrite ($ONOO^-$) [25]. As a group ROS/RNS are short-lived, unstable, highly reactive molecules, which are active in both healthy and toxic reactions. They are essential in normal cellular activity, but appear toxic to the surrounding environment when concentrations become too high. ROS/RNS damage other cells by removing ("stealing") electrons from e.g. structure molecules of the cells, which causes the affected molecule in turn to do the same with another molecule, thus starting a chain reaction of destruction.

Antioxidants (AOs) are the compounds that protect cells from being damaged by ROS/RNS, and in normal function AO-defense is sufficient to keep the amount of ROS/RNS within non-toxic levels. But as production of ROS/RNS increases, the defense becomes inadequate and is overpowered by the radicals, and ROS/RNS begin to damage structures and tissue. This AO-defense consists of both electron donors and enzymes working as catalysts in the process of detoxification (see App. A.2 for a list of antioxidants).

4.2 ROS/RNS

After Yamane et al. (1995) [107] demonstrated cochlear oxidative damage as a result of noise exposure, several studies have tried to assess the effect of oxidative stress in hearing loss (e.g. [36, 44, 45, 52]). These studies have investigated the effects of different drugs on either AO level or ROS/RNS level. Results from such studies show that application of agents promoting ROS-production, or agents inhibiting AO-production, cause an elevation in threshold, while ears treated with agents inhibiting ROS, or agents promoting AO-production, show less damage than untreated ears.

Through such studies one can identify oxidative stress as a source of damage, but one also needs studies that show correlations between noise and effects on ROS/RNS and antioxidants, and damage (e.g. [43, 70, 107, 112]). Through such studies one has found compelling evidence for noise-induced generation of ROS in the cochlea [71, 107], and more specifically in the OHCs [69]. Excessive, and toxic, production of ROS/RNS appear when noise exposure level increases over some limit (not specified in the studies).

ROS/RNS are constantly produced, even during normal metabolism such as in mitochondrial electron transport. However, damage occurs when free radical generation exceeds the capability of the antioxidant systems to control or detoxify them. This imbalance can occur by increasing ROS/RNS levels or by inhibiting antioxidant systems. A number of factors can increase ROS/RNS levels, including inflammation, cigarette smoke, radiation, drugs such as cisplatin and aminoglycides, and cochlear noise exposure [10]. Antioxidant systems can be deactivated when certain enzyme systems are inactivated; antioxidants, such as free radical scavengers are not present in sufficient amounts, or major antioxidant systems, such as the glutathione pathway, are

[1] The unpaired electron in a free radical is denoted by a dot after the chemical formula or atomic symbol ($\cdot$) indicating that this is a free radical, not only a ROS/RNS.

inactivated. For example poor nutrition can diminish many natural antioxidant systems, hence enhancing oxidative stress.

NOTE: Most of these studies have been performed on animals, and it is important to keep that in mind when applying the results to human models. Henderson and Hamernik [37] state that e.g. chinchillas are 10-15 dB more susceptible to noise than humans.

Tutorial box 3.
Sources of ROS.
As stated earlier, ROS comes from many sources in the body. With molecular oxygen (O_2) being in such great supply, there is an abundant supply of oxygen to be converted into various ROS.

1. Mitochondria: Mitochondria are the organelles that use oxygen to metabolize glucose to provide cells with their required energy. Superoxide is used as an intermediate molecule in the Krebs' cycle and the electron transport chain. These molecules are quickly neutralized in a normal functioning mitochondrion by conversion into O_2, CO_2, or H_2O. In an overdriven mitochondrion, superoxide molecules can escape and collect in the cell.
2. Enzymatic reactions: Some enzymes, including xanthine oxidase and NADPH oxidase will catalyze reactions of hydronium ions ($H\cdot$) with O_2 to create superoxide.
3. Ischemia/reperfusion: A state of decreased blood flow to a certain tissue or organ is known as ischemia. Following a period of ischemia, blood flow returns to the deprived population of cells. This is called reperfusion. During ischemia, cells are deprived of oxygen, leading to greater mitochondrial taxation and increased leakage of superoxide. During reperfusion, there is an abundant supply of oxygen to be used in conversion to more superoxide, or to react with the existing superoxide to create other ROS.
4. Excitotoxicity: A condition in which exposure to large amounts of excitatory neurotransmitter (glutamate, in the cochlea) leads to cell death. The excitatory neurotransmitter causes heavy aerobic respiration in the mitochondria, leading to leakage of superoxide from the Krebs' cycle and electron transport chain.

(Adapted from Henderson et al. [36].)

4.2.1 How ROS/RNS are formed

All the different mechanisms, by which ROS/RNS are formed, are not fully understood yet, but some of the mechanisms involved are known.

Mitochondria

As in every other cell there are mitochondria in cochlear cells too, and active cellular processes are dependent on mitochondrial energy production. When the mitochondria are using more and more oxygen to meet increased cellular demands for energy, more and more superoxide is generated as an unwanted byproduct, due to the inefficiency with which the mitochondria must work. The increased level of superoxide can then react with other molecules to generate higher levels of other ROS and radicals in the cochlea (see App. A.4).

Because mitochondria are highly dependent on oxygen supply, even small alterations in blood flow could influence mitochondrial function. If CBF should be decreased, or obstructed in any way, the consequence is that the phosphorylation process[1] in the mitochondria becomes more inefficient, thus producing even more ROS/RNS.

There are also indications that the return of blood flow (reperfusion) after a state of reduced blood flow causes formation of ROS/RNS [36].

In Tutorial box 3 there are listed some sources of ROS which are assumed to be the most important.

4.2.2 Where ROS/RNS are formed

Exact location of ROS/RNS formation is diffuse, but some indications are there however. Le Prell et al. [53] review generation of ROS as a result of noise exposure, and suggest OHCs and cochlea lateral wall tissues to be the main locations for ROS/RNS formation.

Hair cells

Both inner (IHCs) and outer hair cells (OHCs) have mitochondria inside (see Fig. 2.7), thus being potentially vulnerable for increased ROS/RNS formation. The OHCs are, according to Henderson et al. [36], known to be highly demanding of energy, and high level noise exposure places especially high demands on the mitochondria to generate large amounts of energy through aerobic respiration. This would, as mentioned above, lead to excessive formation of ROS/RNS.

As mentioned in Tutorial box 3, glutamate excitotoxicity is also assumed to cause increased levels of ROS. As glutamate is the afferent nerve transmitter substance this would be dangerous primarily in the IHCs, but most studies have found OHCs to be much more vulnerable to ROS injury, hence glutamate excitotoxicity is assumed to be a minor problem.

Stria vascularis and spiral ligament

The pumping of K^+-ions into endolymph is highly dependent on mitochondrial energy. Therefore SV is densely populated with mitochondria, thus being a potential major source of ROS/RNS formation. Both SV and the spiral ligament are full of blood vessels, and the consequence of decreased CBF would have huge implications on ROS/RNS formation in these areas.

Henderson et al. [36] suggest that additional ROS could be produced as normal blood flow is being restored (reperfused) after ischemia (see Tutorial box 3). This would potentially be a bigger problem in areas with many blood vessels, i.e. SV, spiral ligament and spiral limbus.

[1] The process in which ATP is generated from ADP and oxygen

Organ of Corti

According to Takumida and Anniko [97] there is constantly being produced nitric oxide (NO) in the organ of Corti. NO is suggested to play a vital role in normal cell function, but is also capable of damaging cells when the right conditions are met. Takumida and Anniko located NO production to be in the synaptic region beneath inner and outer hair cells and supporting cells, but they also found some NO production in the SV and SL.

4.2.3 How ROS/RNS damage cochlear tissue

As mentioned, ROS/RNS are highly reactive species that attack surrounding molecules and structures. Some structures are more vulnerable than others, in that just small structural changes cause obstruction of vital functionality.

According to Clerici et al. [16] ROS can induce cellular damage by causing

- **lipid peroxidation**[1] – lipid peroxidation is readily initiated by OH radicals, and a single initiating reaction can generate multiple peroxide radicals via a chain reaction.
- **DNA strand breaks** – DNA is particularly susceptible to damage via hydroxyl radicals. Van Campen et al. [101] provides evidence that ROS-induced DNA damage corresponds to auditory dysfunction following an acoustic insult.
- **oxidation of proteins** – ROS damage proteins embedded in e.g. cell membranes. Different ROS/RNS attack protein -SH^2 groups, and hydroxyl radical modifies many amino acid residues [25].
- **perturbed membrane permeability** – Membrane properties are altered. The cells' ability to exchange compounds with extracellular environments over the cell membrane is altered.
- **perturbed ionic transport** – It is suggested that ROS can inhibit Ca^{2+}-activated K^+ channels [94].
- **alteration of membrane-bound enzymes and carbohydrates** – The chemical structure of the membrane can be altered [22].
- **cell death** – ROS induce cell death through both apoptosis and necrosis [36].

ROS can damage biologically critical macromolecules such as DNA, proteins and lipids. Alterations of, or damage to, DNA can lead to mutations, cell death, and sometimes cancer.

[1] Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism.

[2] SH-group – sulphydryl group; a part of many proteins

Damage to OHCs

The above mentioned types of damage can occur at the OHCs, and these are demonstrated *in vitro*, some even *in vivo*. If this occurs in the OHCs, vital functionality is lost. Loss of OHCs in a region will lead to elevated hearing threshold at the corresponding frequencies.

Indications are, however, that the susceptibility to ROS differs in the different regions in the cochlea. Sha et al. [91] found in their study that OHCs in the basal region of the cochlea are more vulnerable to noise than OHCs in more apical regions.

Damage to SV

Shi et al. [93] and Shi and Nuttall [92] report in their studies that NO production and ROS activity was increased as a consequence of loud sound stress, and suggest that this may lead to marginal cell pathology and the dysfunction of cochlear micro-circulation by inducing blood vessel wall damage. The study, performed on mice, showed that noise exposure induces NO (iNOS-mediated[1]) production, which causes subsequent nitrosative (oxidative) stress to the cochlear SV.

Shi and Nuttall found that iNOS-activity can contribute to enhanced ROS production, which may lead to endothelial and marginal cell apoptosis or necrosis (see Tutorial box 2, p.20).

However, the literature differs in this subject; in an article by Clerici and Yang [17] they find no evidence that SV undergoes any change as a result of high levels of ROS.

Hirose and Liberman [39] studied the effect of noise on SV and endocochlear potential (EP). They found massive loss of type II fibrocyes (see Sec. 2.3.3) and degeneration of intermediate and marginal cells with drastic reduction in membrane surface area. Despite all damage to SV and a decrease in EP, Hirose and Liberman found that this had little influence on PTS and TTS.

4.2.4 Different vulnerability of IHCs and OHCs

According to Sha et al. [91], IHCs are less susceptible to oxidative damage than OHCs. Their study was performed *in vitro* where both IHCs and OHCs were placed in a ROS/RNS environment. Sha et al. found that during such exposure IHCs were able to uphold normal function substantially longer than OHCs.

[1] iNOS – inducible nitric oxide synthase

4.2.5 Experimental evidence for ROS-mediated damage

In the following section we will look at some studies that seem to support oxidative stress theory.

Study by Ohinata et al. (2000)

Ohinata et al. [69] found that 8-isoprostane[1] level increased significantly after noise exposure and rapidly decreased after the termination of exposure in guinea pigs. They found that the concentration of 8-isoprostane increased almost linearly with the duration of exposure, and that a 5 hour exposure to OBN centered at 4 kHz with a 115 dB SPL, gave nearly a 30-fold increase in 8-isoprostane level. After exposure the level decreased rapidly for the first 30 minutes, and then less rapidly for the next hours. 8 hours after exposure the pre-exposure level of 8-isoprostane was reached. This exposure gave a 35-40 dB PTS at 4 kHz. Ohinata and colleagues found a limited localization of 8-isoprostane immunoreactivity in hair cells that showed an intriguing correlation with noise-induced morphological changes. Immunoreactivity was heaviest in the second turn coincident with the area of greatest hair cell destruction in a region 10-12 mm from the apex, and in agreement with an elevated threshold shift at 4 and 8 kHz. Ohinata et al. states that their findings suggest a causal relationship between ROS formation (lipid peroxidation) and hair cell damage.

Both supporting cells and IHCs were also found to have increased levels of 8-isoprostane, but the degree of loss among these was significantly less than OHCs. Ohinata et al. also point out that the same is found *in vitro*; IHCs and supporting cells are more capable to resist ROS damage than OHCs.

This study found most ROS-activity in the lateral wall of the cochlea, greater in OHCs than IHCs, and evident in spiral ganglion and supporting cells.

Study by Clerici and Yang (1996)

In a study by Clerici and Yang [17], they find indirect evidence for involvement of ROS in acquired cochlear damage in that administration of ROS directly to the perilymphatic space cause a decrease in threshold sensitivity in vivo. Clerici and Yang instilled guinea pig cochleas with; (1) artificial perilymph, H_2O_2; (2) confirmed generating systems for the superoxide anion, $O_2^- \cdot$, or hydroxyl radical, $OH^- \cdot$; or (3) with a ROS system plus its respective scavenger. In this study they found that cochleas instilled with H_2O_2, $O_2^- \cdot$ or $OH^- \cdot$ generating systems lead to increased compound action potential (CAP) threshold. These elevations in threshold were found at 10 and 120 min post infusion. While cochleas instilled with a ROS system and its respective scavenger did not experience the same increase in CAP threshold.

[1] 8-isoprostane – a lipid peroxidation product, determined biochemically and histochemically as an indicator of ROS.

Clerici and Yang therefore suggest that this rapid degeneration of CAP threshold may provide insight into ROS-mediated cochlear dysfunction and damage following noise.

Study by Chen et al. (2005)

Chen et al. [14] performed a study exploring the role of endogenous nitric oxide (NO) in the temporary threshold shift caused by acoustic trauma. They exposed groups of guinea pigs to white noise at 105 dB SPL for 10 min, which caused the NO concentration to increase nearly a threefold immediately following exposure. Correlation between the NO concentration in lateral wall tissue (SV and SL) and final auditory brainstem responses (ABR) threshold was found to be significant in the noise exposed animals. Chen et al.'s findings indicate that endogenous NO is generated in the noise-induced guinea pig cochleas and its concentration is correlated with the hearing loss.

Study by Hanson et al. (2003)

Hanson et al. [32] applied a nitric oxide donor at the round window of chinchillas, and found a subsequent elevation in perilympatic nitric oxide concentration. The treated animals showed also a significantly elevated ABR threshold.

4.3 Antioxidant defenses

The cochlea employs a complex and sophisticated defense system to protect itself from damage from oxidative molecules. Protective molecules include vitamins (e.g. A, C, E), glutathione (GSH), enzymes (e.g. SOD, CAT, GSH-px/-rd) and reactive transcription factors[1] that can respond to potential threats to cochlear homeostasis [47]. The latter will not be mentioned any further in this paper.

4.3.1 Endogenous antioxidant defense

Glutathione (GSH) is present in tissue throughout our body, and plays an important role in elimination of foreign drugs and substances, but also in synthesis of DNA and proteins, metabolism of nutrients and gene expression [11].

GSH is an agent which serves as an electron donor, hence being very effective in "fighting" ROS. The glutathione peroxidase (GSH-px) enzyme uses GSH as an electron donor to reduce ROS, thus oxidizing GSH to GSSG, which is what it is called when oxidized. The glutathione reductase (GSH-rd) enzyme is then needed to catalyze the reformation of GSH from GSSG.

Antioxidant enzymes are, as mentioned above, superoxide dismutase (SOD), catalase (CAT), and GSH-px and GSH-rd. Enzymes in general are present to initiate or accelerate chemical reactions without being altered themselves. In cochlea SOD converts the superoxide radical anion ($O_2^- \cdot$) into oxygen and hydrogen peroxide (H_2O_2), and CAT converts hydrogen peroxide into water and oxygen (see App. A.3).

Vitamins, such as A, C and E, are all to consider as antioxidants, and they function as electron donors for different enzymes. It should be noted that vitamin C is a good scavenger of ROS, but could appear as dangerous if iron (reducing Fe^{3+} to Fe^{2+}, see App. A.4, Eq. A.8) is present [25].

Different approaches have been tested as to clarify what affects NIHL susceptibility. Experiments with agents that enhance antioxidant systems, and with agents that inhibit ROS production [53], have both lead to attenuated NIHL, which would imply that ROS is at least partially involved in noise-induced cochlear damage.

4.3.2 Where antioxidants are generated

Antioxidants are present everywhere mitochondria are located, but indications are that some areas have a stronger defense and a greater capacity of generating, or a greater supply of, antioxidants than others.

Sha et al. [91] suggest that the basal region of the cochlea has weak antioxidant defenses rel-

[1] A transcription factor – a protein which work together with other proteins to promote or suppress transcription of genes (i.e. DNA to RNA)

ative apical regions, thus making the basal OHCs especially vulnerable. This is based on their experiment *in vitro* where isolated OHCs from base and apex was exposed to ROS, in which they found that basal OHCs are far more susceptible to ROS than apical OHCs. Sha et al. found that this corresponded very well with the level of GSH in the cells, i.e. there was less GSH in basal OHCs than apical cells.

According to both Jacono et al. [43] and Yamasoba et al. [111], stria vascularis GSH content transiently increases after continuous broad-band noise exposure. Bobbin et al. [3] found an increase in perilymphatic GSH levels after sound exposure. Whereas GSH levels increase in the cochlea lateral wall tissue and perilymph, GSH levels in OHCs decrease, especially after exposure to intense sound [47].

4.3.3 Enhancing endogenous AO-defense

Different approaches have been used in attempts to reduce the effect of oxidative stress in noise-induced hearing loss, tryings with both direct application of antioxidants and by stimulation of AO-defense mechanisms.

Oral intake of antioxidants

Animal studies have shown that dietary supplementation of antioxidants have an effect on NIHL. A study by McFadden et al. [61] showed that dietary vitamin C supplementation reduces NIHL in guinea pigs. They found that animals receiving dietary ascorbate developed significantly less PTS than animal on normal or deficient diets, hence suggesting that high levels of vitamin C may be beneficial in reducing susceptibility to NIHL.

A similar study performed by Biesalski et al. [2] give an indirect evidence of antioxidants role in protection from hearing loss. They found that a vitamin A deficiency increases noise susceptibility in guinea pigs, which lead to increased probability of NIHL.

More recently Le Prell et al. [52] performed a study on guinea pigs where a combination of different vitamins (A, C and E) and magnesium was used. The vitamins are known to work as antioxidants in the body, while magnesium is found to reduce noise-induced vasoconstriction (e.g. [1, 88, 89]). According to Le Prell et al. a combination of these vitamins and Mg attenuated NIHL significantly.

Systemic administration of agents

Kopke et al. [48] tried influencing different oxidative or antioxidant mechanisms, and found that agents promoting antioxidant defenses, or free radical scavenger systems, and agents reducing ROS production, both attenuate NIHL. They demonstrated reduction in threshold shift (largest reduction in PTS, but also smaller reductions in TTS) and reduced (both inner and outer) hair

cell death in treated animals compared to control animals[1].

Ohinata et al. [70] performed a similar study in which they injected agents known to inhibit potential sources of ROS formation to guinea pigs. Their result is in accordance with earlier studies which have showed attenuated hearing loss.

A study by Dereköy et al. [21] demonstrated that ascorbic acid treatment inhibited both lipid peroxidation and oxidative damage of proteins in rabbits exposed to noise. They also found that a brief application of ascorbic acid before noise exposure appeared to play a protective role for rabbit cochleas.

Round window administration

Some studies have applied antioxidants directly to the round window of the cochlea [51], and studied the effect of noise on ears getting this treatment (e.g. [38, 41, 96]). It is found that upregulation of antioxidative agents in the perilymphatic space have positive effect on reducing oxidative stress mechanisms in the cochlea, and that such treatment significantly reduces noise-induced hearing loss.

Sound conditioning (preconditioning)

There is strong evidence that sound conditioning attenuates NIHL (e.g. [34, 43]). Sound conditioning, or acquired resistance to noise (ARN), is the name of the effect that low level noise exposure before high level noise exposure decreases the ears susceptibility to noise.

Jacono et al. [43] found increased levels of endogenous antioxidants after sound conditioning and after high level noise exposure. They found that antioxidant levels are increased both in the organ of Corti and the SV, but suggest that SV is the *main* manufacturer of endogenous antioxidants. They find evidence for GSH-transport through the perilymphatic space, and suggest that the Spiral ligament is the source which delivers antioxidants to the Scala tympani. It is therefore assumed that antioxidants can be transported through the perilymphatic space, and gradually reach the OC to help prevent damage to this vital structure.

Harris et al. [34] performed a study using Paraquat (PQ), a potent generator of superoxide, to stress the ears of chinchillas after sound conditioning. Their study aimed to find out if the increase in endogenous antioxidants seen following conditioning could provide protection from such oxidative stress. In this study they also found that conditioned animals showed less susceptibility to ROS-induced damage (i.e. less PTS and less cells were lost) than control animals.

[1] Control animals – animals with no treatment

4.4 Effect of noise on AO-defense mechanisms

As mentioned above Jacono et al. found an increase in endogenous AO-defenses after noise exposure, both in OC and the SV. Yamasoba et al. [111] have pointed out the importance of glutathione as an AO-defense mechanism. In another study Yamasoba et al. [110] investigated the effect of intense noise upon glutathione synthesis in the cochlea. Yamasoba and colleagues found that GSH levels were significantly increased in the lateral wall 2 and 4 hours post-exposure, and that it returned to normal 6 hours post-exposure. GSH levels in the sensory epithelium and modiolus[1] did not show significant changes following noise, according to their study. This would imply that GSH synthesis is markedly upregulated selectively in the lateral wall (i.e. SV) by noise exposure.

4.5 Continuing damage after noise exposure

Some studies have pointed out that damage to the cochlea tissue continues for a period after terminated noise-exposure (e.g. [40, 109]). According to Yamashita et al. [109] there are at least two factors determining delayed damage; (1) the cause of damage could be the slowly progressing pathways of necrotic or apoptotic cell death, or (2) a continuing production of free radicals.

In their study Yamashita et al. found that the noise-induced threshold shift had a maximum right after noise exposure, and that threshold curves decreased with time after noise. Additionally they found that OHCs kept dying for the next 7-10 days after noise exposure, and that this correlated well with the increased levels of ROS and RNS found. In this study Yamashita and colleagues showed a delayed formation of ROS/RNS, which reached a maximum 7-10 days post exposure. For measurements after 7-10 days both threshold shift and number of dead OHCs stabilized.

Yamashita et al. therefore suggest that initial hair cell damage after noise may primarily reflect mechanical events plus transient intense ROS formation, while continued formation of ROS/RNS contributes to the long term hair cell loss.

Also Hu et al. [40] report continuing damage after noise exposure, but they suggest that this is caused by apoptosis and that this process takes time to complete. Under morphological examination of OHC nuclei they found nuclear condensation and fragmentation, which are both indications of an apoptotic process of cell death. According to Hu et al. findings of swollen OHC speaks for a necrotic cell death pathway, and a continuing damage after noise.

Clerici and Yang [17] suggest that chain reactions caused by ROS perpetuate and act for long periods of time and at great distances. They stress the fact that hydrogen peroxide is capable of crossing plasma membranes, and therefore suggest that this chain reaction, at least partly, is causing the continuing damage after noise exposure.

[1] Modiolus – the central, bony core of the cochlea.

4.5.1 Post-exposure treatment reduces NIHL

As described above, indications are that ROS/RNS is generated for a period of time after termination of noise exposure, and therefore treatment with antioxidants during this period of delayed formation of ROS/RNS should also help reducing the extent of oxidative stress. A study performed by Yamashita et al. [108] showed that post-exposure treatment with antioxidants does reduce NIHL, but with less reduction in threshold shifts than pre-exposure treatment.

Sound conditioning (postconditioning)

There is also suggested that lower levels of noise after exposure to high level noise could have a positive effect on recovery from hearing impairment. Niu et al. [68] showed in their study some effect of post-exposure sound condition, but they also found less reduction in NIHL than pre-exposure conditioning.

4.6 Summary

We have now shown, and given reference to literature that indicate a significant role of oxidative stress in noise-induced hearing loss. It should also be clear that antioxidant defenses play a vital role in inhibiting impairment, and that enhancement of such defenses could be a good way to prevent NIHL.

The oxidative stress pathways make the basis for our model, which will be presented in the next chapter (Ch. 5).

Chapter 5

The Model

5.1 Introduction and assumptions

Because of all the different effects of noise on hearing the complexity of a model taking everything into account would be enormous. Our goal is to make a simplified model which will contribute to the understanding and estimation of hearing damage. To do so, several assumptions had to be made.

The main idea of the model is to describe the concentration and flow of damaging compounds in the hair cell with an electric circuit. This will be done using diffusion based principles. One of the most important assumptions we have used is that the concentration of ROS has a proportional correlation to TTS. Another fundamental idea is that production of ROS is located inside the hair cell and the production/supply of antioxidants is located in the lateral wall, because the blood supply in this region is large. This means that the antioxidants have to travel a distance before reaching the hair cell, hence using some time which accumulates ROS in the cell.

As mentioned in Ch. 4 there is always a production of ROS in the ear. At low SPL[1], however, the concentration of antioxidants is large enough to take care of the ROS in the ear. When the SPL increases the production of ROS also increases. If the production of ROS is larger than the hair cells can deal with, there will be an accumulation of damaging compounds in the cell. According to the diffusion principle, which tries to keep a balanced level, this leads to a production of antioxidants in the lateral wall until equilibrium is reached. Because the antioxidants needs some time to get from the lateral wall to the hair cell, the accumulation of ROS is increased even more before this balance is reached.

There are several ways to model the hair cells. One could look at a single hair cell and do estimation for each cell, or one could do estimations for several hair cells at once. We have looked at a group of hair cells, more exactly the area in the ear which responds to frequencies around the 4 kHz band. This means that we describe the concentration for a number of hair cells

[1] SPL < 65 dB is also known as effective quiet (EQ) at the 4 kHz band [106].

and estimate when they start to get damage.

The 4 kHz band was chosen of two reasons. First of all, this area is one of the most delicate areas in the ear. This means that if good estimations are done at this frequency, this estimator can be used at other frequencies without underestimating the damage. In addition, many of the studies found are done at the 4 kHz band, giving more results to base the method on.

An intriguing question is which electrical components to use. Since our goal is to describe a concentration we chose to model the OHCs with a capacitor. Capacitance is defined as the amount of charge, or the concentration of electrons and holes, stored on each plate for a given potential difference. Since holes are positive and electrons are negative, it is natural to choose healing (positive) compounds as holes and damaging (negative) compounds as electrons. Another argument which speaks for the capacitor is the fact that the hair cell has a volume, and therefore an ability to store both antioxidants and ROS. The capacitor has a similar ability to store electrical energy.

Physically the antioxidants render the ROS harmless. A simplified view at this mechanism is that when a ROS is produced it uses one antioxidant to be neutralized. To represent this in the model we have to look at this as positive holes neutralizing negative electrons. This can be modeled by using a current source as a load to the capacitor, where the direction of the current is away from the positive side of the capacitor. The production of ROS also has to change when the SPL is changed, ergo the current source has to be variable.

5.2 The model

In Figure 5.1 the electrical model is shown. The potential difference over the capacitor, V_C, is the size we want to look at. It express the concentration of damaging compounds in the hair cell. When the potential difference is positive it means that the hair cells hold a concentration of antioxidants.

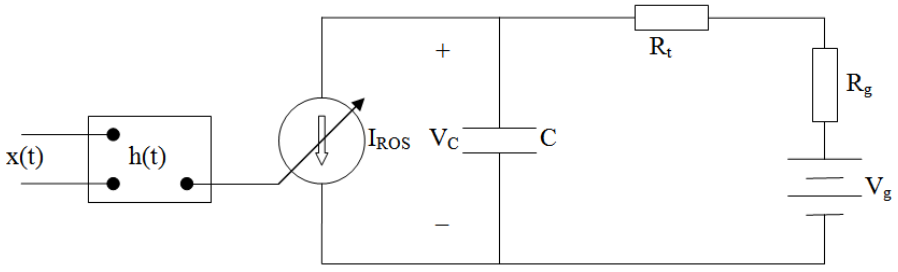


Figure 5.1: Electrical model for estimation of hearing damage.

As an in-parameter to the system the pressure outside the ear is used. In the figure the in-parameter is expressed with $x(t)$ to include that we have used both linear and squared pressure. The choice of using the pressure as an in-parameter was made because of the idea of using the

model in real time measurements, where the pressure is easy available.

The transfer function, $h(t)$, is there to adjust the pressure into production of ROS. Since the pressure is measured outside the ear, the transfer function also has to take into account the changes the outer- and middle ear do to the pressure. This is in our case not important since we look at only one frequency area. If the model should be expanded to include the entire frequency range of the human ear, it would be an important property of the transfer function. The ROS production, expressed as a function of pressure, can then be written as

$$I_{\mathrm{ROS}}(t) = x(t) * h(t). \tag{5.1}$$

In the model the variable current source, I_{ROS}, is the production of damaging molecules. This component represents the production of ROS that we assume to appear inside the hair cell under sound exposure. When ROS is produced, antioxidants are used and the potential decreases.

The hair cells are represented by the capacitor, C, in the model. As mentioned, the hair cells has a volume and therefore a capacity to hold both antioxidants and free radicals.

V_{g} together with the resistance R_{g} represents the main production of antioxidants, which happens in the cochlear lateral wall. The reason we believe that the production is located there, is because this area has a rich blood supply. Whether the generator should be a current or a voltage source was a choice we had to make. We ended up with using a DC voltage source, based on the physiological consideration that wherever the production is located, the concentration of antioxidants at this location is tried to be kept constant. A current source would have implied that the *production* is constant, and not the concentration. The same generating functionality could, however, be made with a current source by finding a Norton equivalent to V_{g} and R_{g}.

When looking at the electrical circuit it may seem like the production of antioxidants (which is represented by the current running through R_{g}) might be infinite. This is, however, a truth with modifications. Since the ROS production is dependent of the SPL, which obviously has an upper limit, the production of antioxidants also has a limit. There is, however, one assumption; we believe that the production of antioxidants is capable of getting as large as the production of ROS.

Since we assume that the main production is located in the lateral wall, the resistor, R_{t}, is placed between the source and the capacitor to represent the resistance the antioxidants most likely experience on the way to the hair cell. In the calculations R_{g} and R_{t} are added and called R.

5.3 Determining the values on the components

5.3.1 The transfer function [$h(t)$]

Since we programmed our model in MatLab, the transfer function did not need to have an ordinary functionality. We could program the current source directly with the pressure as an

in-parameter. However, the transfer function could be looked at as the link between pressure and ROS production, as shown in Figure 5.1, even if the actual processing was done in the source itself. In the next section we describe how the ROS production should be, and the transfer function can be understood as the processing done to the pressure. How this processing was done was in close relation to how the production of ROS was assumed to be. Since we tried several assumptions, the transfer function had to be changed.

5.3.2 The variable current source [I_{ROS}]

How the variable current source was chosen relied mainly on how we wanted to express V_{C}. Based on the assumption that TTS has a correlation to the concentration of damaging compounds in the hair cell, we wanted to express V_{C} as TTS in dB. This lead to some restrictions on how the current source could be chosen.

Table 5.1: The table shows all the different ROS production alternatives used in the model.

Alternative	ROS production (I_{ROS})	Description
Alt. 1	$\alpha_1 \cdot 1.7 \cdot \left[10 \cdot \log\left(\frac{I_{\mathrm{e}}+I_{\mathrm{c}}}{I_{\mathrm{c}}}\right)\right]$	Production assumed to follow the ATS equation
Alt. 2	$\frac{\alpha_2}{R} \cdot p^2(t)$	Production assumed to follow the squared pressure
Alt. 3	$\frac{\alpha_3}{R} \cdot p(t)$	Production assumed to follow the linear pressure
Alt. 4	$\frac{\alpha(\mathrm{SPL})}{R} \cdot p^{\gamma(\mathrm{SPL})}(t)$	Constant and exponent of power assumed to be level dependent

When determining the current source it was helpful to know that V_{C} reaches an asymptote when the current source is on. This asymptote has the value

$$V_{\mathrm{C,asymptote}} = I_{\mathrm{ROS}} \cdot R - V_{\mathrm{g}}. \tag{5.2}$$

In the model the DC voltage source, V_{g}, is large enough to cope with the ROS production generated by 65 dB SPL (EQ). This means that for SPL > 70–75 dB, the DC source starts to be negligible in comparison to $I_{\mathrm{ROS}} \cdot R$, hence the asymptote can be written

$$V_{\mathrm{C,asymptote}} \approx I_{\mathrm{ROS}} \cdot R. \tag{5.3}$$

We tried several ROS production alternatives during the development, and these will be presented later in Ch. 6. In Table 5.1 we have listed all the different alternatives.

5.3.3 RC time constant

When developing the model we wanted, as a first step, to make it as simple as possible. This lead to the use of only one R and one C in the system. Because of this we could only obtain one time constant. Since all the literature read about TTS indicates different time constants for development and recovery, we had to decide what this time constant should be. Mills et al. [64] showed in 1979 that the time development for onset and recovery of TTS can be described by an exponential function with time constants 2.1 hours (126 min) for onset and 7.1 hours (426 min) for recovery. Patuzzi [72] showed in 1998 that the same time development can be described by a multi-exponential function with two time constants for onset and three for recovery. The constants he found was $t_{\text{on1}} = 6.5$ min, $t_{\text{on2}} = 800$ min, $t_{\text{rec1}} = 30$ min, $t_{\text{rec2}} = 240$ min and $t_{\text{rec3}} = 800$ min. Patuzzi's idea, as mentioned in Sec. 3.3.4, is based on inactivation of MET-channels and not on degeneration of the hair cells, and he also emphasize that there might be even more time constants depending on other damaging mechanisms. Because of this we decided not to use his time constants, but instead use one of the two Mills et al. found.

Since a large time constant would underestimate the damage potential from a short exposure sound, we decided to use the time constant Mills et al. found for onset (2.1 hours). Even if this underestimates the damage potential *after* exposure, it is better than the opposite. An alternative would have been to use something in between, but we did not focus on such method.

In RC-circuits the time constant is $\tau = R \cdot C$. Knowing τ we know what the ratio between R and C should be. Since neither of the two variables could be determined, we decided to use $C = 126 \cdot 10^{-3}$ F $= 126$ mF and $R = 1000\ \Omega = 1$ kΩ. This leads to a time constant $\tau = 126$ minutes. It is important to notice that this time constant is in minutes and not in seconds.

5.3.4 Production of antioxidants [V_{g}]

The main production of antioxidants is, as mentioned, assumed to be outside the hair cell. The size of this generator was determined by saying that the hair cell should be able to cope with ROS produced by a SPL at 65 dB. This was done by setting $V_{\text{g}} = 0$ and expose the system to 65 dB SPL for such a long time that the system reached asymptote. The value of V_{g} was then adjusted to the asymptotic level. It was important that the generator was adjusted each time I_{ROS} were changed.

5.3.5 An example of the output from the model

In Figure 5.2 we have plotted a simple example from the model showing the development of the TTS during and after exposure. The exposure is a continuous SPL at 45 dBA for 2 hours followed by 89 dBA SPL for 4 hours and finally 45 dBA for 2 hours. The model used is the one described in Sec. 6.5.

In the output the plot is shown in both linear and logarithmic time scale. This is because short

exposures are better described by a logarithmic scale, while long exposures are best viewed with a linear scale.

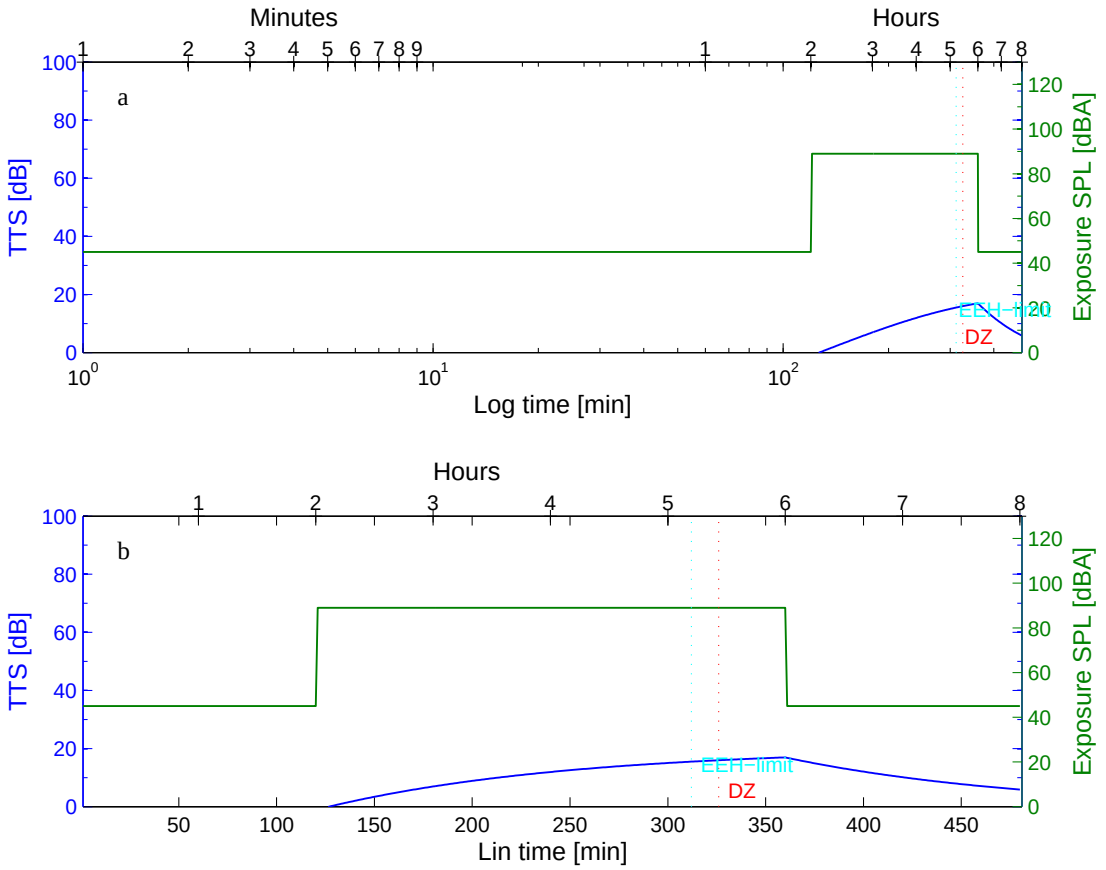


Figure 5.2: An example of the output from the model. DZ is where the danger zone starts. This limit indicates when the model predicts that permanent damage may occur. EEH-limit is the limit where ISO 1999:1990 says that permanent damage may occur. The only difference between (a) and (b) is the x-axis which is logarithmic in (a) and linear in (b).

Chapter 6

Description

Which mechanisms that permanently damages the hair cells, when there is a concentration of ROS inside the cell, is not clear. As mentioned in Sec. 4.2.5 several studies have tried to find a correlation between ROS and PTS and some of them say that increased level of ROS gives an increased amount of damage.

Kraak et al. [50] proposed, in 1974, an idea that noise-induced physiological stress could be measured by a function integrating TTS over time. This expression can be seen in Eq. 6.1.

$$S = \int_{t_0}^{t} \mathrm{TTS}\, \mathrm{d}\tau \tag{6.1}$$

S is the stress the hair cell is exposed to and t_0 is when the exposure starts. When $S > S_0$[1] the hair cell starts to get permanent damage. They make a point out of the fact that the integral should cover the entire period from the start of the exposure (t_0) to the end of the recovery (TTS=0).

Since we assume that the concentration of ROS is equal to the level of TTS, an integral over the concentration, as shown in Eq. 6.2, would be the same as the stress function mentioned in their article. The only difference is that in our model we do not cover the entire period from the start of the exposure, but only from the time when there is a damaging concentration of ROS. For loud SPL, however, the concentration becomes damaging almost instantaneously, and the equations are very similar.

$$S = \int_{t_0}^{t} [ROS]\, \mathrm{d}\tau \tag{6.2}$$

In the equation $[ROS]$ means the concentration of ROS. This is a function of time and t_0 is the time when this concentration becomes large enough to create damage. It is important to notice

[1] They do not say what S_0 should be.

that since the concentration of ROS does not go to zero immediately after the recovery begins, the damage might occur after the end of the exposure.

Since our model is implemented in MatLab the integration has to be converted into a sum. The expression then becomes

$$S = \sum_{n_0}^{N} [ROS] \cdot \Delta t. \tag{6.3}$$

We will in the next section present how we judged our model and describe the different attempts we tested, and discuss the advantages and disadvantages.

6.1 Evaluating conditions

6.1.1 Basis of comparison

Since one of the main assumptions used in the modeling is that the concentration of ROS is proportional to the TTS, we tested the different alternatives against measured ATS values (see Sec. 6.2 for details).

To evaluate the damage estimation we decided to compare the model against two known and used standards.

The international standard ISO 1999:1990, also known as the equal-energy hypothesis (EEH), is commonly used around the world as an estimator of hearing damage. This standard is based on the hypothesis that equal energy causes equal damage. The fundamental idea this standard is based upon is that human ears can tolerate 85 dBA for 8 hours. A doubling or halving of time corresponds to respectively a 3 dB increase or decrease of the SPL.

Another standard in use has a similar approach. OSHA[1] uses a 5 dB exchange, instead of ISO's 3 dB. They base their standard, referred to as the OSHA-rule in this paper, on the idea that human ears can tolerate 90 dBA for 8 hours. It has been proposed questions and recommendations to OSHA to lower their 8 hour level to 85 dBA, because later studies have shown that workers exposed to levels above 85 dBA face significant risk of hearing loss.

6.1.2 Tolerance

When comparing our model against the two standards we had to decide how much difference we could tolerate. Since all of our damaging limits are calculated without the "recovery tail"[2]

[1] OSHA stands for Occupational Safety and Health Administration and is a federal American agency.

[2] This "tail" can be seen in Figure 5.2 and is what happens with the TTS curve after the exposure ends (after 6 hours).

we did not want to be any stricter[1] than the two standards. This is because the tail will add some stress to the ear and adjust the damage potential a bit. The reason we did not include the tail in the calculation of the limits is that this "tail" will change if the SPL during recovery changes. It is important to notice that the calculation of stress includes this "tail".

Because of the indications of the OSHA-rule being to tough, we decided to use this standard as an upper limit and emphasize the ISO standard the most, and the goal is to be closer to the EEH than the OSHA-rule.

It is also important to notice that the two standards are only valid over a limited range of SPLs. ISO says their standard is only valid from 75 dBA to 100 dBA. When comparing the model against the two standards the levels outside these ranges were not emphasized a lot.

Hamernik and Qiu [31] also points out that the ISO standard might be a good estimator for Gaussian, steady state noise, but for more realistic sound exposures the standard has its limitations.

[1] Stricter seen from an employers point of view, but kinder to the ear.

6.2 Alternative 1

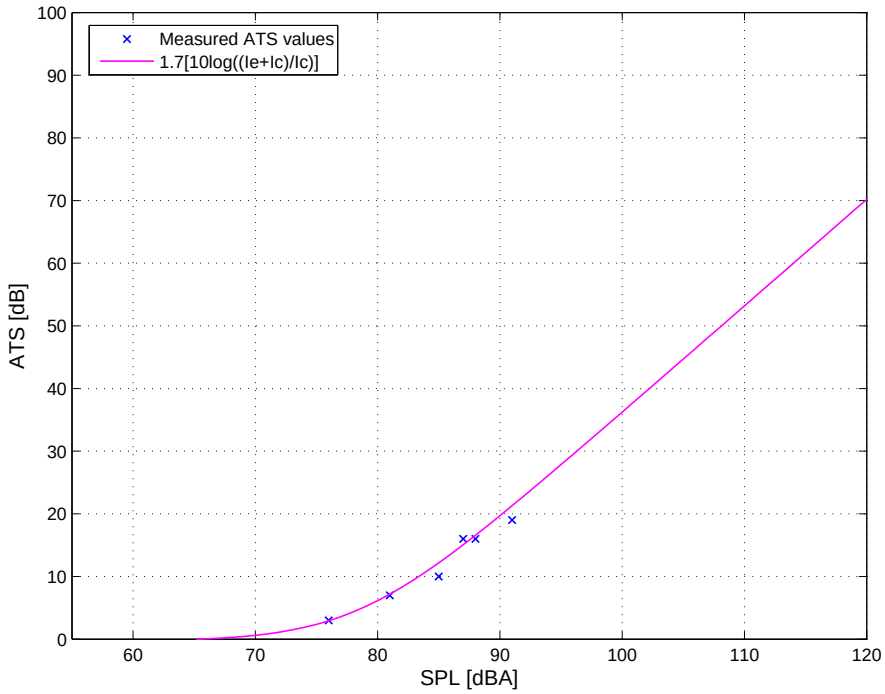


Figure 6.1: Figure showing ATS as a function of SPL. The crosses are the measured ATS values and the line is the equation $1.7 \cdot \left[10 \cdot \log\left(\frac{I_e+I_c}{I_c}\right)\right]$. ATS values from Mills et al. [63].

The first alternative is based on the assumption that the concentration of ROS is proportional to ATS[1]. Mills et al. [64] looked at the ATS persons reached when exposed to a certain SPL. They found a correlation between SPL and ATS following the formula

$$\text{ATS} = 1.7 \cdot \left[10 \cdot \log\left(\frac{I_\text{e} + I_\text{c}}{I_\text{e}}\right)\right], \tag{6.4}$$

where I_e is calculated from the relation SPL = 10log(I_e) and I_c is a frequency dependent constant which can be found from the relation C = 10log(I_c). C is a frequency dependent constant which Mills et al. has estimated empirically to be 78 dBA[2] for broad band noise.

[1]When a person is exposed to loud sounds (SPL>65 dB at 4 kHz) for some time its hearing will experience a TTS. If the exposure is continued for a long time the TTS will reach a plateau. The level this plateau is at is called an asymptotic threshold shift (ATS).

[2]Mills et al. say they used different weightings on the measured values at 87 and 88 dBA in Figure 6.1. Using a LMS algorithm with 0.5 dBA steps and 10 times weighting on the mentioned points in MatLab, we found this constant to be 78.5 dBA. It is this value that is used in Figure 6.1 and that seems to be the one Mills et al. have used also.

In Figure 6.1 the function in Eq. 6.4 is shown. The crosses are the measured ATS values used to develop the function. Mills et al. say, and this can be seen in the figure, that the validity of this mathematical description is limited to $8 \lesssim \text{ATS} \lesssim 30$ dB or $80 \lesssim \text{SPL} \lesssim 96$ dBA. Above these levels there are no measurements supporting the progress. In our model, however, we assume that the equation is valid up to at least 120 dBA SPL.

The current source, which should make the concentration of ROS proportional to the ATS, can then be written as

$$I_{\text{ROS}} = \alpha_1 \cdot \text{ATS} = \alpha_1 \cdot 1.7 \cdot \left[10 \cdot \log \left(\frac{I_\text{e} + I_\text{c}}{I_\text{c}} \right) \right], \tag{6.5}$$

where α_1 is a constant. Keeping Eq. 5.3 and the fact that V_C should be equal to TTS in mind, we find that α_1 has to be equal to $1/R$.

Since SPL can be expressed as

$$\text{SPL} = 10 \cdot \log \left(\frac{p^2(t)}{p_0^2} \right), \tag{6.6}$$

I_e can be written as

$$I_\text{e}(t) = \frac{p^2(t)}{p_0^2}, \tag{6.7}$$

where $p0 = 20 \cdot 10^{-6}$Pa.

The ROS production can then be expressed as:

$$I_{\text{ROS}}(t) = \frac{1.7}{R} \cdot \left[10 \cdot \log \left(\frac{\frac{p^2(t)}{p_0^2} + 10^{\frac{78.5}{10}}}{10^{\frac{78.5}{10}}} \right) \right]. \tag{6.8}$$

This expression is quite complex and makes it difficult to understand how the transfer function, $h(t)$, is. The reason we wanted to use such complex processing was because of the correlation to TTS. If the equation Mills et al. developed is correct, our model estimates this temporary damaging mechanism in a good way.

6.2.1 Linear ROS concentration in damage prediction

This alternative gives the best fitting curve in regard of ATS, according to Mills et al.. However, since we wanted to use a simple sum of ROS concentration to predict potential hearing damage, this alternative meets its limitation.

In Figure 6.2 Alternative 1 is compared against EEH and the OSHA-rule. The first attempt was done using Eq. 6.3 with S_0 equal to the stress from 8 hours with 85 dBA. As the figure shows this

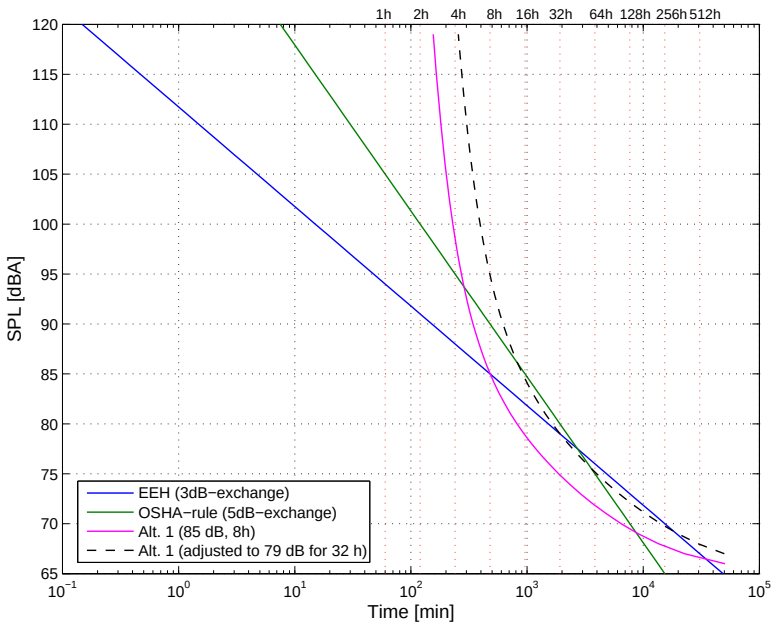


Figure 6.2: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 1 against EEH and the OSHA-rule. The stress, S, has been calculated as the sum of the *linear* ROS concentration over time. The damaging limits were chosen to be 8 hours with 85 dBA and 79 dBA for 32 hours.

first attempt does not give a good estimation of damage, according to our tolerance. Especially at high SPLs the estimate is extremely bad. Since the conformity was bad both at high and low SPLs the second attempt was made to get a better fit, at least at low SPL. This was done by adjusting the damaging limit, S_0, to 79 dBA for 32 hours. As can be seen the estimate gets a bit better at low SPL. For SPL above 90 dBA, however, it gets even worse.

6.2.2 Squared ROS production in damage prediction

To try to cope with the problem at high SPL we tried to square the ROS concentration in Eq. 6.3. The equation then becomes

$$S_2 = \sum_{n_0}^{N} [ROS]^2 \cdot \Delta t. \tag{6.9}$$

In Figure 6.3 we have plotted the result and compared it against the linear approach. From the plot we can see that the squaring affected both the high and low SPL. Nevertheless, the improvement was not large enough.

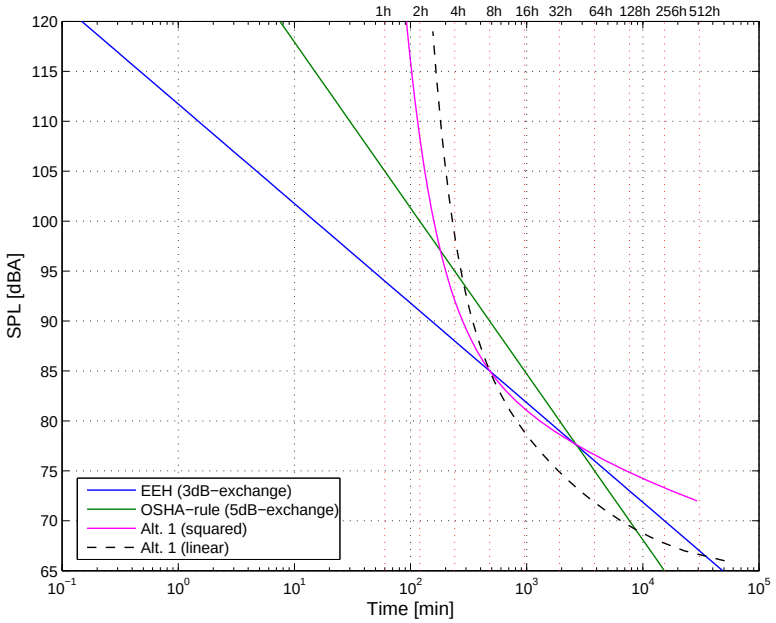


Figure 6.3: Comparison of squared and linear ROS concentration in damage prediction against EEH and the OSHA-rule. The solid line is where stress, S_2, has been calculated as the sum of the *squared* ROS concentration over time. The dashed line is the linear alternative. Damaging limits were chosen to be 8 hours with 85 dBA, as EEH.

The problem is that the high SPLs does not get enough weighting. This means that the concentration of ROS increases too slow in relation to the actual pressure.

6.3 Alternative 2

The second alternative is a similar approach as the international standard ISO 1999:1990. Our approach uses the approximation that the production of ROS follows the equation

$$I_{\mathrm{ROS}}(t) = \frac{\alpha_2}{R} \cdot p^2(t), \tag{6.10}$$

where α_2 is a constant and R is the resistance in the model. $1/R$ is included in the equation of the same reason as in Alternative 1. The production of ROS is in other words proportional to the power of the pressure.

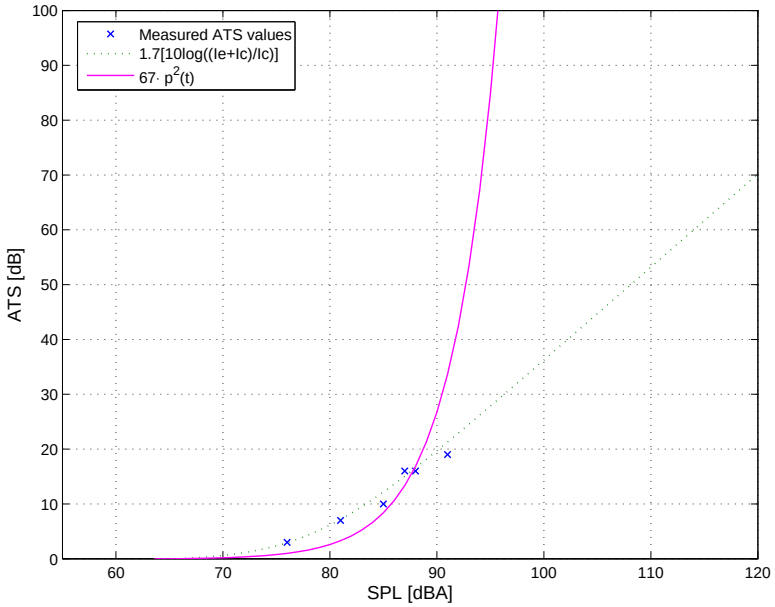


Figure 6.4: Figure showing ATS as a function of SPL. The crosses are the measured ATS values and the line is the equation $67 \cdot p^2(t)$. The dotted line is the line from Alternative 1 described in Eq. 6.4. ATS values from Mills et al. [63].

Using this way of expressing I_{ROS} in Eq. 5.3 we see that the concentration of ROS, after reaching asymptote, can be written as

$$V_{\mathrm{C,asymptote}} \approx \alpha_2 \cdot p^2(t). \tag{6.11}$$

Putting this concentration into the stress integral in Eq. 6.2, the resemblance to EEH is remarkable. In Eq. 6.12 the equation used to calculate the energy in EEH is shown, and in Eq. 6.13 the asymptotic stress calculated for the model is shown.

$$E_{\mathrm{A,T}} = \int_{t_1}^{t_2} p_{\mathrm{A}}^2(\tau)\,\mathrm{d}\tau, \tag{6.12}$$

where $\mathrm{T} = t_2 - t_1$ and p_{A} is the A-weighted pressure.

$$S_{\mathrm{asymptote}} = \int_{t_0}^{t} \alpha_2 \cdot p^2(\tau)\,\mathrm{d}\tau \tag{6.13}$$

It is important to point out that Eq. 6.13 is only an approximation, since it is the asymptotic concentration that is used.

To decide what α_2 should be, we compared the concentration of ROS in the hair cell, after reaching asymptote, against the ATS values used by Mills et al. By using a LMS algorithm on $\alpha_2 \cdot p^2(t)$ we found that $\alpha_2 = 67$ gave the best fit. In Figure 6.4 the result is shown.

As can be seen in Figure 6.4 the equation does not fit very well to the measured ATS values or the equation Mills et al. used in their article. However, the descriptions similarity to EEH makes it an interesting approach.

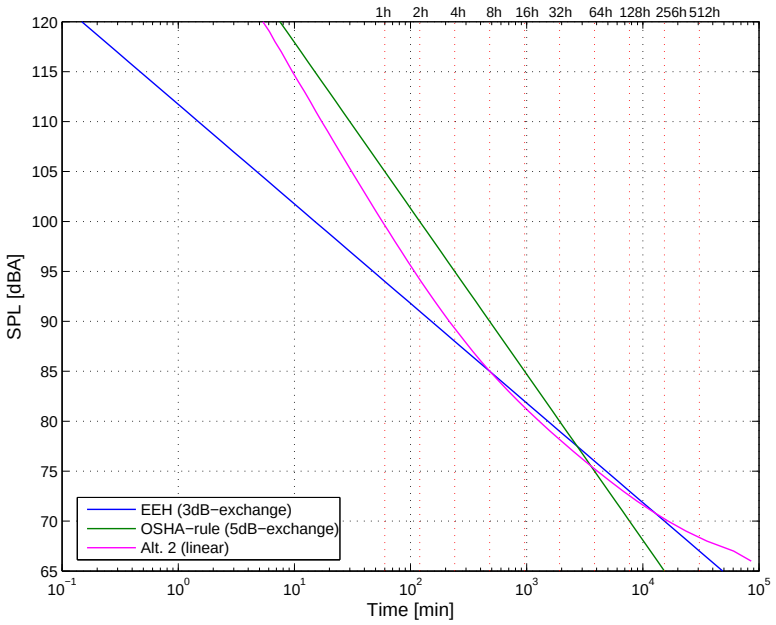


Figure 6.5: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 2 against EEH and the OSHA-rule. The stress has been calculated as the sum of the (linear) ROS concentration over time. Damaging limit was chosen to be 8 hours with 85 dBA, as EEH.

In Figure 6.5 we can see that the estimation of when damage occurs is much closer to the two standards. However, since we wanted to emphasize EEH more than the OSHA-rule the estimation is still to poor at high SPL.

To deal with this the same method was used as in Alternative 1; square the concentration of ROS in the stress-summation. As can be seen in Figure 6.6, the result gets better at high SPL,

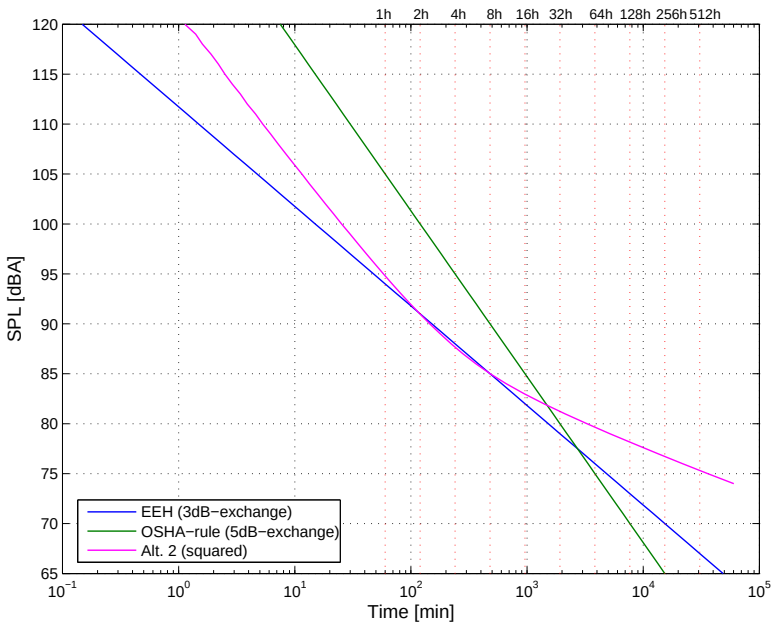


Figure 6.6: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 2 against EEH and the OSHA-rule. The stress has been calculated as the sum of the *squared* ROS concentration over time. Damaging limit was chosen to be 8 hours with 85 dBA, as EEH.

however, at low SPL the result gets worse. This leads to the hypothesis that the development of stress might accelerate above some SPL.

6.3.1 Accelerated development of damage

If such acceleration of the development takes place, it most likely is a continuous process. However, it is not unlikely that things speed up at a certain concentration. Generally this development can be written as

$$S_\beta = \sum_{n_0}^{N} [ROS]^{\beta(\text{SPL})} \cdot \Delta t. \tag{6.14}$$

$\beta(\text{SPL})$ is a function of SPL and might be any number.

Because we did not find any literature saying anything about this, and because we wanted to keep the model simple, we decided to split the estimation of damage into two parts. Based on the two plots in Figure 6.5 and 6.6 we decided to use 85 dBA as the breakpoint. This means that the β function can be written as

$$\beta(\text{SPL}) = \begin{cases} 1 & , \quad \text{SPL} < 85 \text{ dBA} \\ 2 & , \quad \text{SPL} >= 85 \text{ dBA} \end{cases} . \tag{6.15}$$

The result of this combination can be seen in Figure 6.7. It is important to notice that the damaging limit might overestimate the damage from noise fluctuating around 85 dBA.

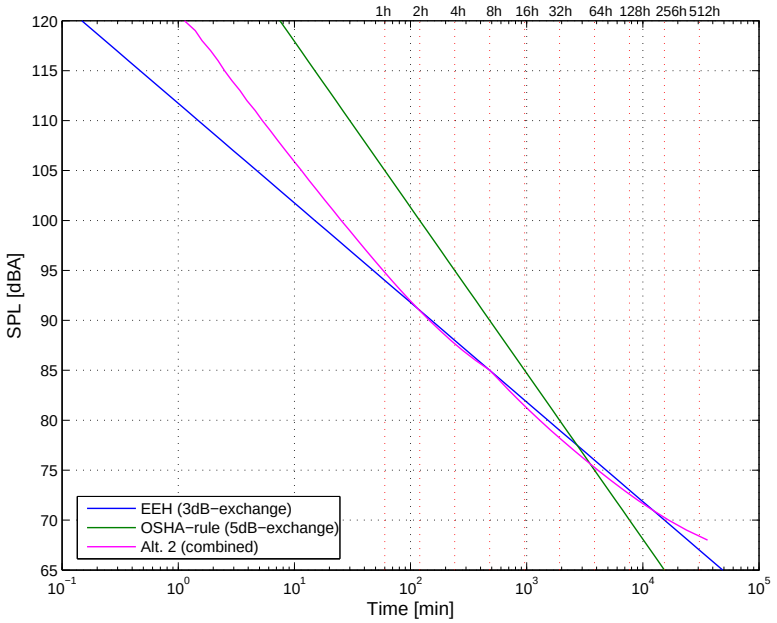


Figure 6.7: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 2 against EEH and the OSHA-rule. The damage prediction has been calculated with two exponents of power. Below 85 dBA the exponent is one, and above the exponent is two.

6.3.2 Drawback

Even if the damage prediction from this alternative is quite good there is one major drawback. This way of expressing the ROS production, and by implication also the TTS, does not fit very well to the ATS curve, as shown in Figure 6.4. This leads to the use of a third alternative; assuming the ROS production to be proportional to the absolute value of the linear pressure.

6.4 Alternative 3

The third alternative was to use the hypothesis that ROS production is proportional to the absolute value of the pressure. The current can then be written as

$$I_{\text{ROS}}(t) = \frac{\alpha_3}{R} \cdot |p(t)|, \tag{6.16}$$

where α_3 is a constant, R is the resistance in the model and $|p(t)|$ is the absolute value of the pressure.

Using the same LMS algorithm as in Alternative 2 we found that α_3 should be 33. In Figure 6.8 the result is plotted together with Alternative 1 and 2. It is clear that the conformity is better for Alternative 3 than Alternative 2.

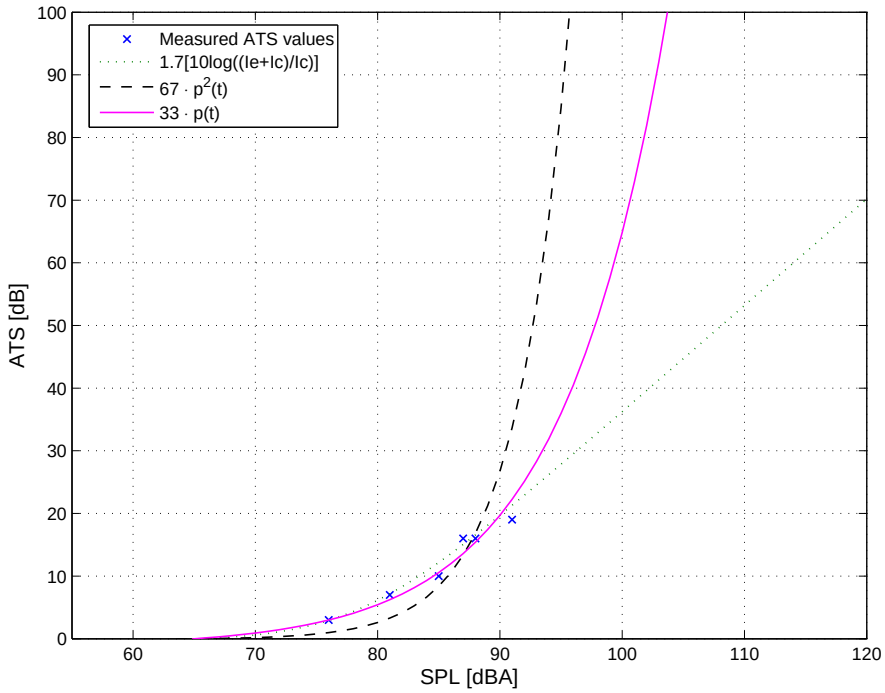


Figure 6.8: Figure showing ATS as a function of SPL. The crosses are the measured ATS values and the line is the equation $33 \cdot |p(t)|$. The dotted line is the line from Alternative 1 and the dashed line is from Alternative 2. ATS values from Mills et al. [63].

This third alternative was evaluated with the same method as the first two. First we tried the linear approach in the damage prediction sum and the result can be seen in Figure 6.9.

As seen the result is a bit worse than Alternative 2 when using 85 dBA as the damaging limit and linear ROS concentration in the summation. It is clear that the result does not fit very well

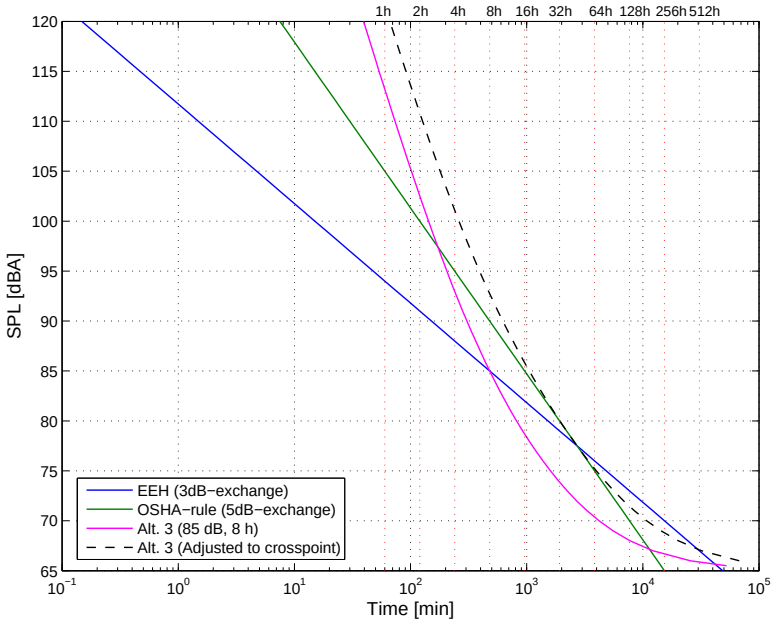


Figure 6.9: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 3 against EEH and the OSHA-rule. The stress has been calculated as the sum of the *linear* ROS concentration over time. Damaging limit was chosen to be 8 hours with 85 dBA, as EEH, in the original plot. In the adjusted one the limit was chosen to be at the crosspoint between EEH and the OSHA-rule.

either at high or low SPL. In the figure we have also adjusted the damaging limit so the curve fits better at low SPL (SPL $<$ 85 dBA). But as can be seen the prediction is bad above this level.

6.4.1 Higher exponent of power

Attempts were made with higher exponents of power, for instance four. In Figure 6.10 we see both the results from using the second and the fourth power in the damage prediction. In the figure it is obvious that the result got better over a larger range (up to about 90–95 dB) when using the 2nd power. At higher SPLs the prediction is still bad. When increasing the exponent of power to four, the result became, as expected, better at higher SPL. The improvement, however, came at the expense of the lower SPLs. Since we in addition wanted to emphasize EEH more than the OSHA-rule, the improvement at higher levels was not sufficient. The use of a higher exponent of power was therefore discarded.

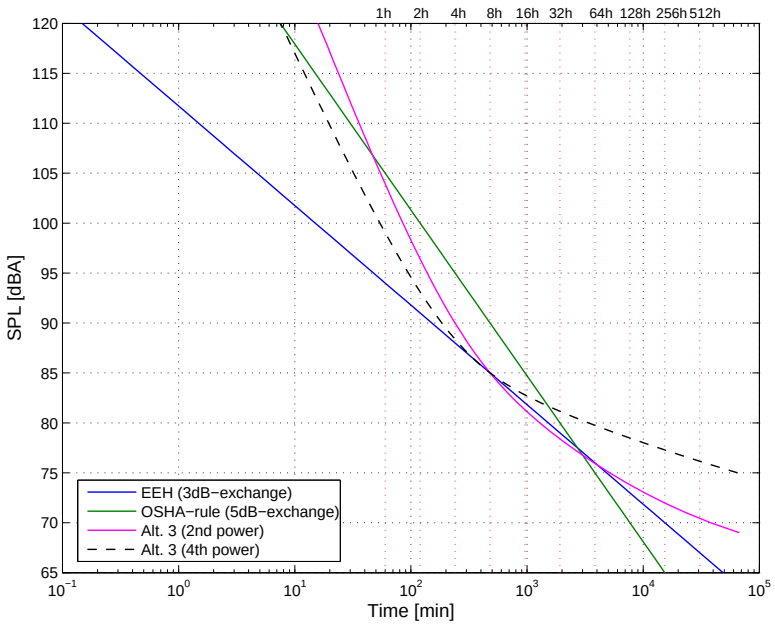


Figure 6.10: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 3, using 2nd and 4th exponent of power in the stress summation, against EEH and the OSHA-rule. Damaging limit was chosen to be 8 hours with 85 dBA, as EEH.

6.5 Alternative 4

Since the previous alternatives have given good results at different SPLs, in accordance to our tolerance, a combination seemed like a reasonable alternative. This alternative combines the best results from Alternative 2 and 3 and is based on the hypothesis that the *production* of ROS accelerates at a certain SPL. Earlier we proposed the hypothesis that the development of damage accelerated and adjusted this by increasing the exponent of power in the stress summation. By adjusting the production of ROS, however, we get a similar effect.

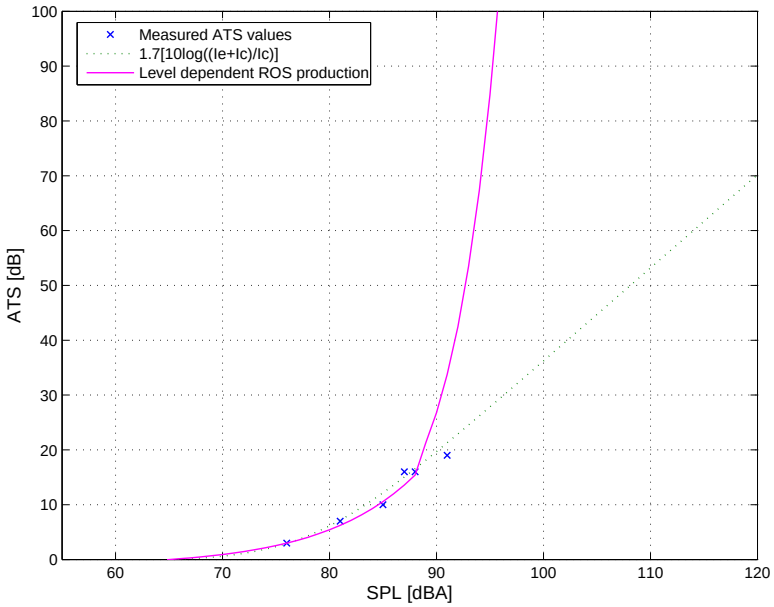


Figure 6.11: Figure showing ATS as a function of SPL. The crosses are the measured ATS values and the line is the result from the level dependent ROS production. ATS values from Mills et al. [63].

In general the hypothesis is that the ROS production follows the equation

$$I_{\mathrm{ROS}}(t) = \frac{\alpha(\mathrm{SPL})}{R} \cdot p^{\gamma(\mathrm{SPL})}(t), \tag{6.17}$$

where $\alpha(\mathrm{SPL})$ and $\gamma(\mathrm{SPL})$ are level dependent constants and R is the model resistance.

Based on the results from the two alternatives we decided to use a ROS production proportional to the absolute value of the pressure below 88 dBA and to the squared pressure above. The ROS production can then be written as

$$I_{\mathrm{ROS}}(t) = \begin{cases} \alpha_2/R \cdot |p(t)| & , \quad \mathrm{SPL} < 88 \text{ dBA} \\ \alpha_3/R \cdot p^2(t) & , \quad \mathrm{SPL} >= 88 \text{ dBA} \end{cases}, \tag{6.18}$$

where α_2 and α_3 are the constants from Alternative 2 and 3, and R is the resistance in the model. The combination of the two alternatives gives an ATS curve which can be seen in Figure 6.11.

If the production of ROS in fact do change with the SPL, the production has almost for sure a continuous increase, as in Eq. 6.17, and not a break point as in our model. This, however, is an error we can tolerate in our simplified model.

6.5.1 Damage estimation

First we tried to calculate the stress with the same exponent of power over the entire range of SPLs. The exponent used was two and the break point was set to be 300 minutes with 88 dBA. This limit was adjusted to a better fit and was mainly determined by the SPL below 88 dBA, since we did not want to go much below EEH.

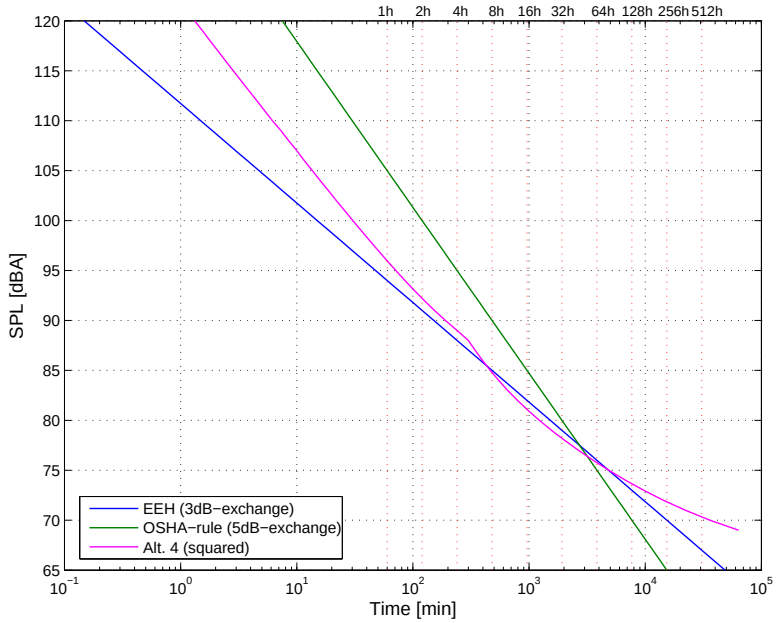


Figure 6.12: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 4 against EEH and the OSHA-rule. The stress has been calculated with the *squared* sum of the ROS concentration over time. The break point was chosen to be 300 minutes with 88 dBA.

Keeping in mind the tolerance we determined in Sec. 6.1.2, we see that this alternative meets the conditions, even if only just.

6.5.2 Divided stress-summation

Keeping in mind the improvement attained in the previous alternatives when dividing the stress-summation, we also tried such method here, using

$$\beta(\text{SPL}) = \begin{cases} 2 & , \quad \text{SPL} < 88 \text{ dBA} \\ 4 & , \quad \text{SPL} >= 88 \text{ dBA} \end{cases} \tag{6.19}$$

in Eq. 6.14. This lead to the result in Figure 6.13. It is obvious that this improved the damage prediction.

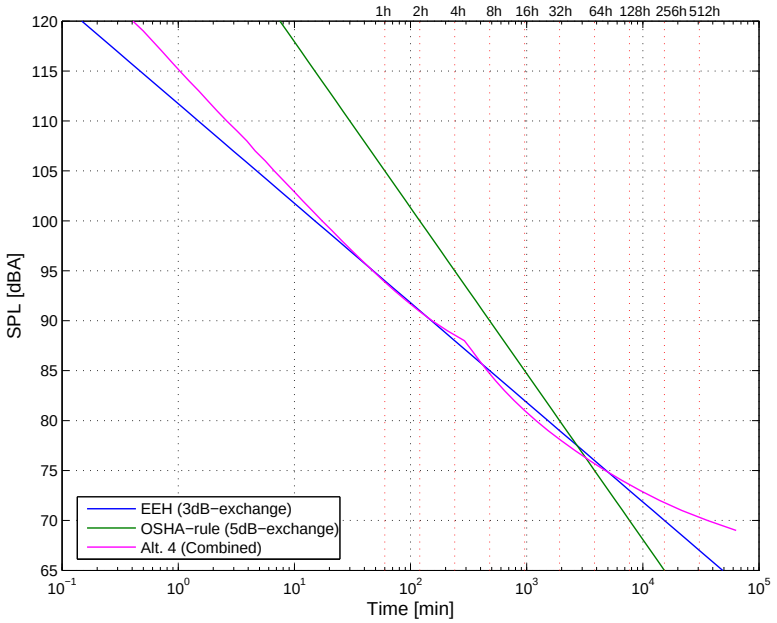


Figure 6.13: The figure shows how long time one can tolerate a SPL before getting damage and compares Alternative 4 against EEH and the OSHA-rule. The stress has been calculated with a combined sum of the ROS concentration over time. Damaging limit was chosen to be 300 minutes with 88 dBA.

6.6 Testing the model with different events

The final step was to test the model with some events. In the following figures two different events are evaluated and will elucidate some of the challenges the model faces. It is Alternative 4 that has been tested with divided stress summation.

6.6.1 Dosimeter inadequacy

The first event we tested was an example of an "intermittent" noise which we believe is *not* damaging. The total energy of the exposure is just above the tolerated limit in the ISO standard and therefore is defined as damaging. However, when looking at the exposure we see that after the first 3,5 hours of exposure there is a 3 hour recovery which should make the ear capable of tolerating more than the following 40 minutes of exposure. Such recovery phase is taken into account in the model. In Figure 6.14 we see that the EEH estimates damage, while our model does not.

6.6.2 More realistic pressure as in-parameter

In all the previous examples and tests the pressure into the system has been continuous and none fluctuating. Figure 6.15 shows that the pressure might be a varying size. This means that one may use a real measurement (absolute value) as an input, and still get a reasonable result. If, however, the real measurement has a high sampling rate the simulation will be time consuming. A possible solution to this would be to calculate the RMS-value of small pieces, for instance one minute, and then run the simulation on this signal. The signal used in this example is similar to the one in Figure 5.2, except this one has a random fluctuating amplitude.

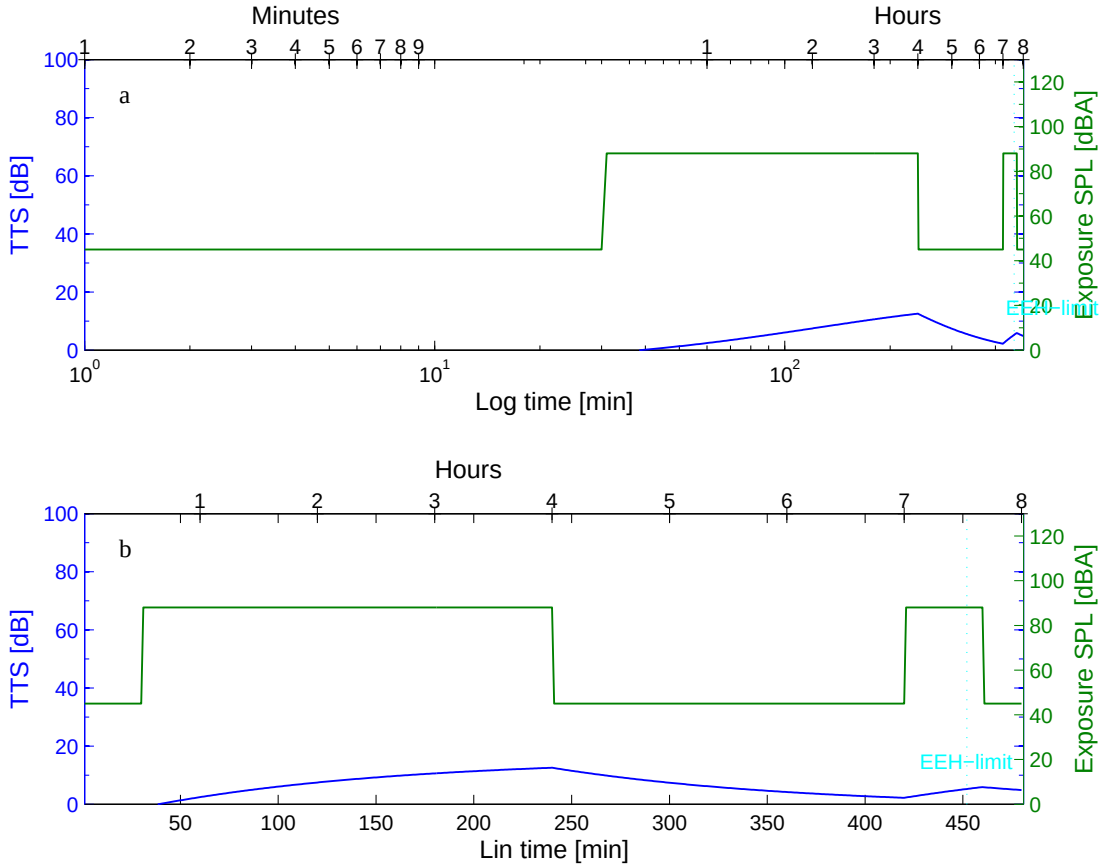


Figure 6.14: Test of the model with "intermittent" noise. The exposure is 30 min in 45 dBA followed by 88 dBA for 3,5 hours, recovery in 45 dBA for 3 hours, 40 min with 88 dBA and finally 20 minutes of recovery. The only difference between (a) and (b) is the x-axis which is logarithmic in (a) and linear in (b).

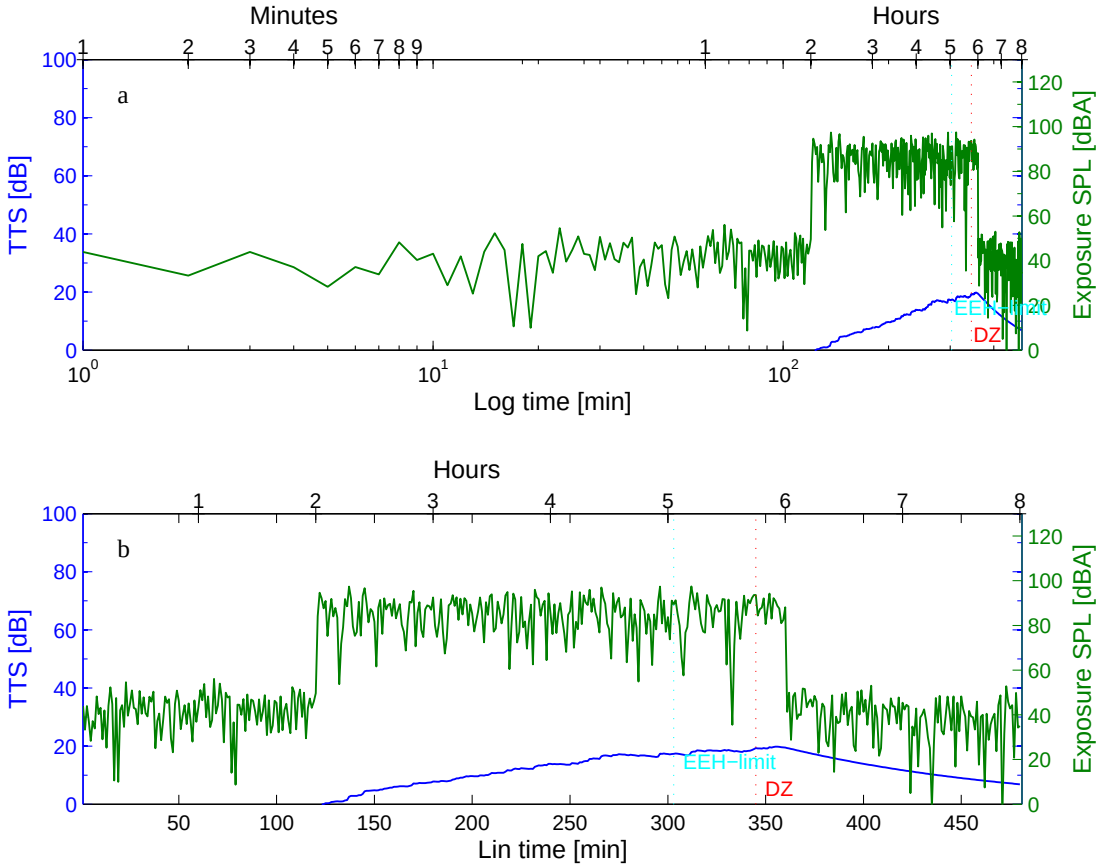


Figure 6.15: Test of the model with random noise as input. The exposure is 2 hours in approximately 45 dBA followed by approximately 89 dBA for 4 hours and finally approximately 45 dBA for 2 hours. The only difference between (a) and (b) is the x-axis which is logarithmic in (a) and linear in (b).

Chapter 7

Discussion

7.1 Assumptions

Since it would be very complex to model all the damaging mechanisms in the ear, we had to do some assumptions and aim for a simplified model. First of all we limited the model to look at the damaging compounds in the hair cell. This means that we excluded many possible damaging mechanism already. Second, the main assumption we base our model on, is the idea that concentration of ROS has a proportional relation to TTS. Since we could not find any literature saying anything about the sizes of neither production nor concentration of ROS, we tested several hypotheses. To evaluate the different alternatives we ran two tests on them. Based on measured values and accepted standards (see Sec. 6.1 for details) we judged whether the hypotheses could be a possible solution.

When mechanical damage occurs was another question. Since we only try to model damaging oxidative stress mechanisms, we do not include SPLs creating mechanical damage. As we saw in Sec. 3.2 there are literature saying that mechanical damage might occur from 125 dB, but also others saying that severe mechanical damage first occurs at levels exceeding 150 dB. Even if we say that the model does not include SPLs which creates mechanical damage (SPL $\gtrsim$ 125 dB), this is not entirely true. The model is estimating damage in this region too, but since other mechanisms start to be the main reason for hearing damage, the total damage is underestimated.

7.2 The alternatives

Since all the alternatives had advantages and disadvantages, it was difficult to choose one of them.

Alternative 1 had a very good estimation of TTS, if Mills et al.'s findings are correct, but estimates the damage poorly (see Sec. 6.2). The reason for this was that the production of ROS

increased to slow compared to the increase in pressure.

Alternative 2, which assumed a ROS production following the squared pressure, did not estimate TTS very good, but estimates the damage better. This was not very surprising, since the resemblance between the stress summation the model used and the equation calculating the total energy in the EEH, was close (see Sec. 6.3).

The third alternative, in Sec. 6.4, was made to get a better fit to TTS than Alternative 2. Since this was a thing in between Alternative 1 and 2, the result was sort of a compromise. The TTS estimation was better than Alternative 2, but not as good as Alternative 1. The damage estimation was the opposite way around.

As a last attempt we tried a combination of Alternative 2 and 3. This was done to get better results at both TTS and damage estimation. In Sec. 6.5 the results from this alternative are shown. The hypothesis used in this alternative is that the production of ROS has an accelerated development when rising above some SPL.

To get a better result the idea of using an accelerated development of the stress emerged. The assumption used is that above a certain SPL (or concentration of ROS), the stress accelerates. In the model we have divided this acceleration into only two parts, but the general equation for such development is shown in Eq. 6.14.

Indications are that the correlation between concentration of ROS and either TTS and/or PTS is more complex than we assume. This is, however, difficult to prove in any way and needs more research to conclude.

7.3 Electrical components

Another interesting point is whether the electrical components used are the best. As mentioned in Sec. 3.3.4, noise can alter the membrane properties, leading to increased permeability of the cells. Taking this into account one could argue that the R_t in the model should be a variable resistance. This would certainly change the behavior of the model, but has not been tested since we did not manage to quantify such variability.

The ROS production might also be represented in another way. As mentioned in Sec. 4.5 there has been reported a continued production of ROS *after* the noise exposure has ended. In our model we have used a variable current source to represent the ROS production. We have, however, said that the production is level dependent, which means that when the exposure ends, the production ends as well. Whether this is true is hard to say, but we assume that if the production continues, it has to decrease. This would give the antioxidants opportunity to get the upper hand, causing less damage.

As mentioned in Sec. 5.2 we assume that the production of antioxidants has the ability to get as large as the production of ROS. We base this assumption on the fact that the lateral wall has a great amount of blood vessels, providing the ability to either produce antioxidants itself

or receive antioxidants from the blood. If the production of antioxidants have an upper limit, this would have catastrophic consequences for the ear if the production of ROS is increased further. This is the reason we believe that the production of antioxidants can get as large as the production of ROS.

7.4 Further studies

It is beyond all doubt that our model is a bit too simple to give a good estimation of the dangers of noise. Our model has, however, two very important qualities: it includes recovery and has the ability of individual calibration.

Common dose measurements does not take the noise characteristics into account. This is especially a problem for noise exposures including long recovery phases. The example in Sec. 6.6.1 shows such exposure and explains the problem. Since we use the concentration of damaging compounds in the estimation of damage, this inadequacy is eliminated.

When estimating damage individual differences are also a big challenge. None of the estimators we have found has any ability for individual calibration. Our model, on the other hand, might be calibrated if there exists TTS data (or PTS data when this is included in the model) to a known exposure. By running the exposure in the model, the constants or limits can be adjusted to fit each person. This might be a time consuming job to do, but can improve the estimation of damage for each person a lot. This is specially important for those with delicate ears and can be used to prevent damage.

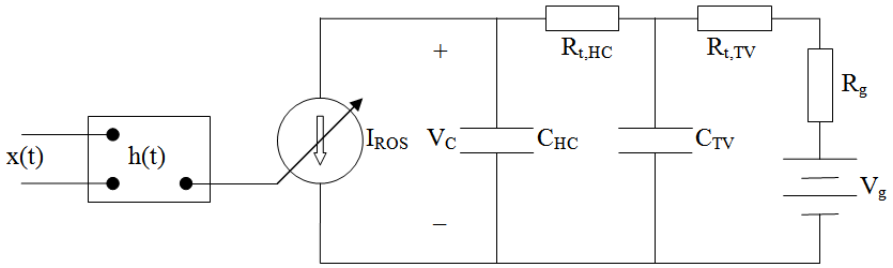


Figure 7.1: Expansion of the model. The change from the model used in this study is the second RC circuit. HC=Hair Cell, TV=Traveling Volumes.

One improvement of the model would be to include sound conditioning, mentioned in Sec. 4.3.3. It is an accepted fact that sound conditioning (both pre- and post-) may improve the ears tolerance against damaging noise. Since it is difficult to quantify how much such sound conditioning may affect the damage, we have not tried to implement such behavior.

Another important improvement would be to include the effects from impulsive noise. It has been thoroughly shown by several studies (e.g. Hamernik and Qiu [31]) that the EEH does not handle impulsive noise in a good way. Short impulses does not contain enough energy to be

detected as dangerous by the EEH, but they have been shown to be very hazardous. Since these impulses often, if not always, have very high SPLs, we have not given any effort to include this in our model. Low level impulses, however, might be more dangerous than continuous noise as well, but such statement can not be supported by any literature we have found, and this has therefore not been included in the model.

The quantification of PTS is also a possible improvement. We have limited our estimator to estimate *when* damage might occur, without saying anything about the size of the PTS. Since we have not found any literature providing information on correlation between TTS and PTS, or concentration of ROS and PTS, we have chosen to not quantify this size. There is, however, most likely a correlation between some of these factors which might be included in the model.

One possible expansion of the electrical circuit is also a improvement for future work. In Figure 7.1 a possible expansion is shown. The change from the model used in this study is the second RC circuit. Since we believe the antioxidants have to travel from the lateral wall to the hair cells, there are several volumes the antioxidants have to pass through. These volumes (for instance scala tympani) can be described in the same way as the hair cell with a capacitance. In the figure we have chosen to call the capacitance C_{TV}, where TV is an abbreviation for Traveling Volumes, since we do not know exactly which volumes should be included. This expansion would also include a second time constant in the model, an inclusion supported by the literature.

Chapter 8

Conclusion

There are compelling evidences for noise-induced formation and accumulation of oxidative species in the cochlea. These oxidative species have a negative effect on hearing functionality and cause damage to vital tissue and structures of the cochlea. As a consequence of this degeneration, hearing is impaired, which results in elevated hearing threshold.

We therefore suggest that modeling of waste product accumulation would give a better picture of how NIHL develops, and that such approach would have a broader applicability as to assess hearing impairment from noise, than today's methods.

Our model did not give unambiguous results. It did, however, show some very interesting qualities. First of all, recovery is included in the estimation of damage. This is an important improvement from the standards used today. Such recovery is important to include since most situations of occupational noise contain periods of silence.

Another important property is the ability to include individual calibration. There are big individual differences when it comes to what harms the ear. By using TTS data from known exposures, our model can be calibrated for each person. Even if this might be a time consuming and difficult job, there is no doubt that this would improve estimations of damage.

In real life the damaging mechanisms are quite complex, and can most likely not be expressed in such simple way as our model. A very interesting approach for future work would be to include a second RC circuit in the electrical model. This expansion would include the volumes on the pathway from the cochlear lateral wall to the hair cell. A second RC circuit would also introduce a second time constant, which is backed up by the literature.

If some of the assumptions used are correct, the model can give new insight to hearing damage. As mentioned, the reality is quite complex, and small pieces of insight is important to get the total picture.

Appendix A

Chemical approach to oxidants and antioxidants

A.1 ROS/RNS/Free Radicals

This section (A.1) is adapted from Evans and Halliwell, 1999 [25].

A free radical is any atom, molecule, or ion that contains one or more unpaired electrons.

ROS is a collective term that includes both oxygen radicals and certain nonradicals that are oxidizing agents and/or are easily converted into radicals.

Superoxide radical, $O_2^- \cdot$, hydroxyl radical, $OH \cdot$, and hydrogen peroxide, H_2O_2, known as reactive oxygen metabolites, are produced in the reduction of molecular oxygen to water during oxidative phosphorylation. Oxygenderived free radicals (FOR) are a highly reactive chemical species involved in a variety of clinical disorders.

In healthy cells and tissues, any free radicals generated are likely to encounter and react with nonradicals since most cellular constituents are nonradicals and chances of most radicals meeting are low. Such encounters often (depending on the reactivity of the free radical) perpetuate free radicals in reactions of the type:

$$X \cdot \quad + \quad HY \quad \longrightarrow \quad HX \quad + \quad Y \cdot \tag{A.1}$$

or,

$$X \cdot \quad + \quad Y \quad \longrightarrow \quad [X\text{ - }Y] \cdot . \tag{A.2}$$

For this reason, free radicals can act as initiators and propagators of chain reactions. One biological example of a free-radical chain reaction is lipid peroxidation. Another is the addition of hydroxyl radical to the DNA base guanine to make the 8-hydroxyguanine radical.

A.2 Antioxidants

This section is adapted from Evans and Halliwell, 1999 [25], and Henderson et al., 2006 [36].

The body's defense against oxidative stress, the antioxidant system, consists of two different compounds; (1) enzymes, which are mostly intracellular, and (2) low-molecular-mass antioxidants, which are located both inside and outside the cells. Table A.1 lists the most important of these antioxidant compounds.

Table A.1: Different antioxidants which are common in the body in general, and found to be active in the cochlea in special. Adapted from Evans and Halliwell, 1999 [25].

Enzymes (mainly intracellular) removing $O_2^- \cdot$ and H_2O_2
Superoxide dismutase (SOD): Used in transition of $O_2^- \cdot$ to O_2 and H_2O_2.
Glutathione peroxidase (GSH-px): Used when GSH neutralizes H_2O_2.
Glutathione reductase (GSH-rd): Used to make GSH from oxidized GSH (GSSG).
Catalase (CAT): Used to transition of H_2O_2 to O_2 and H_2O.
Low-molecular-mass antioxidants (intra- and extracellular)
Glutathione (GSH): Scavenges $OH \cdot$, singlet oxygen, HOCl, $ONOO^-$, $RO \cdot$, $RO_2 \cdot$
Vitamins (A, C, E): Reducing agent that reacts with $O_2^- \cdot$, $OH \cdot$, $HO_2 \cdot$, HOCl, $ONOO^-$, $RO \cdot$, $RO_2 \cdot$, and singlet oxygen. May detoxify other radicals by reducing them.
α-Tocpherol: Terminates chain reaction of lipid peroxidation, thought to be recycled by ascorbic acid or ubiquinol.
Uric acid: Scavenges singlet oxygen, $OH \cdot$, HOCl, O_3, $ONOO^-$, and peroxyl radicals. Urate radical may be recycled by ascorbate.

A.3 Scavenging enzymes

This section (A.3) is adapted from Campbell, 2003 [10].

An enzyme is a complex protein substance, produced in living cells. Enzymes cause or accelerate other chemical reactions within an organism, but are not altered themselves in the process. Basically, enzymes are organic catalysts. Two major enzymes involved in detoxifying ROS are:

- **Superoxide dismutase (SOD)**, which converts the superoxide radical anion, $O_2^- \cdot$, into oxygen (O_2) and hydrogen peroxide (H_2O_2).

$$2O_2^- \cdot \quad + \quad 2H^+ \quad \xrightarrow{\text{SOD}} \quad O_2 \quad + \quad 2H_2O_2 \tag{A.3}$$

- **Catalase (CAT)**, which converts the hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2).

$$2H_2O_2 \quad \xrightarrow{\text{CAT}} \quad O_2 \quad + \quad 2H_2O \tag{A.4}$$

And no rocket science is needed to understand the consequences of one of these enzymes being inhibited, or missing.

A.4 Redox-reactions

Redox-reactions are reactions in which the involved compounds are reduced or oxidized. A compound is reduced when getting, or "stealing" an electron, since pairing of electrons in orbitals represent a lower energy state than the same electrons unpaired. On the other hand a compound is oxidized when loosing an electron, thus having an unpaired electron.

In this way antioxidants reduce radicals, but being oxidized themselves.

ROS and RNS are generated via several different reactions in the body, and below we have listed the most important ones.

This first reaction is the process in which oxygen is used in normal respiration in the mitochondria to generate ATP from ADP, and the second reaction is a detoxifying process using the SOD enzyme.

$$O_2 \xrightarrow[\text{enzymatic reaction others}]{\text{Mitochondria Krebs' cycle}} O_2^- \cdot \xrightarrow{\text{Superoxide dismutase (SOD)}} H_2O_2 \qquad \text{(A.5)}$$

But with NO present, super oxide can combine with this agent to generate peroxynitrite:

$$O_2^- + NO \cdot \longrightarrow ONOO^- \cdot . \qquad \text{(A.6)}$$

Hydrogen peroxide and super oxide can react to form hydroxyl radical through the reaction known as the Haber-Weiss reaction:

$$H_2O_2 + O_2^- \cdot \longrightarrow O_2 + OH \cdot + OH^- \qquad \text{(Haber-Weiss reaction).} \qquad \text{(A.7)}$$

Hydroxyl radicals can also be formed through reaction between hydrogen peroxide and ferrous iron molecule:

$$H_2O_2 \xrightarrow{Fe^{2+} \rightarrow Fe^{3+}} OH \cdot + OH^- \qquad \text{(Fenton reaction).} \qquad \text{(A.8)}$$

Transition metals (e.g. iron) act as catalysts in converting poorly reactive species into more damaging forms. However, according to Evans and Halliwell [25], it is only in disease states such as fulminant hepatic failure and hemochromatosis that such metal ions become available in quantities sufficient to cause tissue damage.

Bibliography

[1] ALTURA, B. M., ALTURA, B. T., GEBREWOLD, A., ISING, H., AND GUNTHER, T. Noise-induced hypertension and magnesium in rats: relationship to microcirculation and calcium. *J. Appl. Physiol. 72*, 1 (1992), 194–202.

[2] BIESALSKI, H. K., WELLNER, U., AND WEISER, H. Vitamin a deficiency increases noise susceptibility in guinea pigs. *J. Nutr. 120*, 7 (1990), 726–737.

[3] BOBBIN, R. P., FALLON, M., LEBLANC, C., AND BABER, A. Evidence that glutathione is the unidentified amine (unk 2.5) released by high potassium into cochlear fluids. *Hear. Res. 87* (1995), 49–54.

[4] BOHNE, B. A., AND RABBITT, K. D. Holes in the reticular lamina after noise exposure: Implication for continuing damage in the organ of corti. *Hear. Res. 11* (1983), 41–53.

[5] BOHNE, B. A., YOHMAN, L., AND GRUNER, M. M. Cochlear damage following interrupted exposure to high-frequency noise. *Hear. Res. 29* (1987), 251–264.

[6] BRÜEL, P. V. Episodes and archievements within acousticts before 1954. Published in connection to The Danish Acoustical Society's 50 year anniversary, 2005. In danish.

[7] BROOKES, P. S., YOON, Y., ROBOTHAM, J. L., ANDERS, M. W., AND SHEU, S.-S. Calcium, atp, and ros: a mitochondrial love-hate triangle. *Am. J. Physiol. Cell Phyiol. 287* (2004), 817–833.

[8] BROWNELL, W. E., BADER, C. R., BERTRAND, D., AND DE RIBAUPIERRE, Y. Evoked mechanical responses of isolated cochlear outer hair cells. *Sci. 227*, 4683 (1985), 194–196.

[9] BROWNELL, W. E., SPECTOR, A. A., RAPHAEL, R. M., AND POPEL, A. S. Micro- and nanomechanics of the cochlear outer hair cell. *Ann. Rev. Biomed. Eng. 3* (2001), 169–194.

[10] CAMPBELL, K. Ototoxicity: understanding oxidative mechanisms. *J. Am. Acad. Audiol. 14*, 3 (2003), 121–123.

[11] CAMPBELL, K. Oxidants vs. antioxidants: The war within – and our cells are at stake. *Hear. J. 57*, 5 (2004).

[12] CANLON, B. Acoustic overstimulation alters the morphology of the tectorial membrane. *Hear. Res. 30* (1987), 127–134.

[13] CHAN, E., SUNESON, A., AND ULFENDAHL, M. Acoustic trauma causes reversible stiffness changes in auditory sensory cells. *Neurosci. 83*, 3 (1998), 961–968.

[14] CHEN, Y.-S., TSENG, F.-Y., LIU, T.-C., LIN-SHIAU, S. Y., AND HSU, C.-J. Involvement of nitric oxide generation in noise-induced temporary threshold shift in guinea pigs. *Hear. Res. 203* (2005), 94–100.

[15] CLARK, W. W., AND BOHNE, B. A. Effects of noise on hearing. *JAMA 281*, 17 (1999), 1658–1659.

[16] CLERICI, W. J., HENSLEY, K., DIMARTINO, D. L., AND BUTTERFIELD, D. A. Direct detection of ototoxicant-induced reactive oxygen species generation in cochlear explants. *Hear. Res. 98* (1996), 116–124.

[17] CLERICI, W. J., AND YANG, L. Direct effects of intraperilymphatic reactive oxygen species generation on cochlear function. *Hear. Res. 101* (1996), 14–22.

[18] COOPER, N. P., AND GUINAN, J. J. Efferent-mediated control of basilar membrane motion. *J. Physiology 576*, 1 (2006), 49–54.

[19] DALLOS, P. The active cochlea. *J. Neuroscience 12*, 12 (1992), 4575–4585.

[20] DALLOS, P., AND FAKLER, B. Prestin, a new type of motor protein. *Nat. Rev. Mol. Cell Bio. 3*, 2 (2002), 104–111.

[21] DEREKÖY, F. S., KÖKEN, T., YLIMAZ, D., KAHRAMAN, A., AND ALTUNTAS, A. Effects of ascorbic acid on oxidative system and transient evoked otoacoustic emissions in rabbits exposed to noise. *Laryngoscope 114*, 10 (2004), 1775–1779.

[22] DUAN, M. L., ULFENDAHL, M., LAURELL, G., COUNTER, A. S., PYYKKÖ, I., BORG, E., AND ROSENHALL, U. Protection and treatment of sensorineural hearing disorders caused by exogenous factors: Experimental findings and potential clinical application. *Hear. Res. 169* (2002), 169–178.

[23] DULON, D., ZAJIC, G., AND SCHACHT, J. Increasing intracellular free calcium induces circumferential contractions in isolated cochlear outer hair cells. *J. Neu.Sci. 10*, 4 (1990), 1388–1397.

[24] ENGSTRÖM, B., FLOCK, Å., AND BORG, E. Ultrastructural studies of stereocilia in noise-exposed rabbits. *Hear. Res. 14*, 2 (1983), 251–264.

[25] EVANS, P., AND HALLIWELL, B. Free radicals and hearing: Cause, consequence, and criteria. *Ann. N.Y. Acad. Sci. 884* (1999), 19–40.

[26] EYBALIN, M. Neurotransmitters and neuromodulators of the mammalian cochlea. *Physiol. Rev. 73*, 2 (1993), 309–373.

[27] FARBER, J. L. Mechanisms of cell injury by activated oxygen species. *Env. Health Perspec. 102*, Suppl. 10 (1994), 17–24.

[28] FLOCK, Å., FLOCK, B., FRIDBERGER, A., SCARFONE, E., AND ULFENDAHL, M. Supporting cells contribute to control of hearing sensitivity. *J. Neurosci. 19*, 11 (1999), 4498–4507.

[29] FRIDBERGER, A., FLOCK, Å., ULFENDAHL, M., AND FLOCK, B. Acoustic overstimulation increases outer hair cell ca^{2+} concentrations and causes dynamic contraction of the hearing organ. *Proc. Natl. Acad. Sci. USA 95* (1998), 7127–7132.

[30] FROLENKOV, G. I. Regulation of electromotility in the cochlear outer hair cell. *J. Physiol. 576*, Pt 1 (2006), 43–48.

[31] HAMERNIK, R. P., AND QIU, W. Energy-independent factors influencing noise-induced hearing loss in the chinchilla model. *JASA 110*, 6 (2001), 3163–3168.

[32] HANSON, J. B., RUSSELL, P. T., CHUNG, A. T. A., KAURA, C. S., KAURA, S. H., JOHN, E. O., AND JUNG, T. T. K. Effect of round window membrane application of nitric oxide on hearing and nitric oxide concentration in perilymph. *Int. J. Ped. Otorhinolaryn. 67* (2003), 585–590.

[33] HARDING, G. W., BAGGOT, P. J., AND BOHNE, B. A. Height changes in the organ of corti after noise exposure. *Hear. Res. 63* (1992), 26–36.

[34] HARRIS, K. C., BIELEFELD, E., HU, B. H., AND HENDERSON, D. Increased resistance to free radical damage induced by low-level sound conditioning. *Hear. Res. 213* (2006), 118–129.

[35] HEINRICH, U. R., MAURER, J., AND MANN, W. Ultrastructural evidence for protection of the outer hair cells of the inner ear during intense noise exposure by application of the organic calcium channel blocker diltiazem. *ORL 61*, 6 (1999), 321–327.

[36] HENDERSON, D., BIELEFELD, E. C., HARRIS, K. C., AND HU, B. H. The role of oxidative stress in noise-induced hearing loss. *Ear and Hearing 27*, 1 (2006), 1–19.

[37] HENDERSON, D., AND HAMERNIK, R. P. Biologic bases of noise-induced hearing loss. *Occ. med. 10*, 3 (1995), 513–534.

[38] HIGHT, N. G., MCFADDEN, S. L., HENDERSON, D., BURKARD, R. F., AND NICOTERA, T. Noise-induced hearing loss in chinchillas pre-treated with glutathione monoethylester and r-pia. *Hear. Res. 179* (2003), 21–32.

[39] HIROSE, K., AND LIBERMAN, M. C. Lateral wall histopathology and endocochlear potential in the noise-damaged mouse cochlea. *JARO 4*, 3 (2003), 339–352.

[40] HU, B. H., HENDERSON, D., AND NICOTERA, T. M. Involvement of apoptosis in progression of cochlear lesion following exposure to intense noise. *Hear. Res. 166* (2002), 62–71.

[41] HU, B. H., ZHENG, X. Y., MCFADDEN, S. L., KOPKE, R. D., AND HENDERSON, D. R-phenylisopropyladenosine attenuates noise-induced hearing loss in the chinchilla. *Hear. Res. 113* (1997), 198–206.

[42] ISO 1999:1990 - Acoustics – Determination of occupational noise exposure and estimation of noise-induced hearing impairment, 1990. 2nd edition.

[43] JACONO, A. A., HU, B. H., KOPKE, R. D., HENDERSON, D., WATER, T. R. V. D., AND STEINMAN, H. M. Changes in cochlear antioxidant enzyme activity after sound conditioning and noise exposure in the chinchilla. *Hear. Res. 117* (1998), 31–38.

[44] KARLIDAG, T., YALCIN, S., ÖZTÜRK, A., ÜSTÜNDAG, B., GÖK, ., KAYGUSUZ, I., AND SUSAMAN, N. The role of free oxygen radicals in noise induced hearing loss: effects of melatonin and methylprednisolone. *Auris Nasus Larynx 29*, 2 (2002), 147–152.

[45] KAYGUSUZ, I., ÖZTÜRK, A., ÜSTÜNDAG, B., AND YALCIN, S. Role of free oxygen radicals in noise-related hearing impairment. *Hear. Res. 162* (2001), 43–47.

[46] KIKUCHI, T., KIMURA, R. S., PAUL, D. L., TAKASAKA, T., AND ADAMS, J. C. Gap junction systems in the mammalian cochlea. *Brain res. rev. 32* (2000), 163–166.

[47] KOPKE, R., ALLEN, K. A., HENDERSON, D., HOFFER, M., FRENZ, D., AND WATER, T. V. D. A radical demise: Toxins and trauma share common pathways in hair cell death. *Ann. N.Y. Acad. Sci. 884* (1999), 171–191.

[48] KOPKE, R. D., COLEMAN, J. K. M., LIU, J., CAMPBELL, K. C. M., AND RIFFENBURGH, R. H. Enhancing intrinsic cochlear stress defenses to reduce noise-induced hearing loss. *Laryngoscope 112* (2002), 1515–1532.

[49] KOPKE, R. D., AND DANIELSON, R. W. Antioxidants – good for your health, good for your hearing. *CAOHC 15*, 1 (2003).

[50] KRAAK, W., ERTEL, H., FUDER, G., AND KRACHT, L. Risk of hearing damage caused by steady-state and impulsive noise. *J. of Sound and Vibration 36*, 3 (1974), 347–359.

[51] LAURELL, G., TEIXEIRA, M., STERKERS, O., BAGGER-SJÖBÄCK, D., EKSBORG, S., LIDMAN, O., AND FERRARY, E. Local administration of antioxidants to the inner ear: Kinetics and distribution. *Hear. Res. 173* (2002), 198–209.

[52] LE PRELL, C. G., HUGHES, L. F., AND MILLER, J. M. Free radical scavengers vitamins A, C and E plus magnesium reduce noise trauma. *Free Rad. Bio. and Med. 42*, 9 (2007), 1454–1463.

[53] LE PRELL, C. G., YAMASHITA, D., MINAMI, S. B., YAMASOBA, T., AND MILLER, J. M. Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. *Hear. Res. 226* (2007), 22–43.

[54] LIBERMAN, M. C., AND DODDS, L. W. Acute ultrastructural changes in acoustic trauma: Serial-section reconstruction of stereocilia and cuticular plates. *Hear. Res. 26* (1987), 45–64.

[55] LIBERMAN, M. C., GAO, J., HE, D. Z. Z., WU, X., JIA, S., AND ZUO, J. Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. *Nature 419*, 6904 (2002), 300–304.

[56] LIM, D. J., AND MELNICK, W. Acoustic damage of the cochlea. a scanning and transmission electron microscopic observation. *Arch. Otolaryngol. 94*, 4 (1971), 294–305.

[57] LYNCH, E. D., AND KIL, J. Compounds for the prevention and treatment of noise-induced hearing loss. *DDT 10*, 19 (2005), 1291–1298.

[58] MAMMANO, F., AND NOBILI, R. The cochlea homepage. Website. Last visited June 9., 2007. `http://www.vimm.it/cochlea/`.

[59] MANDAL, A., KALTENBACH, J. A., AND QUIRK, W. S. Changes in dorsal cochlear nucleus blood flow during noise exposure. *Hear. Res. 106* (1997), 1–8.

[60] MANN, M. D. The nervous system in action. Website. Last visited June 9., 2007. `http://www.unmc.edu/Physiology/Mann/mann4b.html`.

[61] MCFADDEN, S. L., WOO, J. M., MICHALACK, N., AND DING, D. Dietary vitamin c supplementation reduces noise-induced hearing loss in guinea pigs. *Hear. Res. 202* (2005), 200–208.

[62] MILLER, J. M., REN, T., AND NUTTALL, A. L. Studies of inner ear blood flow in animals and human beings. *Otol. Head and Neck surg. 112*, 1 (1995), 101–113.

[63] MILLS, J. H., ADKINS, W. Y., AND GILBERT, R. M. Temporary threshold shift produced by wideband noise. *J. Acoust. Soc. Am. 70*, 2 (1981), 390–396.

[64] MILLS, J. H., GILBERT, R. M., AND ADKINS, W. Y. Temporary threshold shift in humans exposed to octave bands of noise for 16 to 24 hours. *J. Acoust. Soc. Am. 65*, 5 (1979), 1238–1248.

[65] MØLLER, A. R. *Hearing – Anatomy, Physiology, and Disorders of the Auditory System*, 2nd ed. Elsevier Inc., 2006.

[66] MOM, T., CHAZAL, J., GABRILLARGUES, J., GILAIN, L., AND AVAN, P. Cochlear blood supply: an update on anatomy and function. *Fr. ORL 88* (2005), 81–88.

[67] NAKASHIMA, T., NAGANAWA, S., SONE, M., TOMINAGA, M., HAYASHI, H., YAMAMOTO, H., LIU, X., AND NUTTALL, A. L. Disorders of cochlear blood flow. *Br. Res. Rev. 43*, 1 (2003), 17–28.

[68] NIU, X., TAHERA, Y., AND CANLON, B. Protection against acoustic trauma by forward and backward sound conditioning. *Audiol. Neu.-Otol. 9*, 5 (2004), 265–273.

[69] OHINATA, Y., MILLER, J. M., ALTSCHULER, R. A., AND SCHACHT, J. Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea. *Brain Res. 878* (2000), 163–173.

[70] OHINATA, Y., MILLER, J. M., AND SCHACHT, J. Protection from noise-induced lipid peroxidation and hair cell loss in the cochlea. *Brain Res. 966* (2003), 265–273.

[71] OHLEMILLER, K. K., WRIGHT, J. S., AND DUGAN, L. L. Early elevation of cochlear reactive oxygen species following noise exposure. *Audiol. Neuro-Otol. 4* (1999), 229–236.

[72] PATUZZI, R. Exponential onset and recovery of temporary threshold shift after loud sound: evidence for long-term inactivation of mechano-electrical transduction channels. *Hear. Res. 125* (1998), 17–38.

[73] PATUZZI, R. A four-state kinetic model of the temporary threshold shift after loud sound based on inactivation of hair cell transduction channels. *Hear. Res. 125* (1998), 39–70.

[74] PICKARD, D. Image:humanear.jpg. Website. Last visited June 9., 2007. `http://en.wikipedia.org/wiki/Image:HumanEar.jpg`.

[75] PICKLES, J. O., OSBORNE, M. P., AND COMIS, S. D. Vulnerability of tip links between stereocilia to acoustic trauma in the guinea pig. *Hear. Res. 25* (1987), 173–183.

[76] POURBAKHT, A., AND YAMASOBA, T. Cochlear damage caused by continuous and intermittent noise exposure. *Hear. Res. 178* (2003), 70–78.

[77] PUJOL, R., AND PUEL, J.-L. Excitotoxicity, synaptic repair, and functional recovery in the mammalian cochlea: A review of recent findings. *Ann. N.Y. Acad. Sci. 884*, 1 (1999), 249–254.

[78] QUIRK, W. S., AVINASH, G., NUTTALL, A. L., AND MILLER, J. M. The influence of loud sound on red-blood-cell velocity and blood-vessel diameter in the cochlea. *Hear. Res. 63* (1992), 102–107.

[79] RASK-ANDERSEN, H., EKVALL, L., SCHOLTZ, A., AND SCHROTT-FISCHER, A. Structural/audiometric correlations in a human inner ear with noise-induced hearing loss. *Hear. Res. 141* (2000), 129–139.

[80] ROBERTSON, D. Functional significance of dendritic swelling after loud sounds in the guinea pig cochlea. *Hear. Res. 9*, 3 (1983), 263–278.

[81] RUAN, R.-S. Possible roles of nitric oxide in the physiology and pathophysiology of the mammalian cochlea. *Ann. N.Y. Acad. Sci. 962* (2002), 260–274.

[82] RUCKENSTEIN, M. J. Cochlear function, general. Website. Last visited June 9., 2007. `http://www.emedicine.com/Ent/topic361.htm`.

[83] SANTI, P. A., LAKHANI, B., AND BINGHAM, C. The volume density of cells and capillaries of the normal stria vascularis. *Hear. Res. 11*, 1 (1983), 7–22.

[84] SAUNDERS, J. C., COHEN, Y. E., AND SZYMKO, Y. M. The structural and functional consequences of acoustic injury in the cochlea and peripheral auditory system: A five year update. *JASA 90*, 1 (1991), 136–146.

[85] SAUNDERS, J. C., DEAR, S. P., AND SCHNEIDER, M. E. The anatomical consequences of acoustic injury: A review and tutorial. *JASA 78*, 3 (1985), 833–860.

[86] SCHARF, B., MAGNAN, J., AND CHAYS, A. On the role of the olivocochlear bundle in hearing: 16 case studies. *Hear. Res. 103* (1997), 101–122.

[87] SCHARF, B., MAGNAN, J., COLLET, L., ULMER, E., AND CHAYS, A. On the role of the olivocochlear bundle in hearing: A case study. *Hear. Res. 75* (1994), 11–26.

[88] SCHEIBE, F., HAUPT, H., AND ISING, H. Preventive effect of magnesium supplement on noise-induced hearing loss in the guinea pig. *Eur. Arch. Otorhinolaryngol. 257*, 1 (2000), 10–16.

[89] SCHEIBE, F., HAUPT, H., AND ISING, H. Preventive magnesium supplement reduces ischemia-induced hearing loss and blood viscosity in the guinea pig. *Eur. Arch. Otorhinolaryngol. 257*, 7 (2000), 355–361.

[90] SEIDMAN, M. D., QUIRK, W. S., AND SHIRWANY, N. A. Mechanisms of alterations in the microcirculation of the cochlea. *Ann. N.Y. Acad. Sci. 884* (1999), 226–232.

[91] SHA, S., TAYLOR, R., FORGE, A., AND SCHACHT, J. Differential vulnerability of basal and apical hair cells is based on intrinsic susceptibility to free radicals. *Hear. Res. 155* (2001), 1–8.

[92] SHI, X., AND NUTTALL, A. L. Upregulated inos and oxidative damage to the cochlear stria vascularis due to noise stress. *Brain Res. 967* (2003), 1–10.

[93] SHI, X., REN, T., AND NUTTALL, A. L. The electrochemical and fluorescence detection of nitric oxide in the cochlea and its increase following loud sound. *Hear. Res. 164* (2002), 49–58.

[94] SOTO, M. A., GONZÁLEZ, C., LISSI, E., VERGARA, C., AND LATORRE, R. Ca^{2+}-activated k^{+} channel inhibition by reactive oxygen species. *Am. J. Physiol. Cell Physiol. 282* (2002), 461–471.

[95] SZÖNYI, M., HE, D. Z. Z., RIBÁRI, O., SZIKLAI, I., AND DALLOS, P. Intracellular calcium and outer hair cell electromotility. *Brain Res. 922* (2001), 65–70.

[96] TAKEMURA, K., KOMEDA, M., YAGI, M., HIMENO, C., IZUMIKAWA, M., DOI, T., KURIYAMA, H., MILLER, J. M., AND YAMASHITA, T. Direct inner ear infusion of dexamethasone attenuates noise-induced trauma in guinea pig. *Hear. Res. 196* (2004), 58–68.

[97] TAKUMIDA, M., AND ANNIKO, M. Direct evidence of nitric oxide production in the guinea pig organ of corti. *Acta. Otolaryngol. 121*, 3 (2001), 342–345.

[98] THORNE, P. R., AND NUTTALL, A. L. Laser doppler measurements of cochlear blood flow during loud sound exposure in the guinea pig. *Hear. Res. 27*, 1 (1987), 1–10.

[99] ULFENDAHL, M., AND FLOCK, Å. Outer hair cells provide active tuning in the organ of corti. *News Physiol. Sci. 13* (1998), 107–111.

[100] VALKO, M., LEIVFRITZ, D., MONOCOL, J., CRONIN, M. T. D., MAZUR, M., AND TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. *IJBCB 39*, 1 (2007), 44–84.

[101] VAN CAMPEN, L. E., MURPHY, W. J., FRANKS, J. R., MATHIAS, P. I., AND TORAASON, M. A. Oxidative DNA damage is associated with intense noise exposure in the rat. *Hear. Res. 164* (2002), 29–38.

[102] WADA LABORATORY. Analysis of outer hair cell motility and force production using shell theory. Website. Last visited March 19., 2007. `http://www.wadalab.mech.tohoku.ac.jp/OHCmodel-e.html`.

[103] WANG, Y., HIROSE, K., AND LIBERMAN, M. C. Dynamics of noise-induced cellular injury and repair in the mouse cochlea. *JARO 3*, 3 (2002), 248–268.

[104] WANGEMANN, P. K^+-cycling and the endocochlear potential. *Hear. Res. 165* (2002), 1–9.

[105] WANGEMANN, P. Supporting sensory transduction: cochlear fluid homeostasis and the endocochlear potential. *J. Physiology 576*, 1 (2006), 11–21.

[106] WARD, W. D., CUSHING, E. M., AND BURNS, E. M. Effective quiet and moderate TTS: Implications for noise exposure standards. *J. Acoust. Soc. Am. 59*, 1 (1976), 160–165.

[107] YAMANE, H., NAKAI, Y., TAKAYAMA, M., KONISHI, K., IGUCHI, H., AND NAKAGAWA, T. The emergence of free radicals after acoustic trauma and strial blood flow. *Acta Otolaryngol Suppl. 519* (1995), 87–92.

[108] YAMASHITA, D., JIANG, H. Y., LE PRELL, C. G., SCHACHT, J., AND MILLER, J. M. Post-exposure treatment attenuates noise-induced hearing loss. *Neurosci. 134* (2005), 633–642.

[109] YAMASHITA, D., JIANG, H. Y., SCHACHT, J., AND MILLER, J. M. Delayed production of free radicals following noise exposure. *Hear. Res. 1019* (2004), 201–209.

[110] YAMASOBA, T., HARRIS, C., SHOIJ, F., LEE, R. J., NUTTALL, A. L., AND MILLER, J. M. Influence of intense sound exposure on glutathione synthesis in the cochlea. *Brain Res. 804* (1998), 72–78.

[111] YAMASOBA, T., NUTTALL, A. L., HARRIS, C., RAPHAEL, Y., AND MILLER, J. M. Role of glutathione in protection against noise-induced hearing loss. *Brain Res. 784* (1998), 82–90.

[112] YAMASOBA, T., SCHACHT, J., SHOJI, F., AND MILLER, J. M. Attenuation of cochlear damage from noise trauma by an iron chelator, a free radical scavenger and glial cell line-derived neurotrophic factor in vivo. *Brain Res. 815* (1999), 317–325.

[113] ZENK, J., SCHEFFLER, D., SCHEFFLER, P., DELB, W., AND IRO, H. The influence of noise on blood flow in the basilar artery (ba) - measurements with transcranial color-coded duplex sonography (tccd). *Hear. Res. 140* (2000), 157–164.

[114] ZHENG, X.-Y., HENDERSON, D., MCFADDEN, S. L., AND HU, B.-H. The role of the cochlear efferent system in acquired resistance to noise-induced hearing loss. *Hear. Res. 104* (1997), 191–203.

[115] ZHENG, X.-Y., MCFADDEN, S. L., DING, D. L., AND HENDERSON, D. Cochlear de-efferentation and impulse noise-induced acoustic trauma in the chinchilla. *Hear. Res. 144* (2000), 187–195.