Mammalian Inner Ear Homeostasis
Out of Balance?

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DISSERTATION

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Abstract

In our daily lives, we take for granted that we are able to perceive our surrounding acoustically and detect faint sounds as well as stand intense noises, which are often separated by several orders of magnitude. Furthermore, we are able to move through our surroundings without problems, although our perception of them is constantly changing. We are so used to our abilities that we often forget how strongly we rely on highly sensitive sensory systems in order to perform even the most basic of our daily activities. The two sensory systems enabling our hearing and balanced movement are the auditory system and the vestibular system, respectively. Both of them are located in our inner ear. These sensory systems process information extremely fast and accurately as long as efficient feedback processes and self-regulatory mechanisms are in place. The interplay of all processes and mechanisms provide a constant environment within the inner ear with regards to fluid composition, fluid volume and ion concentration. However, this so called homeostasis can get out of balance. When inner ear homeostasis collapses and feedback mechanisms do not function anymore, it results in disabling symptoms like hearing loss, tinnitus and vertigo.

In this thesis, I present three experimental studies investigating the effects of transiently and permanently challenged inner ear homeostasis in humans and in an animal model.

In the first study, human participants reported the pitch and loudness of their tinnitus percepts by matching pure tones and noises to their tinnitus. The participants were Menière's disease patients with chronic tinnitus, who were contrasted with healthy individuals experiencing a transient tinnitus after intense exposure to a low-frequency tone. Based on previous literature, an excessive build-up of inner ear fluid as well as "roaring" tinnitus were proposed to occur in both study groups. The findings of this first study showed that there was considerable heterogeneity in the tinnitus quality within and between the two groups; ranging from high-pitched pure tones to low-pitched noises. Therefore, it was considered unlikely that a single mechanism was underlying tinnitus generation in the two groups. Several alternative mechanisms for tinnitus generation were discussed.

In the second study, I was particularly interested whether electrical stimulation known to activate the vestibular periphery was likewise affecting the auditory system. In order to investigate this, the inner ears of human participants were electrically stimulated with surface electrodes placed behind the pinnae. Comparable to previous studies, the sinusoidal current stimulation caused a side-to-side illusion by activating the vestibular system. At the same time, the current

stimulation modulated the perception of pure tones and of auditory illusions, presumably either indirectly via vestibular projections to auditory nuclei or directly by activating the auditory periphery. Although the cochlea was the most probable structure within the auditory system to be activated by a transcutaneous vestibular stimulation, I did not find any influence of the current stimulation on the outer hair cell activity within the cochlea. Findings, therefore, suggested that the electrical stimulation affected different, presumably retrocochlear, structures of the auditory system.

The third study was conducted in Mongolian gerbils, an established model organism for the human auditory system. The study focused on temporary cochlear changes after intense low-frequency sound exposure for which the term Bounce phenomenon was coined. In this study,
I tested whether transient changes would transition into permanent cochlear damage by increasing exposure time. The probability of eliciting a Bounce phenomenon decreased with increasing duration of the sound exposure. Nevertheless, the oscillatory changes linked to electromotility of outer hair cells were uniform regarding time course and size for all exposure durations. Permanent cochlear damage could not be observed and the Bounce phenomenon was proposed to be an optimal tool to challenge and investigate cochlear homeostasis under intact conditions.

The three studies I present in this thesis substantially contribute to the increase of our knowledge about homeostatic mechanisms in the inner ear. By understanding how these mechanisms are impacted in different disorders, it will be possible to diagnose and ultimately treat underlying pathologies more accurately in the future.

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List of Abbreviations

ATP	Adenosine Triphosphate
BM	Basilar Membrane
BP	Bounce Phenomenon
DPOAEs	Distortion Product Otoacoustic Emission
EL	Endolymph
ELH	Endolymphatic Hydrops
EP	Endocochlear Potential
HCs	Hair Cells
IHCs	Inner Hair Cells
LF	Low Frequency
MD	Menière's Disease
MET	Mechano-Electrical Transduction
OAEs	Otoacoustic Emissions
OHCs	Outer Hair Cells
OP	Operating Point
SOAEs	Spontaneous Otoacoustic Emissions
SPL	Sound Pressure Level
SV	Stria Vascularis

CHAPTER 1

GENERAL INTRODUCTION

To survive in a complex environment, animals and humans rely on sensory information which they acquire via their different senses. The auditory and the vestibular system are two of those senses. While the auditory system is highly specialized in hearing and enables acoustic communication as well as detection, identification and localization of objects in the surrounding, the vestibular system provides a sense of balance, body motion and orientation in space.

Both systems are crucial for survival, but only if they process information accurately. Processing sensory information, however, can be disturbed due to external perturbations or in case of pathologies. The inner ear as the peripheral part of the auditory and the vestibular system is a delicate structure and often the site where disturbances occur. This thesis focuses on different scenarios in which the inner ear is off balance. For those scenarios, the physiological state of the inner ear and perceptual consequences for the organism are elaborated. In the following, the inner ear is disturbed either by overstimulation with intense sounds, which can lead to transient or even permanent changes of the sound detection system, or transcutaneous electrical stimulation, which excites the vestibular system simulating body motion. From the long list of inner ear pathologies, this thesis focuses on Menière's disease. This disorder is particularly interesting, as the vestibular and the auditory system are both affected at the same time. Menière's disease shows a combination of symptoms such as hearing loss, a sensation of ringing in the ears in the absence of sound (tinnitus) and a feeling of spinning around in the absence of motion (vertigo). This introduction aims to familiarize the reader with the inner ear structure and function of the auditory and vestibular system under normal, healthy conditions. Furthermore, I will elaborate on the disturbances and pathologies potentially occurring in the inner ear.

1.1 Common Features of the Auditory and the Vestibular System in Mammals

Although the auditory and the vestibular system are often examined separately and treated as independent entities , there is significant overlap regarding structure and function (Carey and Amin, 2006). Both systems form one single organ, the inner ear, protected by a bony capsule, the bony labyrinth. Within the bony labyrinth a second labyrinth, the fluid-filled membraneous labyrinth is surrounded by perilymph (see Fig. 1.1). Perilymph, one of the two inner ear fluids, was suggested to be an ultrafiltrate of blood plasma (Schnieder, 1974; Sterkers et al., 1988) and a derivate of cerebrospinal fluid (Kellerhals, 1979), depending on its location. Perilymph has an ionic composition typical for extracellular body fluids with high sodium and low potassium ion concentration ($[K^+]$) (Peterson et al., 1978; Smith et al., 1954). In contrast, the membraneous labyrinth is filled with endolymph (EL) containing an unusual extracellular composition with a high amount of potassium ions (K^+) and only few sodium ions (Na^+) (Smith et al., 1954; Peterson et al., 1978). EL originates in the cochlear wall and is reabsorbed by the endolymphatic sac which is located in the subdural space around the cerebellum and is connected with the vestibular system via the vestibular aqueduct (Ekdale, 2016).

Furthermore, the two systems share underlying anatomical features like mechanosensitive hair cells (HCs) which have their origin in a common ancestor over 400 million years ago (Coffin et al., 2004). HCs are characterized by microvilli, commonly called stereocilia, protruding from their cell bodies ("hairs"). In fish and amphibians, HCs can also be found on the body surface within the lateral line system. In their function as sensory cells of the lateral line system, HCs detect water motion and currents surrounding the animals. In terrestrial vertebrates, auditory and vestibular HCs are internalized, but they detect fluid motion within the fluid-filled inner ear.

Pathologies simultaneously affecting the auditory and the vestibular system sometimes reveal the close connection between the two sensory systems. For instance, the so called superior canal dehiscence syndrome, caused by a defect of the bony structure of the labyrinth, can trigger, amongst other symptoms, sound-induced vertigo. The inner ear disorder known as Menière's disease (MD) shows symptoms like hearing loss and concomitant vertigo possibly related to a disturbance of the inner ear fluid homeostasis (see section 1.4.2.2).

1.2 Auditory System in Mammals

The auditory system detects and processes sound. Sound is a wave of alternating pressure that travels through air or other elastic media. These vibrations can be perceived by humans and all other animals with auditory systems, when the wave amplitude (measured in sound pressure level [dB SPL]) not only exceeds a certain threshold, but also oscillates with frequencies (measured in Hertz [Hz]) that lie within the species-specific hearing range (see Fig. 1.4). The hearing range is determined by mechanical and neuronal filters on several levels of the hearing

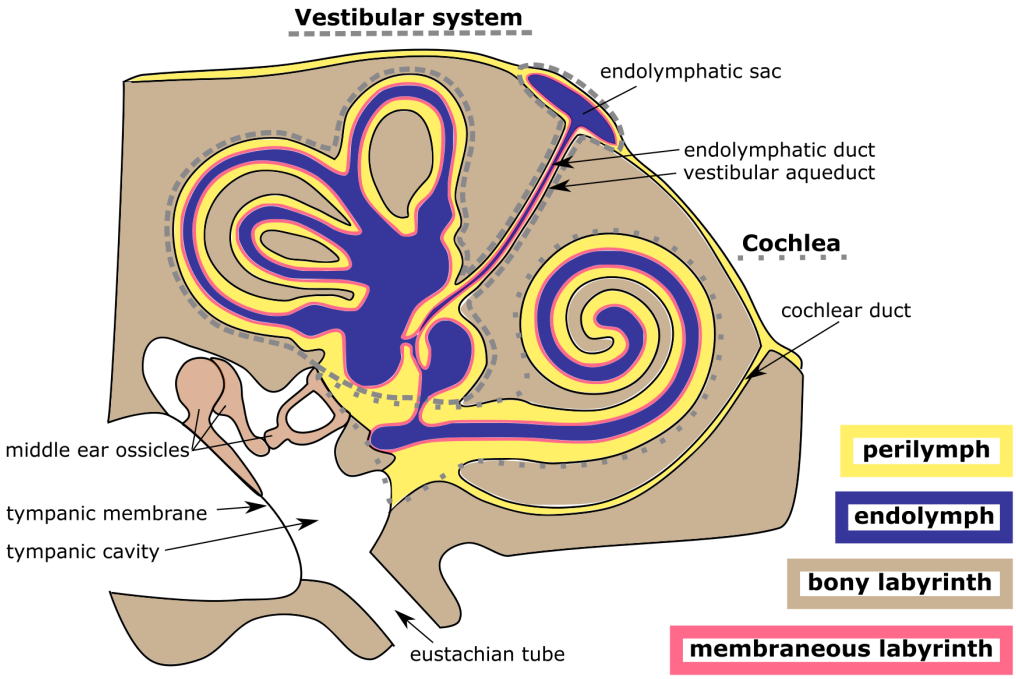

FIGURE 1.1. Cross-Section through the Mammalian Inner Ear. Cross-section through the skull showing the vestibular endorgans and the auditory periphery including tympanic cavity with middle ear ossicles and the cochlea. Blue indicates endolymph, yellow indicates perilymph. The membraneous labyrinth is highlighted with a fine red line, the bony labyrinth is colored in brown. *Own work re-drawn from and inspired by Kipp and Radlanski (2017), Fig.5.33b*

system from the periphery to the brain. Examples for such filters are the middle ear transfer function, the shape of the inner ear and the tuning of sensory cells and neurons. Based on the frequency range of human hearing between 20 Hz and 16 kHz (ISO 28961, 2012) (termed audible sound), sounds with frequencies lower than 20 Hz are called infrasound, whereas for sounds higher than 16 kHz the term ultrasound is used.

The auditory system comprises a central part with neural structures located in the brainstem, midbrain and the cortex and a peripheral part. The periphery consists of an outer, middle and inner ear. This thesis mainly focuses on the inner ear of mammals with the term mammal, as used here, including extant therians only.

Besides traditional mammal-like features (mammary glands, hair, secondary palate, cranial innervation etc.), mammals can also be described by ear-specific characteristics regarding the inner and middle ear. Middle ears of mammals consist of three ossicles (malleus, incus, stapes) transferring sound pressure from the external environment of the animal to its fluid-filled inner ear (for review see Manley, 2010). Inner ears of mammals show merging of soft tissue with the surrounding bony labyrinth (Manley, 2012). But the most prominent feature of mammalian inner ears is their coiled structure due to which mammalian inner ears are termed cochleae (lat. snail).

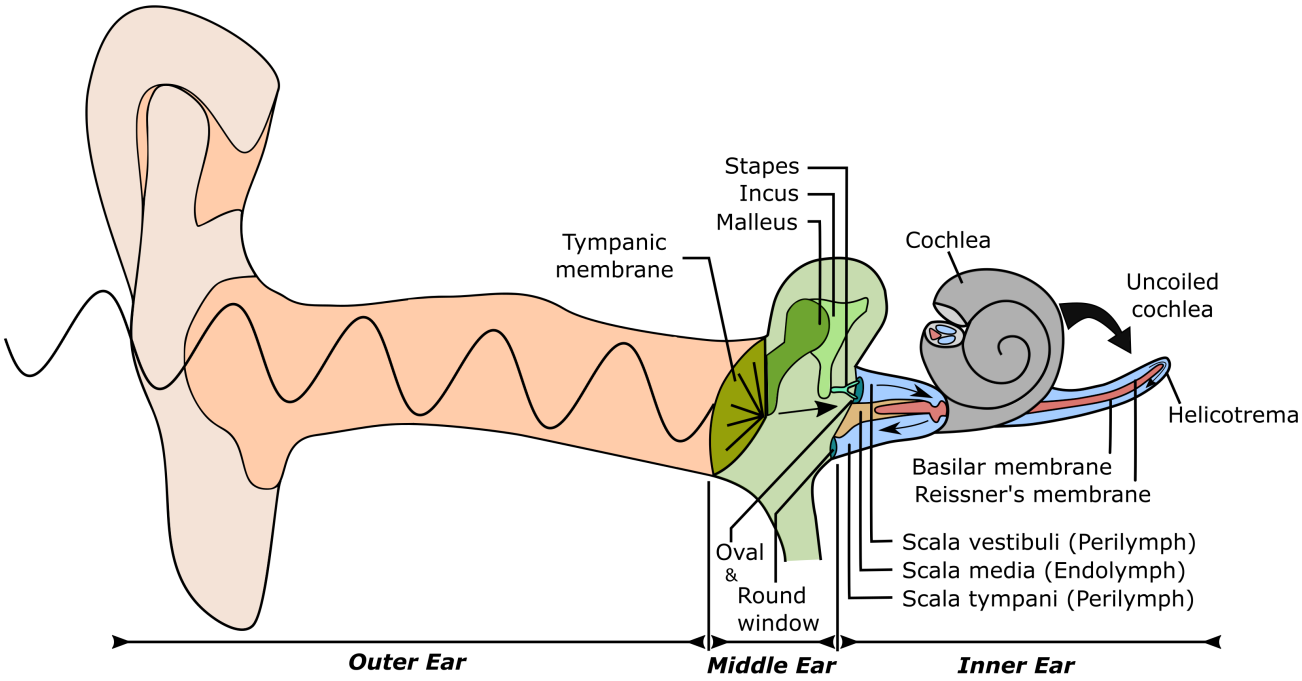

FIGURE 1.2. Anatomy of the Human Ear. Human ear including outer ear with pinna and ear canal, middle ear with tympanic membrane, tympanum and ossicles, and cochlea coiled and uncoiled (for better illustration). *Own work re-drawn from and inspired by Zenner (2007), Fig. 16.4 and 16.5, and Chittka and Brockmann (2005), Fig.1*

The cochlea is considered to be the most sensitive pressure sensor in the mammalian body. It can detect air pressure oscillations down to a few μPa. The following section describes the most important concepts regarding anatomy and physiology of the cochlea.

1.2.1 The Cochlea

The cochlea consists of three coiled tube-like compartments lying on top of each other (see Fig. 1.2). These fluid-filled compartments narrow gradually from the cochlear base (end of the middle ear cavity) towards the cochlear apex (tip of coil). The upper and the lower compartments, called scala vestibuli and scala tympani, are filled with perilymph and are connected via an opening at the apex, termed helicotrema. The middle compartment, scala media, is filled with EL and contains the organ of Corti with the sensory HCs. As mentioned before, EL and perilymph differ in their ion concentrations (see section 1.4) with EL containing an unusual high $[K^+]$. At the base of the cochlea, there are two membrane-covered openings towards the middle ear, the round window terminating scala tympani and the oval window of scala vestibuli (see Fig.1.2). The oval window is the contact point between the ossicular chain of the middle ear and the inner ear. The stapes footplate moves the window membrane of the oval window.

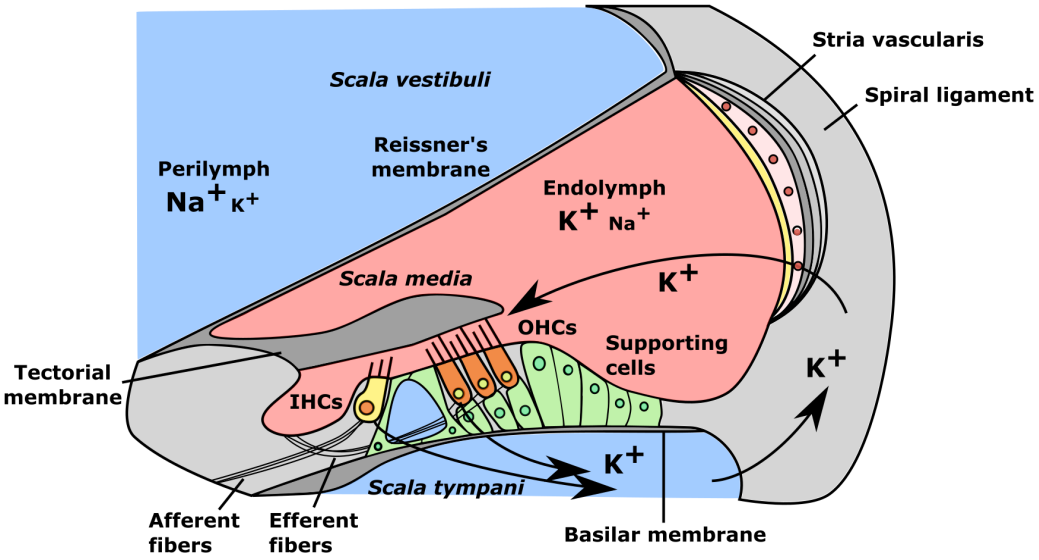

FIGURE 1.3. Cross-Section of Scala Media. Cross-section of cochlea focusing on the middle compartment, scala media, and adjacent structures like the multi-layered epithelium, the stria vascularis (SV), in the lateral wall of the endolymphatic duct. Red indicates endolymph, blue indicates perilymph, arrows show direction of recycling of potassium ions (K^+). Cells of the organ of Corti are highlighted: inner hair cells (IHCs) are colored in yellow, outer hair cells (OHCs) in orange, supporting cells in green. *Own work re-drawn from and inspired by Zdebik et al. (2009), Fig.3, and Lang et al. (2007), Fig.2a*

The three scalae are separated by membranes (see Fig. 1.3). Reissner's membrane separates scala vestibuli from scala media. The basilar membrane (BM) separates scala media from scala tympani. Resting on the BM, the actual sensory organ, the organ of Corti, can be found. The organ of Corti consists of different cell types: several supporting cells and, most importantly, sensory HCs of two types, inner (IHCs) and outer hair cells (OHCs). There is typically one row of IHCs and three rows of OHCs in humans. IHCs are responsible for transforming mechanical vibration into electrical neural signal. Thus, IHCs have excitatory synaptic contacts with afferent fibers of the spiral ganglion neurons. Those afferent fibers merge as they ascend to form the eighth cranial nerve. In contrast, OHCs amplify the incoming sound and have mainly connections to efferent neurons of the medial olivocochlear complex that serve as modulators of the amplification process (Rasmussen, 1946; Warr and Guinan, 1979).

OHCs in mammals amplify the mechanical displacement of the BM by elongating and contracting their cell bodies, thereby enhancing the BM displacement. Cellular length changes are triggered by the motorprotein prestin (Dallos and Fakler, 2002; Zheng et al., 2000). This protein is found in the lateral cell walls of the OHCs. The amplification process is essential for detecting sounds with minimal sound pressure level.

Along the length of the cochlea, properties of IHCs and OHCs change. From the cochlear base to the apex, stereocilia become taller but the number of stereocilia per cell is reduced (Lim, 1986; Davis, 2003). At the cochlear base with shorter stereocilia and higher number of cilia, the organ of Corti is more sensitive to mechanical displacement than in the apex. As a result the stiffness of the stereocilia is highest at the base and decreases towards the apex. Apart from mechanical properties of stereocilia, stiffness of the tectorial membrane and stiffness of the BM also decrease from the base to the apex. Especially prominent is the mechanical tuning of the BM. The BM widens from the base to the apex of the cochlea causing an increase in mass. Each segment of the BM, together with its surrounding fluid, can be considered a "mass-spring" system with different resonant properties: the BM at the cochlear base is characterized by high stiffness and low mass, hence shows high resonance frequencies. On the contrary, the BM at the cochlear apex, with its low stiffness and high mass, shows low resonance frequencies.
The presence of mechanical and structural gradients along the length of the cochlea is important with respect to sound propagation and frequency analysis. The cochlea is arranged in a tonotopical order meaning that each longitudinal position in the cochlea is tuned to a certain frequency, its characteristic frequency. Therefore, sound can be decomposed spatially into its constituent frequencies. Determined by the resonance frequencies, the base of the cochlea responds maximally to high-frequency (HF) sound, and the apex with its more compliant part of the BM responds maximally to low-frequency (LF) sound.

1.2.2 Anatomical Differences in Mammalian Cochleae

Although the first section described a typical bauplan of the cochlea, mammalian inner ears cannot be considered uniform (West, 1985). On the contrary, cochleae show high interspecific variability. The diversity of mammalian cochleae enables different functionalities and can be explained by different selection pressures, which are linked to various life styles (Vater and Kössl, 2011). For instance, adaptations were made to enable LF hearing. Subterranean animals like golden moles specialize on LF sounds below 20 Hz to detect ground vibrations (Narins et al., 1997; Mason et al., 2018). Golden moles possess highly coiled cochleae. The coiling of the cochlea was originally suggested to have evolved in order to conserve space inside the skull (Manley, 2012). Conserving space became necessary when mammals expanded their hearing ranges to higher frequencies, causing an elongation of BM during evolution (Manley, 1972). Another theory proposed that coiling of the cochlea also enhanced LF hearing. Like in whispering galleries of old churches, where curved walls carry even faint sounds to another distant location, acoustic energy is redistributed toward the outer wall of the coiled cochlea (Cai et al., 2005; Manoussaki et al., 2008). Thereby, the spiral form enhances pressure wave propagation towards the cochlear apex, where LF sounds are detected. However, the underlying reason for the coiled structure of cochleae is not conclusively clarified yet and still actively debated in the field of hearing research (Pietsch et al., 2017).

Rodents like chinchilla or the Mongolian gerbil that spend a lot of time underground show various adaptations to LF hearing. Regarding the inner ear, the size of the helicotrema can be optimized for LF hearing. The helicotrema connecting scala tympani and scala vestibuli at the apex shows a shunting effect for static pressure and LF sound (Marquardt and Jurado, 2011). When LFs are detected at the apical end right before the helicotrema or virtually beyond the apical end, perilymph is forced through the helicotrema without generating a pressure difference between compartments (Marquardt et al., 2007). A pressure difference between compartments, however, is ultimately necessary to detect sound. A smaller helicotrema allows LFs to be detected more easily (Dallos, 1970; Manoussaki et al., 2008). Moreover, middle ear features reducing the acoustic impedance at LFs are favored in LF specialists. For instance, middle ear adaptations like enlarged middle ear cavities in chinchillas (Rosowski et al., 2006) and gerbils (Ravicz and Rosowski, 1997; Cox and Hautier, 2015) translate into better LF hearing.
Independent from adaptations to life style (e.g., LF hearing), there are variations due to physical and anatomical constraints (size of tympanic membrane, head size etc.) (Ekdale, 2016). BM length in mammalian cochleae shows a positive correlation with body weight (e.g., 6-7 mm in mice, 34 mm in human) (Lewis et al., 1985). However, the number of cochlear turns does not follow any simple principle (West, 1985). Within rodents, inner ears differ, for instance not only regarding the number of sensory cell rows (3-4), but also regarding the number of cochlear turns, from 1.5 to 2 turns in mice (Keen, 1939) to 3.5 to over 4 turns in guinea pigs (Keen, 1939; Ekdale, 2016).
The mammals that were used as study objects in the current work are humans and Mongolian gerbils (*Meriones unguiculatus*). Gerbils are able to hear LF sounds down to 50 Hz due to enlarged middle ear cavities and increased BM width compared to other rodents of similar size (Webster and Plassmann, 1992). Within the field of auditory research, gerbils serve as a popular and extensively used animal model for the human auditory system, as the hearing ranges of humans and gerbils largely overlap (see Fig. 1.4).

1.2.3 The Hearing Process

Hearing is a complex process comprising multiple steps such as transmitting sound waves, conversion of the mechanical sound wave into a receptor potential and neural signalling.

1.2.3.1 From the Outer to the Inner Ear

Sound waves are funneled by the external part of the ear, a cartilagineous conic structure called pinna. The pinna contains several structural elements which are the first site along the auditory pathway where frequency-selective filtering occurs (Batteau, 1967; Koka et al., 2011).
Sound travels through the ear canal (meatus) to the tympanic membrane. The tympanic membrane separates the outer ear canal from the air-filled middle ear cavity, which in some animals like Mongolian gerbils widens into an enlarged, bulbous, bony cavity, called auditory bulla.

A

Cochlea in Numbers	Length of...	Number of...		
	Basilar membrane	Cochlear turns	IHCs	OHCs
Homo sapiens	30 – 35 mm [1,2]	2.75 [3,4]	3,000	12,000
Meriones unguiculatus	11 – 12.7 mm [5,6]	2.5 – 3 [7]	1,420 [6]	4,140 [6]
Mus musculus	5 – 6 mm	2 [10]	765 [8,9]	2526 [8,9]

B

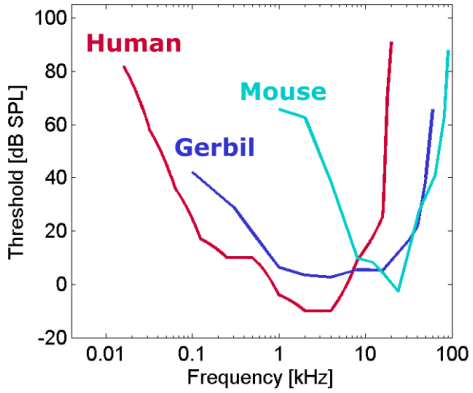

FIGURE 1.4. Comparison Hearing Thresholds and Cochlear Data between Humans and Gerbils. Hearing Comparison of humans (*Homo sapiens*), gerbils (*Meriones unguiculatus*) and mice (*Mus musculus*). A: Cochleae in numbers with the following references [1]Keen, 1940; [2]Liu et al., 2015; [3]Hardy, 1938; [4]Biedron et al., 2009; [5]Muller, 1996; [6]Risoud et al., 2017; [7]Jones-Mumby and Axelsson, 1984; [8]Ehret and Frankenreiter, 1977; [9]Burda and Branis, 1988; [10]Keen, 1939. B: Hearing threshold comparison: humans (Jackson et al., 1999), gerbils (Ryan, 1976), mice (Radziwon et al., 2009).

The tympanic membrane is a three-layered membrane in the shape of an inward pointing cone. Sound waves cause the tympanic membrane to oscillate. Oscillations of the tympanic membrane initiate movement of the middle ear ossicles (malleus, incus, stapes). The malleus touches the tympanic membrane directly and is connected via the incus to the stapes, which sits on the oval window at the base of the cochlea. Two middle ear muscles (stapedial muscle and tensor tympani) are attached to the ossicles and can attenuate sound transmission in the form of a protective reflex (Møller, 1974). The oscillatory ossicular movement is transmitted onto the oval window inducing a fluid pressure wave within the cochlea. The main function of the middle ear is to overcome the impedance mismatch between low impedance in air (outer and middle ear) and high impedance in fluid (inner ear). There are two processes that ensure sound transmission across the air-fluid boundary: First and foremost, focusing force collected by the large area of the tympanic membrane onto the small area of the oval window increases the pressure along the transmission process. Second and related to the first process, the three connected ossicles serve as a compound lever amplifying the force that moves the oval window. In this way, even faint sounds causing only minute movements of the tympanic membrane can be transformed into pressure waves within the cochlea.

1.2.3.2 Sound Traveling within the Cochlea

A differential pressure arises at the oval window of scala vestibuli, where the stapes motion is detected, versus at the round window, where pressure is relieved. This differential pressure is the origin of the pressure waves traveling from the cochlear base towards the apex. The pressure wave within the inner ear fluid travels at the speed of sound in water (1,500 m/s) and causes energy transfer onto the sensory cells. Two prominent theories, that might either exclude or complement each other in a hybrid model, have been hypothesized to drive auditory transduction (Bell, 2004). In the traveling wave theory, as suggested by Von Békésy and Wever (1960), cochlear fluid and the BM with the sensory cells atop are hydrodynamically coupled. The pressure wave leads to a significantly slower (hundreds of m/s) forward-moving wave passing on energy within the membranous structure of the BM. The mechanical displacement of the BM including the organ of Corti increases gradually with its peak at the characteristic frequency. Traveling waves are considered to slow down (tens of m/s) when approaching the characteristic frequency place and to die out quickly after reaching their peak. In contrast, the resonator theory proposed by Von Helmholtz (1912) and supported by Gold's finding (Gold, 1948) proposes a bank of uncoupled resonators, like the discrete strings of a piano, that can vibrate side to side in synchrony (Bell, 2004). In the cochlea, these resonators are the sections of the organ of Corti from base to apex, which show different resonances depending on their mechanical properties (see section 1.2.1). If the resonators respond to the fast pressure wave directly, the resonator theory is an alternative to Békésy's theory. If the resonators are set off by a traveling wave, the two theories merge to a hybrid model.

1.2.3.3 Mechanotransduction by Hair Cells

OHCs and IHCs detect sound with their stereocilia protruding from the apical part of their cell bodies. The stereocilia of the OHCs are attached to the gelatinous tectorial membrane covering the organ of Corti, the stereocilia of IHCs are viscously coupled to the EL (Dallos et al., 1972; Gummer et al., 1996; Guinan, 2012; Lim, 1980). This slight difference might be a possible reason why OHCs are more sensitive to LF sound (see section 1.4.2.1) than IHCs. IHCs detect fluid velocity (Patuzzi and Yates, 1986), which is low at LFs. In contrast, OHCs are linked to two membranes: the cell bodies of OHCs sit on the BM and the sterecilia are attached to the tectorial membrane. Therefore, OHCs might sense mechanical displacement induced by LFs reasonably well (Dallos et al., 1982).

Regardless the adequate stimulus, both hair cell populations employ essentially the same mechano-electrical transduction (MET) mechanism: when the pressure difference between scala vestibuli and scala tympani displaces the BM relative to the tectorial membrane, a shearing motion between BM and tectorial membrane deflects the stereocilia. Stereocilia are bundled via interstereociliary links. Ankle links connect adjacent cilia at the bases (Michalski et al., 2007) and horizontal top connectors connect them at the shafts (Goodyear et al., 2005).

As a result, cilia form a cohesive unit and move in parallel (Kozlov et al., 2007; Hudspeth, 1983). Among the fibrous links, the so-called tip links connect the tip of one stereocilium with the adjacent taller stereocilium. Movement of the stereocilia into either direction changes the tip link tension. Tip links induce conformational changes of mechanosensitive ion channels at the tip of the stereocilia. Those ion channels are also called mechano-electrical transduction (MET) channels. When undergoing conformational changes, the opening probability of MET channels changes as well (Pickles et al., 1984; Hudspeth, 1989). Deflection towards the largest stereocilium causes increased opening probability of MET channels and increased ion influx, cell membranes depolarize. Deflection into the opposite direction causes decreased opening probability of MET channels and thus decreased ion influx, HCs hyperpolarize. Through the ion channels mainly K^+-ions flow into the HCs. K^+-ions are driven into the cells by the endocochlear potential (EP). In mammals, the EP is positive compared to the cytosol of the HCs (see section 1.4.1) (Schmidt and Fernández, 1962; Davis, 1965; Russell, 1983; Fettiplace, 2017). During repolarization, K^+-ions exit the HCs via somatic channels in the basal membrane.

1.2.3.4 Cochlear Amplifier

OHCs are responsible for amplification of faint sounds. This OHC-triggered amplification contributes to the high sensitivity and sharp frequency selectivity of hearing. Amplification relies on a process termed electromotility (Brownell, 1984; Brownell et al., 1985). Changes of OHC membrane potentials result in molecular conformational changes of the trans-membrane protein, prestin (Dallos and Fakler, 2002; Zheng et al., 2000). As conformational changes are correlated with cross-sectional area changes of many tightly packed prestin molecules (Oliver et al., 2001), they ultimately lead to cellular length changes. OHCs contract their cell bodies during depolarization and elongate their cell bodies during hyperpolarization. Thereby, OHCs are thought to increase the displacement amplitude of the organ of Corti (Murakoshi et al., 2015).
Apart from these rather fast length changes - with high-frequency limits up to 79 kHz (Frank et al., 1999) - the OHCs have been observed to undergo slow length changes - over several seconds or even minutes - which can be elicited by different mechanisms. These mechanisms include osmotic effects, mechanical stimulation, efferent innervation and increase of intracellular calcium ion (Ca^{2+}) levels (Dulon et al., 1990; Dulon and Schacht, 1992).
While OHCs are the key elements of the cochlear amplifier (for review Ashmore et al., 2010), IHCs serve as the classical sensory cells. They transduce the mechanical stimulus into a neural signal.

1.2.3.5 Mechanotransduction: Transfer Function and Operating Point

The MET in HCs describes the mechanism how HCs convert the sound pressure wave, a mechanical stimulus, into electrochemical activity like inward ion currents and subsequent depolarization. In IHCs and OHCs, this mechanotransduction works slightly different. The transduction process

can be described by a so called input/output transfer function. Transfer functions illustrate the relationship between mechanical input, e.g. stereocilia displacement, and electrical output, e.g. depolarizing current or open probability of transducer channels. In general, transfer characteristics of HCs are non-linear and compressive. This means that an increase in sound pressure level might only result in a small change of electrochemical signal. The compressive behavior relates to saturation of transducer currents at high sound pressure levels (Lukashkin and Russell, 1998) and conductances of basolateral K^+-channels involved in outward currents (Dallos, 1996). The fact that the auditory system is compressive becomes obvious, as a wide range of audible sound pressure levels are mapped into a limited range of neural signalling rates (Lopez-Poveda and Eustaquio-Martín, 2006). The compressive, non-linear character is responsible for the sigmoidal shape of HC transfer functions (see Fig.1.5). The transfer function of OHCs is highly symmetrical with approximately 50% of the transducer channels being open at rest (Dallos, 1992). In contrast, IHCs have a rectified response. Only approximately 20% of the transducer channels are open at rest (Russell et al., 1986) resulting in a less sensitive behavior when stereocilia are displaced in the hyperpolarizing direction.
To describe the system's current state and to identify where on the transfer curve the transduction process currently occurs, the concept of the operating point (OP) is often applied. The OP located on the transfer function of HCs is known as transducer OP. If the OP marks the location on the transfer function in the absence of any input stimulus or at the zero-crossings of sinusoidal stimulation, the OP is called resting OP. In most IHCs, the resting OP lies on the hyperpolarizing side from the inflection point (see Fig.1.5), and therefore not in the region with the largest slope. In contrast in OHCs, the resting OP can normally be found around the inflection point of the response curve and therefore around the maximum sensitivity. This explains why OHCs are hugely affected in case the OP is shifted away from this point. OP shifts can be temporarily achieved in so called biasing experiments using current injection into scala media or LF sound stimulation (Frank and Kossl, 1997). However, OP shifts also happen due to pathological changes like dysfunctional HCs (Bian and Chertoff, 1998), endolymphatic hydrops (Mrowinski et al., 1996; Brown and Gibson, 2011) etc. (see section 1.4.2). Indicators of OP shifts are modified hearing thresholds (Mrowinski et al., 1995) and otoacoustic emissions (OAEs) (see section 1.2.4) (Bian, 2004; Bian and Watts, 2008).

1.2.3.6 Afferent and Efferent Innervation of Hair Cells

In depolarized IHCs, voltage-gated-Ca^{2+} influx at the base of the cells induces glutamate release into the synaptic cleft. Glutamate stimulates the afferent auditory nerve endings by docking onto ionotropic glutamate receptors at the postsynaptic side of primary afferents.
The primary afferents, the so called spiral ganglion neurons, react to the transmitter with excitatory postsynaptic potentials, which can result in action potentials. 90-95% of primary afferents are linked to IHCs. Each IHC is innervated by 10 to 20 myelinated, unbranched neurites (type I).

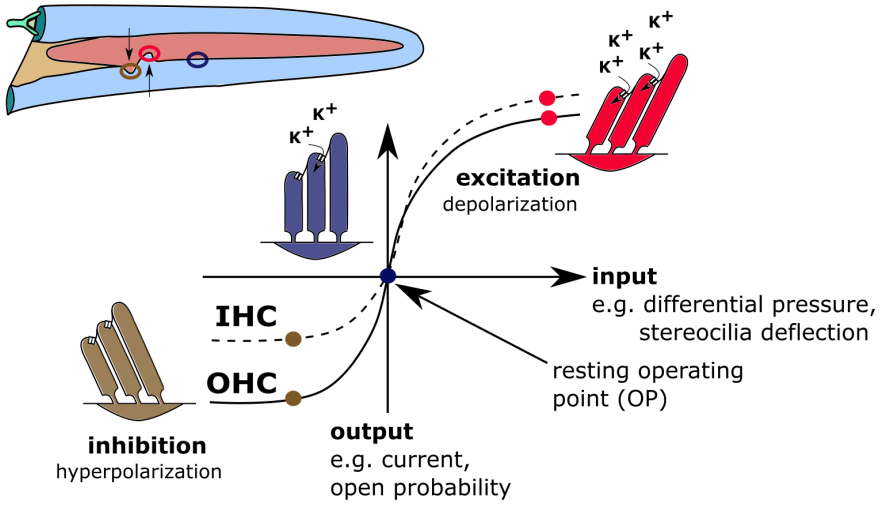

FIGURE 1.5. Explanatory Scheme for Operating Point and Input/Output Transfer Functions. Operating points (OP) are indicated by filled circles on non-linear transfer functions of inner hair cells (IHCs, dashed line) and outer hair cells (OHCs, solid line). Changes in stereocilia angle due to basilar membrane (BM) displacement (see uncoiled cochlea upper left) cause opening (excitatory - red) or closure (inhibitory - brown) of mechano-electrical transduction (MET) channels through which potassium ions (K^+) enter the cell. *Own work re-drawn from and inspired by Russell et al. (1986), Fig.1, and Gillespie (1995), Fig.1*

The remaining 5-10% of primary afferents are type II spiral ganglion neurons. They send their unmyelinated neurites to OHCs. One neurite innervates several OHCs.

Efferent innervation of the OHCs is much denser than efferent innervation of the IHCs. Myelinated efferents are neurons, which originate from the medial olivocochlear complex (see Fig. 1.6) from the same (ipsilateral) or the opposite (contralateral) body side like the respective ear and end directly on the HC body of OHCs. Efferent activity can thus modulate the gain of the amplification process. Classically, contralateral stimuli reduce the ipsilateral output indicators such as cochlear microphonics (Fex, 1959; Mountain, 1980), compound action potential (Siegel and Kim, 1982), and OAEs (see section 1.2.4).

Efferent nerve fibers associated with IHCs are unmyelinated and originate from the ipsilateral lateral olivary complex. Those efferents do not innervate the IHCs directly, but end onto the auditory nerve fibers below IHCs where they alter the firing pattern of the auditory nerve fibers. Efferent innervation on sensory cells is restricted to HCs in the inner ear and is mostly involved in the so called efferent acoustic reflex to protect the cochlea against high sound pressure levels (Guinan, 2006). Furthermore, efferents are proposed to play a role in cochlear amplifier control (Mountain, 1980; Siegel and Kim, 1982), improvement of signal-to-noise ratio (Dolan and Nuttall, 1988; Kawase et al., 1993) and dynamic range increase (Geisler, 1974).

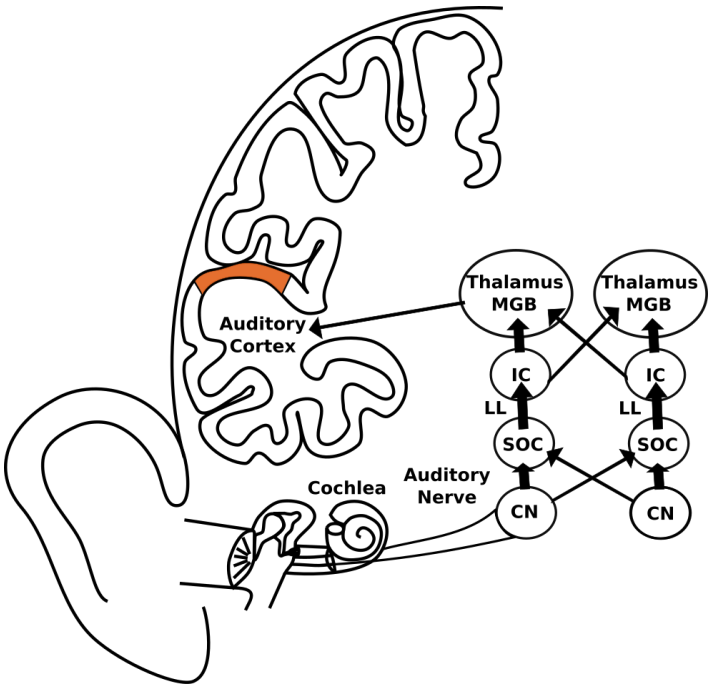

FIGURE 1.6. The Ascending Auditory Pathway. The auditory pathway from the periphery to the central brain areas; the complete ipsilateral pathway is shown. The analogous contralateral pathway is partially shown to illustrate connections with the contralateral side; first relay station after the auditory nerve is the cochlear nucleus (CN) projecting to the superior olivary complex (SOC). The SOC is connected via the lateral lemniscus (LL) to the inferior colliculus (IC). The next brain structure involved in auditory processing is the thalamus with the medial geniculate body (MGB) as gateway to the cortical centers of auditory perception. The primary auditory cortex is indicated in orange. *Own work re-drawn from and inspired by Davies and Sugano (2018), Fig.1*

1.2.3.7 Auditory Pathway beyond the Cochlea

Once the sensory cells have changed the mechanical input into an electrical output, the auditory information is transmitted via action potentials from the afferent nerve fibers of the spiral ganglion neurons to higher brain areas (for review see Pickles, 2015). The ascending auditory pathway connects the periphery with the auditory cortex (see Fig.1.6). The first important relay station is the cochlear nucleus, which can be further divided into the dorsal, the anteroventral and the posteroventral cochlear nucleus. Cochlear nuclei are tonotopically organized with LF auditory nerve fibers terminating ventrally and those of high frequencies dorsally. Neurons from the cochlear nucleus send their axons to the brainstem, specifically the superior olivary complex with its multiple nuclei. The superior olivary complex is the first brain structure receiving binaural input from cochlear nuclei from the ipsi- and contralateral side and is therefore heavily involved in azimuthal sound localization. From the superior olivary complex, information is passed on via

the lateral lemniscus to the inferior colliculus.
The central inferior colliculus produces a tonotopic map with lateral neurons responding to LF sound. In the inferior colliculus, patterns of sound can be extracted. After the inferior colliculus, auditory information travels to the medial geniculate body in the thalamus where tonotopy is maintained in isofrequency layers in the ventral division. The ventral division of the medial geniculate body is the main projection site to the primary auditory cortex, which is also organized in isofrequency layers from LFs in the rostral end to high frequencies caudally.
At all levels of either the periphery (middle ear, cochlea, auditory nerve) or the ascending pathways (cochlear nucleus, superior olivary complex, inferior colliculus, medial geniculate body, auditory cortex) a pathological change can interfere with hearing ability and cause hearing loss and tinnitus. This thesis focuses mainly on the periphery. The main causes for peripheral hearing loss are mainly due to sensory HC loss caused by noise exposure, ototoxic drugs, or hereditary hearing loss (for review see Morrill and He, 2017).

1.2.4 Otoacoustic Emissions

Already in 1948, Gold suspected that the high sensitivity of the cochlea might be due to active amplification in the form of a cochlear feedback system (Gold, 1948). In case this feedback system was of non-linear nature, the cochlea, normally responsible for sound detection, would generate sound waves as a by-product of the non-linear process. Proof for such a non-linear cochlear amplifier was found by Kemp (1978). Kemp discovered faint sounds that were emitted by the cochlea of healthy humans. Today those sounds are known as otoacoustic emissions (OAEs). OAEs can be recorded with a sensitive microphone in the ear canal of humans and various mammalian species (Faulstich et al., 1996; Kössl, 1994; Withnell et al., 2003). OAEs are widely used to non-invasively examine the integrity of the cochlear amplifier and of its main components, the OHCs (for review see Probst et al., 1991; Lonsbury-Martin and Martin, 1990). OHCs feed energy back into the system by actively contracting and elongating their cell bodies in synchrony with the incoming sound wave. This somatic contraction and elongation enhances BM motion.
Dependent on the experimental paradigm, different types of OAEs can be distinguished: spontaneous OAEs, transiently evoked OAEs, stimulus-frequency OAEs and distortion product OAEs (for review see Probst et al., 1991). Spontaneous OAEs and distortion product OAEs were used in our studies (chapter 2-4) and are explained in more detail in the following.

Spontaneous OAEs (SOAEs) occur without any sound stimulation. Therefore, SOAEs are not masked by stimuli or stimulus artifacts when they are recorded. SOAEs can be measured in a few mammals, e.g. humans (Penner et al., 1993; Bilger et al., 1990). As their name suggests, SOAEs originate from spontaneous activity of OHCs. Due to the varying open probability of ion channels, the membrane potential within the OHCs fluctuates and leads to spontaneous prestin-driven cell length changes. Spontaneous activity of all OHCs cancels out, given a perfect

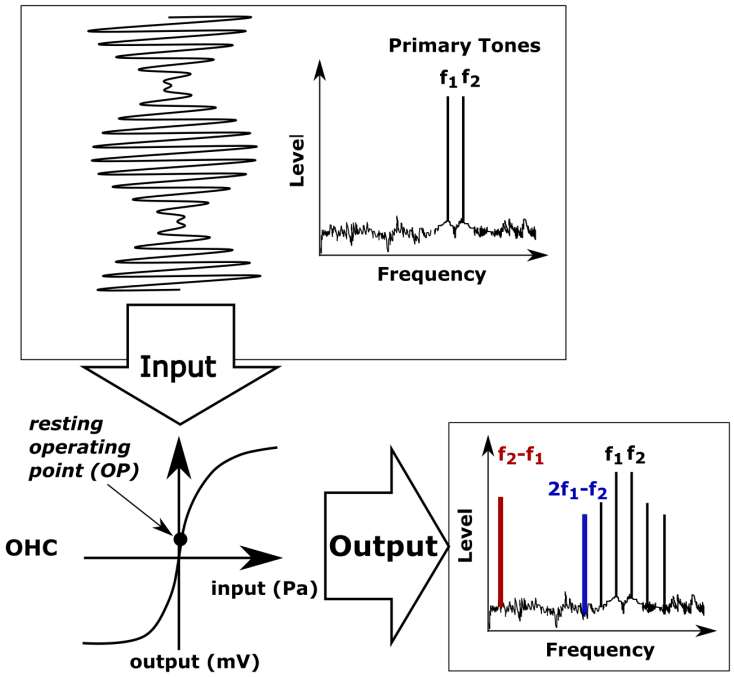

FIGURE 1.7. Scheme of Non-Linear Amplification of Two Pure Tones. Outer hair cells (OHCs) non-linearly amplify two sinusoidal pressure waves with frequencies f_1 and f_2. Input to non-linear transfer function is shown as waveform of two sinusoids with corresponding frequency spectrum. Output is shown as frequency spectrum with distortion frequencies; the quadratic (f_2-f_1) and cubic distortion product otoacoustic emission (DPOAE) ($2f_1$-f_2) are indicated in red and blue. *Own work re-drawn from and inspired by Abel (2008), Fig.1.2*

cochlea. However, in case of inhomogeneities some cells might show coupled spontaneous activity leading to a coordinated BM motion. Coordinated BM displacement causes a pressure wave that travels back to the oval window. This pressure wave moves the middle ear ossicles and tympanic membrane, which can in turn can be measured as sound in the ear canal.

Sound pressure levels of SOAEs are often very faint. In experiments, it is easier to use distortion product OAEs (DPOAEs) which show higher intensities.
DPOAEs are physiological correlates of cochlear non-linearity. The cochlea shows characteristics of a non-linear system at numerous levels, e.g. non-linear BM vibration, non-linear extra- and intracellular electrical potentials in OHCs, non-linear cochlear amplifier, non-linear IHC potentials (Rhode, 1971; Durrant and Dallos, 1972; Russell and Sellick, 1978; Cheatham and Dallos, 1982; Zagaeski et al., 1994).
Nevertheless, DPOAEs with their distinctive amplitude and phase patterns can be described as the output of a single saturating non-linearity (Lukashkin and Russell, 1998) (see Fig. 1.7). The MET of the OHCs is a well-suited candidate for DPOAE generation, as it is often consid-

ered to show the strongest non-linearity in the periphery of the auditory system (see Fig. 1.5) (Patuzzi et al., 1989; Santos-Sacchi, 1993). The MET transfer function can be approximated by a two-exponential Boltzmann function and is sufficient to explain DPOAE generation. However, it must be remembered that other sources of non-linearity will contribute to DPOAEs.

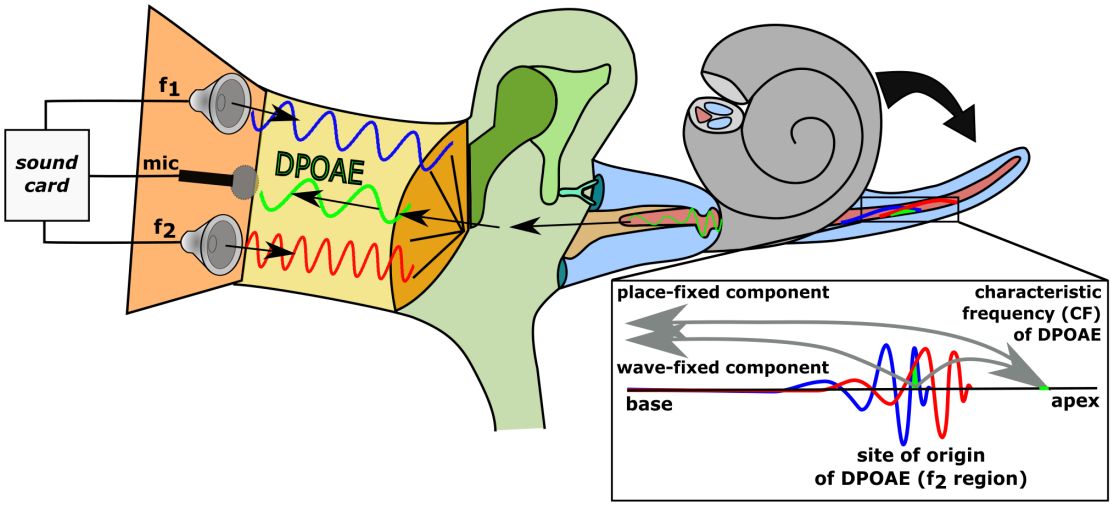

FIGURE 1.8. Distortion Product Otoacoustic Emission Generation. Distortion product otoacoustic emissions (DPOAEs) are generated by two primary tones of different frequencies (f_1 and f_2) emitted by transducers in the outer ear canal and illustrated in blue and red, respectively. Within the cochlea, DPOAEs have two sites of origin (shown in the insert), known as the place-fixed and wave-fixed component. DPOAEs (exemplary DPOAE indicated in green) travel back to the ear canal and are recorded by a microphone. *Own work re-drawn from and inspired by Janssen (2004), Fig.1, and Knight and Kemp (2001), Fig.6*

DPOAEs are triggered by two simultaneously presented pure tones called primary tones or primaries. Primaries have two different frequencies f_1 and f_2. f_1 and f_2 are chosen with a frequency ratio slightly bigger than 1, so that traveling waves of the two tones, peaking at the characteristic frequency sites on the BM, overlap. The frequency ratio and also the ratio of sound pressure levels of the two primaries (L_1 and L_2) determine whether emissions are elicited. DPOAEs are not echoes of the two presented tones, but are tones with frequencies different from both primary tone frequencies (f_1 and f_2). Presentation of two primary tones triggers the generation of several DPOAEs with various frequencies (see Fig. 1.7). These frequencies can be mathematically calculated (see appendix 6). DPOAEs with frequencies at f_2-f_1 called quadratic emissions and at $2f_1$-f_2 called cubic emissions are most prominent and most often used experimentally.

Generation of DPOAEs is based on a combination of two mechanisms (see Fig.1.8) (Shera and Guinan, 1999). In the region where the two traveling waves overlap, basal to the characteristic

frequency of f_2, intermodulation occurs due to the non-linear opening probability of MET channels of OHCs. As a result, electromechanical transduction by OHCs induce distortion. This distortion gives rise to two traveling waves: a reverse traveling wave (non-linear distortion component, wave-fixed) that directly travels back to the middle ear and a forward traveling wave. This forward wave travels to the characteristic frequency place of the distortion component and is reflected at sites of mechanical irregularities (coherent-reflection component, place-fixed).

1.3 Vestibular System in Mammals

The vestibular system is essential for keeping balance and orientation in space as well as perception of motion and maintaining stable visual perception during motion (Cullen and Sadeghi, 2008; Goldberg et al., 2012). The vestibular end organs sense acceleration of the body and more specifically the head (units: [m/s^2]). Acceleration is measured in linear and angular direction and depends on body orientation in space.
In contrast to the auditory system, which gives rise to hearing - a sense on its own, the sensory information processed by the vestibular system converges with multiple inputs of other sensory systems like the visual or somatosensory system. Therefore, the vestibular system does not provide a distinct conscious sensation. Nevertheless, the vestibular system as one of the evolutionary oldest structures in the animal kingdom (Graf, 2009) is crucial for survival in a three-dimensional world. This becomes apparent by its involvement in many different reflexes and body perception (Angelaki and Cullen, 2008) or by the severe effects of pathologies of the vestibular system causing balance disabilities and navigational problems (e.g. Brandt et al., 2005).

1.3.1 Anatomy and Physiology

The vestibular system and the cochlea share the same temporal cavity consisting of the perilymph filled bony labyrinth and the EL filled membranous labyrinth. Three semicircular canals (horizontal, anterior vertical, and posterior vertical canal) are oriented towards each other in an orthogonal way. They span the three spatial dimensions and code for angular velocity (Wilson and Jones, 1979) or more generally rotation. Their sensitivity is estimated to be around 0.1°/s² (Shumway-Cook and Woollacott, 2007) and they sense movements of the fluid relative to the surrounding bony structure.
The so called otolith organs, utricle and saccule, are also oriented in an orthogonal manner to each other. Utricle and saccule sense linear acceleration including gravity or translation in horizontal and vertical direction, respectively (Goldberg et al., 2012).

Receptor cells of the vestibular system are HCs, which additionally to stereocilia possess one true kinocilium per cell. Like in the cochlea, there are ion channels at the tips of all cilia that open and close depending on the direction of deflection. For the semicircular canals, the HCs sit

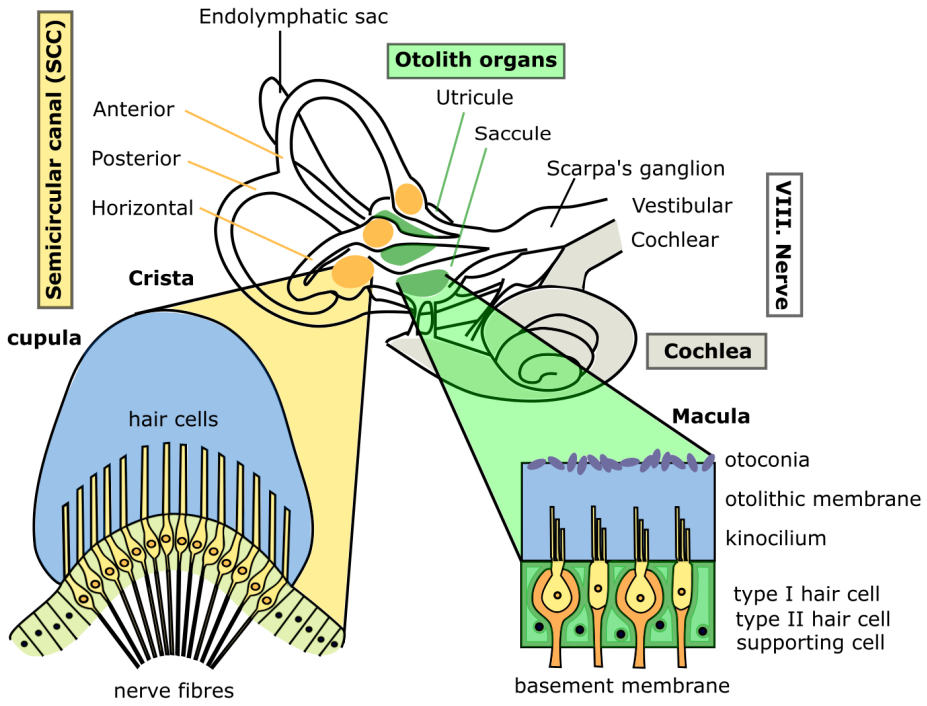

FIGURE 1.9. Anatomy of the Vestibular System. The membraneous labyrinth of the vestibular endorgans contains sensory epithelia, cristae within the ampullae of the semicircular canals (left) and maculae of the otolith organs, utricle and saccule (right). *Own work re-drawn from and inspired by Rudge (2019), figure in section "The vestibular system"*

in the so called ampullae at the base of each canal (see Fig. 1.9). HCs in the ampullae are covered by gelatinous structures, the cupulas, which are coupled to the EL. Head rotations cause relative EL motion and consequently cupula motion. The cupula motion bends the stereocilia of the HCs which reach into the cupula.
For utricle and saccule, HCs and supporting cells form so called maculae. In the macula, there is a polysaccaride layer, the otolithic membrane on top of the HCs. Calcit crystals termed otoconia lie on top of the layer adding weight and increasing inertia. Otolith organs measure linear acceleration as the sum of gravity and inertia, gravito-inertial force.

In both systems, in the ampullae of the semicircular canals and in the maculae of the otolith organs, surrounding fluid or overlying membrane move the stereocilia relative to the HCs' bodies causing changes in ion influx and in the membrane potential of the HC (Hudspeth, 2005). Similar to the IHCs of the cochlea, deflection of stereocilia towards the largest kinocilium causes depolarisation with increased vesicle release. Deflection of cilia in the opposite direction leads to hyperpolarisation and a decrease in synaptic vesicle release. Vesicles are released into the synaptic cleft between HCs and primary vestibular afferents. Spontaneous vesicle release causes

a resting firing rate of around 100 Hz. Vestibular afferents converge to the eighth cranial nerve which also contains auditory nerve fibers. Afferents carry the vestibular signal to the central nervous system as firing rate changes.
The two vestibular systems from both ears (left and right) work together to encode stimuli. For instance, each anterior canal is paired with the posterior canal of the contralateral ear. If head movement triggers excitation on one side, the other side is inhibited (Straka and Dieringer, 2004). However, the vestibular system is not capable of coding for continuous rotation (net fluid motion in semi-circular canals = 0), neither can it differentiate between head and whole body movement.

The vestibular system is involved in postural reflexes, the vestibular-ocular reflex which stabilizes gaze during externally induced head movements, and balance control via vestibulospinal connections (McCrea et al., 1987).
Although the vestibular system and the cochlea are for the most part functionally different entities, some parts of the vestibular system like the saccule and utricle are able to detect sound in the frequency range up to 500 Hz, or even above 1 kHz when considering newest results with phase-locking (Jones et al., 2010; Young et al., 1977; Curthoys et al., 2019). From an evolutionary perspective, the saccule was involved in auditory processing (Fay and Popper, 2000) and has retained its function in different mammalian species (cats: McCue and Guinan, 1994; McCue and Guinan Jr, 1995; guinea pigs: Cazals et al., 1983; Didier and Cazals, 1989; mice: Jones et al., 2010; monkeys: Young et al., 1977).
In humans, excitation of the vestibular system by acoustic stimulation triggers muscle twitches, e.g. in cervical muscles, as part of a vestibular reflex (Bickford et al., 1964; Ferber-Viart et al., 1999). This is exploited when measuring vestibular-evoked myogenic potentials (VEMPs) in the clinical routine. Even though not only LFs but audible frequencies up to 2000 Hz are used in VEMP measurements, the vestibular system and not the auditory system triggers a positional reflex (e.g. for ocular VEMPs (Chihara et al., 2009)). This becomes obvious, when successfully recording VEMPs in patients with hearing loss (Sheykholeslami and Kaga, 2002; Wang and Young, 2003) and absence of VEMPs in patients without intact vestibular system (Murofushi et al., 1996).

1.4 Mammalian Inner Ear Homeostasis

The concept of homeostasis was introduced in the late 19th century by Bernard (1878) and the term was first applied by Cannon (1929). Homeostasis describes the tendency of a physiological system to sustain a stable steady state, even in response to a perturbing stimulus or situation. A classical homeostatic challenge would be that cells have to sustain their intracellular milieu while communicating with the external environment. Homeostasis is often linked to regulatory feedback processes. The complexity of the interplay of various feedback processes is usually overlooked until a failed homeostasis manifests in a pathophysiology (Wangemann and Schacht, 1996). As suggested by Hawkins (1973) the inner ear has various homeostatic mechanisms that are essential to ensure integrity and sensitivity. Normal cochlear and vestibular function requires a homeostatically stable environment regarding volume and composition of cochlear fluids, specifically EL with its exceptional extracellular ion concentration resulting in an endolymphatic potential (Wangemann, 2006; Köppl et al., 2018). Anatomical structures that are involved in cochlear homeostasis are the fluid filled scalae of the organ of Corti and the stria vascularis (SV).

1.4.1 Stria Vascularis and Endocochlear Potential

The stria vascularis (SV) in the outer wall of the scala media is responsible for EL production and for generating the endocochlear potential (EP). The EP is highly positive (between +80mV to +120mV) relative to other extracellular fluids like perilymph and blood plasma (Tasaki and Spyropoulos, 1959; Köppl et al., 2018) and therefore unique within the mammalian organism (Hibino and Kurachi, 2006). Consequently, integrity of the SV is crucial for normal hearing function.

Structure and Function of SV

The SV is a vascularized structure providing the cochlea with ions, nutrients and fluids, but shielding it from blood-born toxic substances (Shi, 2016). Blood supply within the SV is required, as the strial cells are involved in several energy consuming mechanisms, such as ion transports against the gradient. But as those mechanisms are focused within the SV, the cochlea does not need additional blood capillaries adjacent to the sensory cells. Thus, sensory cells and blood supply are spatially separated which has the advantage that blood flow vibrations are not sensed (Wangemann, 2006).

Multiple epithelial layers of the SV enable EL production (Köppl et al., 2018; Nin et al., 2008; Wangemann, 2002b) (see Fig. 1.10). On the outside, strial basal cells touch the fibrocytes of the spiral ligament. Further to the inside, basal cells are electrically coupled to intermediate cells followed by an intrastrial space containing capillaries. The epithelial layer forming a barrier between the intrastrial space and EL filled scala media consists of marginal cells (Jahnke, 1975). Intrastrial space and all small extracellular spaces within SV are filled with intrastrial fluid.

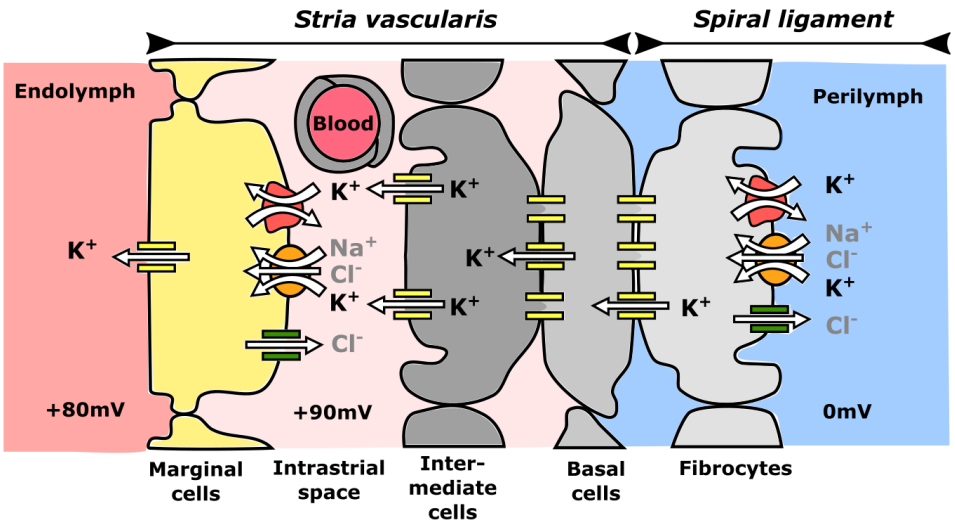

FIGURE 1.10. Structure of the Stria Vasularis. Scheme of the stria vascularis (SV) with several cell layers with ion transporters shown as circles with arrows and gap junctions shown as yellow channels. Endolymph is colored in red, perilymph in blue. *Own work re-drawn from and inspired by Zdebik et al. (2009), Fig.3*

Basal cells of SV are linked by tight junctions. Furthermore, basal cells are connected via gap junctions to spiral fibrocytes on one side and to intermediate cells on the other side (Kikuchi et al., 1995, 2000b; Lautermann et al., 1998; Forge, 1984).
Apart from proteins forming gap and tight junctions (Kikuchi et al., 1995; Kitajiri et al., 2004; Wilcox et al., 2001; Nin et al., 2008), the different cells within the SV express ion channels like K^+-channel and energy dependent ion pumps like Na^+-K^+-ATPase (Kuijpers and Bonting, 1969). The EP essentially depends on active transportation of K^+. K^+-ions are abundant in the EL and carry the transduction current of the HCs.
Within the different layers of SV, [K^+] and K^+-flows are regulated such that strial marginal cells continuously secret K^+ into scala media (Takeuchi et al., 2000; Marcus et al., 2002; Schulte and Adams, 1989; Mizuta et al., 1997; Crouch et al., 1997). Decreased functionality of the SV directly causes a decline in the EP (Gratton et al., 1997).
At the same time, K^+ is steadily removed from EL by several mechanisms predominantly through stereociliary transducer channels of HCs, which show an open probability of around 20-50% in the absence of stimulation (Wangemann, 2002a). After its removal from the EL, e.g. by transduction currents through the HCs, K^+ is recycled via a complicated process, so that it can again be secreted by SV into the EL (Weber et al., 2001; Spicer and Schulte, 1998; Kikuchi et al., 2000a). Additional to the EP, which has to be sustained to ensure MET, the EL volume has to be regulated, in order to maintain the normal activity in the inner ear (Salt, 2001; Takumida et al., 2008). The regulatory mechanism of EL volume is still not fully understood, but the endolymphatic sac (ES) (see section 1.3) probably plays a significant role (Salt, 2001). Anatomy and histology suggest that

the ES takes over functions regarding pressure and volume regulation, ion transport, secretion and uptake of molecules and immune responses (Tomiyama and Harris, 1986, 1987; Altermatt et al., 1990; Salt, 2001).
For volume and composition of the EL not only ion flows but also the right amount of water has to be controlled. Therefore, aquaporins are present in the cochlear walls which mediate osmotic water fluxes across membranes (Mhatre et al., 2002; Li and Verkman, 2001). Aquaporins and sensor proteins in the inner ear resemble those in the collecting duct of the kidney. Consequently, a similar water regulation system was suggested (Beitz et al., 1999).

1.4.2 Disturbance of Homeostasis

The importance of homeostasis becomes obvious when gene mutations of aquaporins and gap junctions, of K^+-channels and Na^+- and Ca^{2+}-transporters cause hearing impairment or deafness in humans and mice (aquaporins: Li and Verkman, 2001; Christensen et al., 2009; K^+-channels: Kubisch et al., 1999). These gene mutations are often also accompanied by volume changes of the inner ear compartments. In mice that lack K^+-channels, the cochlear endolymphatic space collapses (Marcus et al., 2002). But also the opposite scenario can happen: an excessive build-up of EL can affect the auditory and the vestibular structures of the inner ear. A substantial enlargement of EL volume at the expense of the perilymphatic compartments is called endolymphatic hydrops (ELH) (see Fig. 1.11). Within a hydropic cochlea, Reissner's membrane bulges into the scala vestibuli. There are several mechanisms that were suggested to induce an increased water influx in the EL volume, and thus ultimately to ELH: for instance, trauma (Shea et al., 1995), absence of ES (Kimura, 1967; Kimura and Schuknecht, 1965), ES tumor (Butman et al., 2007) and pathologies like Menière's Disease (MD) (see section 1.4.2.2). ELH was also shown to be induced by exposing ears to LF sound at high sound pressure levels (Salt, 2004; Flock and Flock, 2000). An extreme form of such a sound exposure is a blast which contains a wide range of frequencies including LFs. In experiments, blast traumas were also associated with ELH. While blasts often induced destruction of HCs and afferent synapses directly, ELH was hypothesized to be a surrogate for imminent synaptopathy with reduction of the ELH preventing loss of synapses (Kim et al., 2018).

1.4.2.1 Low-Frequency Sound Induced Changes

Low-Frequency Sound

Low frequencies (LFs) usually lie in the range of 20 Hz to 200 Hz, although the concept of LFs does not have precise lower and upper limits. Depending on the literature, upper limits lie at 100, 200 or 250 Hz (Berglund et al., 1996; Leventhall, 2009). 20 Hz is usually seen as the lower limit. However, LFs can be extended to the infrasonic range down to 5 Hz, as "infrasonic" frequencies also become audible at high sound pressure levels (Yeowart et al., 1967; Watanabe and Møller, 1990; Jerger et al., 1966; Moller and Pedersen, 2004; Leventhall, 2007).

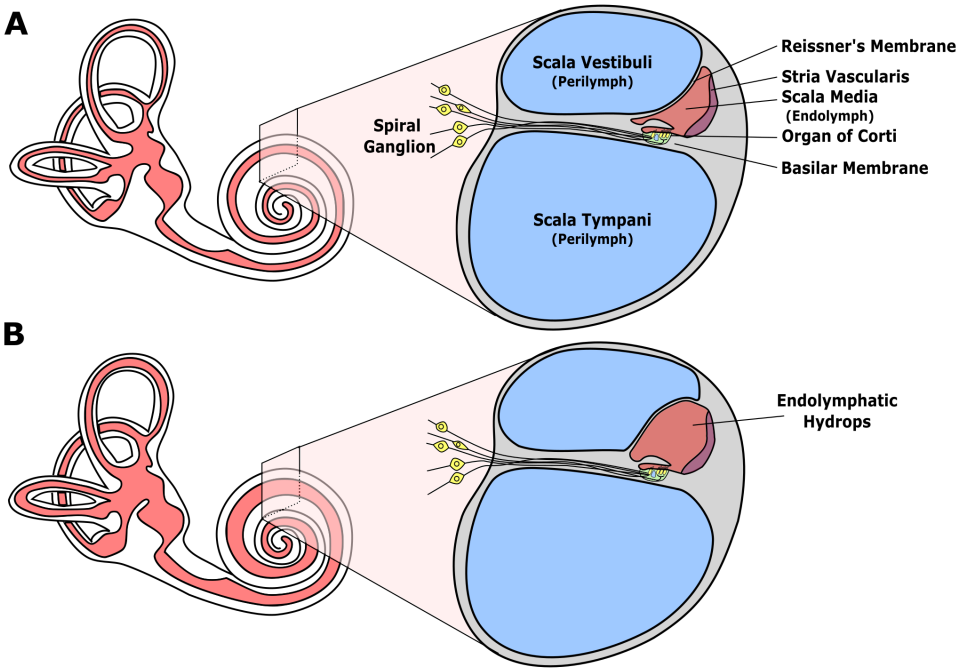

FIGURE 1.11. Endolymphatic Hydrops (ELH). Normal (A) and increased (B) endolymphatic volume indicated in red in the cochlea and in the vestibular endorgans. Cross-section through the cochlea shows increased diameter of scala media. *Own work re-drawn from and inspired by Nakashima et al. (2016), Fig.4, and Zenner (2007), Fig. 16.6*

LF sound is suggested to be detected not only by the hearing system, but also by the vestibular system (see section 1.3), the skin (Pacinian corpuscles) (Sato, 1961), and the whole body (Berglund et al., 1996). However, compared to the ear, frequency ranges and sound pressure levels that can be sensed by all these other systems are very limited and unlikely to be present in environmental sound. Therefore, the ear is seen as the most sensitive organ regarding LF sound.

In auditory research, studies on LF sound and LF processing in mammals are underrepresented due to several reasons. First, humans are perceptually rather insensitive to LF sound, so LFs have long been underestimated with regards to their physiological impact. Second, in mammalian cochleae the LFs are processed at the apex which is a lot harder to access than the base with its direct connection to the middle ear. Third, to control for sound pressure level of LFs, especially at higher intensities, acoustic systems have to be tightly sealed.

However, it turned out that "infrasound" and LF sound have similar or even larger impact on the inner ear physiology than sound of higher frequencies at equal loudness (Drexl et al., 2016). LF sound waves produce large fluid movements within the cochlea (Salt and DeMott, 1999) when traveling from the base to their apical characteristic frequency sites. Thereby, LF sounds can cause pathological changes like rupture of tectorial or Reissner's membrane, HC loss etc. (Lim et al., 1982; Von Gierke and Parker, 1976). Because of the possible impact of LF sound and its

presence in our daily lifes, research on LF processing is highly relevant.

Low-Frequency Sound Induced ELH

Endocochlear potential changes and even development of ELH (see section 1.4) were shown in guinea pigs after intense sound stimulation with frequencies from infrasound up to 1 kHz (Flock and Flock, 2000; Salt, 2004). In vitro, Flock and Flock (2000) directly stimulated the cochleae of guinea pigs with tone bursts of 140 Hz . Under the confocal microscope, they could observe bulging of the Reissner's membrane indicating ELH. Salt (2004) found a 20-40% increase of the cross-section of the endolymphatic compartment by measuring ion marker concentrations within the scala media in vivo.

The Bounce Phenomenon

The bounce phenomenon (BP) is one example for the impact of LF sound on the inner ear homeostasis and cochlear activity.
After presenting an intense, however non-traumatic LF sound, the mammalian cochlea undergoes slow, mostly biphasic oscillations over a time course of 1.5 to 4 min. As oscillations comprise states of hyper- and hypoactivity, the term bounce has been coined. The presented non-traumatic LF sounds eliciting the BP differ in frequency, intensity and duration in several studies.

In humans, transient-evoked (click-evoked) OAEs and DPOAEs (specifically quadratic distortion products) were shown to slowly oscillate with an amplitude increase and a subsequent amplitude decrease after exposure to intense LF sounds (below 1 kHz, 80-105 dB(A)) (Kemp, 1986; Kevanishvili et al., 2006; Kemp and Brill, 2009; Drexl et al., 2014).
SOAEs in humans, typically stable over a lifetime (Burns, 2009), behaved similarly to evoked OAEs with slow, cyclic changes in level and frequency (Kemp, 1986; Kugler et al., 2014; Jeanson et al., 2017). SOAEs increased and decreased their frequency, finally returning to their pre-exposure frequency. Additionally, some SOAEs, dormant SOAEs, just appeared after LF offset, because they temporarily increased in amplitude, thereby exceeding the noise floor and becoming recordable (Kemp, 1986; Kugler et al., 2014).
As OAEs are by-products of amplification by OHCs (see section 1.2.4), slow cyclic changes originate from LF-induced changes in the OHCs.

Even before OAEs had been detected, the first 'two minute bounce' evoked by LF tones (e.g. 500 Hz, 3 min, 120 dB SPL) was found by Hirsh and Ward (1952) who could show that human hearing thresholds fluctuated with a sensitisation period followed by a desensitisation. Further hearing threshold studies also observed sensitisation (Noffsinger and Olsen, 1970) even up to 6-8 dB after 1 min post-exposure (Hughes, 1954). The bimodal nature including a sensitisation contrasted with the typical temporary threshold shift seen after traumatic sound exposure in the

mid- and high-frequency range (Melnick, 1991).
While early studies suggested that the BP with its sensitisation component was probably of neural origin (Hughes, 1954; Hughes and Rosenblith, 1957), Zwicker and Hesse (1984) investigating pure tone threshold during and after LF sound presentation were the first to propose a cochlear origin for the BP. The cochlear origin is still seen as the most probable explanation today.

Another indicator for altered cochlear activity was reported in some of the BP studies. A temporary roaring tinnitus fading out after 2 min was described by many human subjects (Hirsh and Ward, 1952; Patuzzi and Wareing, 2002; Kemp, 1986; Drexl et al., 2014).
The origin for this tinnitus might be an elevated firing rate of the auditory nerve during and after LF sound presentation (Patuzzi, 2002). Hints for this mechanisms were shown by Kirk and colleagues (Kirk and Patuzzi, 1997; Kirk et al., 1997). Kirk and Patuzzi (1997) invasively measured compound action potentials (CAP) of auditory nerve fibers in guinea pigs and found a temporary sensitisation following LF sound exposure.
Furthermore, they showed a decrease in cochlear microphonic amplitudes and an increase of EP with a time course comparable to the BP described in humans. But most importantly, Kirk and Patuzzi (1997) could prove the theory that BP is not of neural origin but cochleogenic. They treated guinea pigs with tetrodotoxin, a Na^+ channel blocker. Although auditory nerve activity and middle ear muscle activity was thereby inhibited, the typical BP changes still appeared after LF sound offset.

1.4.2.2 Menière's Disease

In 1861, the physician Prosper Menière described for the first time an inner ear disorder with symptoms affecting the auditory and the vestibular system (Ménière, 1861). Nowadays, the disorder is known as Menière's disease (MD). It is classically defined by a triad of symptoms: rotatory vertigo, hearing loss and tinnitus. Sometimes aural fullness as an additional symptom is listed to describe MD. In the beginning of the disease, all of these symptoms fluctuate and only appear during recurrent episodes described as attacks. In some cases, attacks are characterized by symptoms appearing in a temporal order with a sense of fullness, increasing tinnitus level and decreasing hearing threshold preceding the vertigo attack. In later stages of MD, HCs degenerate leading to a permanent hearing loss, permanent tinnitus but absence of vertigo attacks (Huppert et al., 2010). At the beginning of the disease, MD is mostly found to occur unilaterally (Kitahara, 1991), but it develops into a binaural disease in later stages in about 30% to above 40% of MD patients (Kitahara, 1991; Yazawa and Kitahara, 1990; Huppert et al., 2010).

Epidemiology and Diagnosis

In epidemiological studies, prevalence of MD differs between 17 and 513 cases per 100,000 (Stahle et al., 1978; Kotimaki et al., 1999; Watanabe et al., 1995; Bruderer et al., 2017; Havia et al., 2005) with incidence of MD of around 4 to 13 cases per 100,000 (Kotimaki et al., 1999; Bruderer et al., 2017). In general, women seem to be affected more often than men (Lopez-Escamez et al., 2015; Bruderer et al., 2017) and age plays a critical role with a peak incidence between 30 and 69 years of age (Van Esch et al., 2016; Lopez-Escamez et al., 2015; Bruderer et al., 2017).

Especially in early stages of the disease, MD is difficult to diagnose and can be confounded with vestibular migraine, stroke, transient ischemic attack and several other diseases (for current standardized criteria see Lopez-Escamez et al., 2015).

Pathogenesis and Etiology

The exact etiology and pathogenesis of MD is still under debate (Oberman et al., 2017). But MD is most likely of multifactorial origin and thought to arise from disturbed ion homeostasis in inner ear fluids (Trune, 2010).

The anatomical correlate most often mentioned and proposed to be the origin of MD is the ELH. ELH was first correlated to MD in temporal bone studies of cadavers in 1938 (Hallpike and Cairns, 1938) and findings were confirmed in several studies (Lindsay, 1944; Day and Lindsay, 1949; Yazawa and Kitahara, 1990; Morita et al., 2009). The ELH mostly occured in the cochlea and the saccule (Morita et al., 2009). In the past, the ELH associated with MD was often classified as idiopathic ELH because no underlying cause could be found to explain the development of the ELH and symptoms of MD (Paparella, 1985). Nowadays, a lot of research focuses on the development of ELH and a large variety of factors were suggested to trigger ELH development (for review see Oberman et al. (2017)), e.g. decreased EL absorption (Paparella, 1983, 1991), genetic abnormalities (Arweiler et al., 1995; Koyama et al., 1993; Paparella, 1985), viral infection (Arnold and Niedermeyer, 1997), autoimmune reactions (Greco et al., 2012), hormonal aspects (Takeda et al., 2000, 2009) etc..

ELH is often suggested to be responsible for cochlear and vestibular dysfunction (Foster and Breeze, 2013; Gürkov and Hornibrook, 2018; Naganawa and Nakashima, 2014). One possible explanation how the ELH could be causative is the theory of Reissner's membrane rupture, which was postulated 50 - 60 years ago (Lawrence and McCabe, 1959; Koskas et al., 1983; Valk et al., 2006). According to this theory, the dilated endolymphatic volume puts pressure onto the Reissner's membrane until the membrane ruptures and a symptomatic attack is triggered. The rupture allows mixture of EL and perilymph with the perilymph becoming richer in K^+-ions. K^+ intoxicates the first-order neurons (Brown et al., 1988). As a result, sensory processing is inhibited and is only restored, when the membrane seals again and pressure equalizes between endolymphatic and perilymphatic compartment. Over the course of the disease, however, the HCs suffer from these recurrent ionic insults and degenerate, which explains why MD "burns out" (Olson and Wolfe, 1981). Doubts of the membrane rupture theory were raised, when Brown et al.

observed nystagmus patterns in MD patients suffering from an attack. These patterns showed the opposite of what would be expected in case of a membrane rupture (Brown et al., 1988).
Furthermore, several studies have not only shown that ELH can be found in healthy subjects or subjects with other disorders like vestibular schwannoma (Naganawa et al., 2011) but also that patients with MD typical symptoms do not always show ELH (Rauch et al., 1989). This leads to the conclusion that ELH might be just a consequence or epiphenomenon (Merchant et al., 2005). ELH is certainly not a sufficient cause of MD (Foster and Breeze, 2013).

Menière's disease in Animals?

The largest hurdle in order to gain a better understanding for MD is the lack of appropriate animal models (Brown et al., 2018). Therefore, most studies are done on patients or postmortem in temporal bones which limit investigations regarding the pathophysiology.
MD has neither been found in animals nor is it inducible covering all typical symptoms like re-occuring attacks. ELH was induced in numerous animal models, for instance in guinea pigs by surgically destroying the endolymphatic sac and obliterating the endolymphatic duct (Kimura, 1967; Kimura and Schuknecht, 1965). Consequences were hearing loss and histopathology similar to that in MD patients. But this procedure has two drawbacks: first, animals do not suffer from any balance disorder after the surgery, therefore MD can not be mimicked accurately. Second, in contrast to a destroyed endolymphatic sac in the animal model, MD patients show an intact endolymphatic sac with increased volume (Bloch and Friis, 2011). Other procedures to induce ELH are injections of artificial EL into the cochlea (Rask-Andersen et al., 1999; Valk et al., 2005) or of distilled water into the middle ear cavity (Kimura, 1982; Akagi et al., 2008), again only showing the ELH without any vestibular attacks (Kimura, 1982). Transient vestibular dysfunction accompanied with a mild to moderate ELH could so far only be shown by a mouse model injected with lipopolysaccharide and aldersterone (Takumida et al., 2008). Instead of inducing ELH mechanically, it is probably more clinically relevant to use immune responses for an adequate animal model with ELH (Tomiyama, 1992).

1.5 Overview - About this Thesis

We have seen that the mammalian auditory and vestibular system are highly sensitive and specialized. At the same time the two systems are prone to disturbances.

Intense LF sound stimulation was suggested to transiently evoke similar symptoms like in MD, for instance tinnitus, fluctuating hearing loss and slowly oscillating OAE levels (Drexl et al., 2014). Therefore, the first study in chapter 2, was a psychophysical study to examine whether tinnitus in MD patients and temporary tinnitus after LF sound stimulation show any resemblance. Healthy participants undergoing the BP and MD patients matched an externally presented stimulus regarding sound intensity and pitch/timbre to their tinnitus. Results between the two study groups were compared.

In the second study (chapter 3), the transient tinnitus of BP was not examined, but used as a measure in order to investigate whether transcutaneous electrical stimulation of the vestibular system has an impact on the auditory periphery. Healthy study participants exposed to electrical stimulation, known as galvanic vestibular stimulation, provided feedback about their perception of a presented pure tone, of BP tinnitus, and of an auditory afterimage. Comparable to the tinnitus matching procedure, participants matched an external stimulus to their percepts during galvanic vestibular stimulation. Additionally, effects of galvanic vestibular stimulation on the auditory periphery were investigated by recording OAEs.

While the first two studies were of psychophysical character and were conducted on healthy human participants and MD patients, the third study was focused on more basic research questions, which were addressed in gerbils. This third study investigated whether a temporarily induced BP eventually manifests in permanent cochlear damage and which factors influence this development. To this end, OAEs were recorded in gerbils exposed to intense LF sound presentations of different durations up to one hour.

All studies were motivated by research questions in the frontier zone between basic and clinically relevant research dealing with inner ear physiology and either artificially biased or pathologically altered homeostasis.

CHAPTER 2

TINNITUS - INDICATOR OF COCHLEAR HOMEOSTASIS IMPAIRMENT

The following content was originally published under the title "Tinnitus in Normal-Hearing Participants after Exposure to Intense Low-Frequency Sound and in Ménière's Disease Patients" in the Journal Frontiers in Neurology by Margarete Anna Ueberfuhr, Lutz Wiegrebe, Eike Krause, Robert Guerkov, Markus Drexl on January 5th 2017 (doi: 10.3389/fneur.2016.00239). Frontiers does not require any formal permission for reprint.

2.1 Author Contribution

The author of this thesis contributed to study design, was responsible for data acquisition, statistical analysis and interpretation of data, as well as drafting, revising and approving the manuscript. The other authors contributed as follows:
Markus Drexl conceptualized and designed the study with Lutz Wiegrebe contributing to the study design. Markus Drexl also interpreted the data and drafted the manuscript. Markus Drexl, Lutz Wiegrebe, Eike Krause and Robert Gürkov provided support during data acquisition. They critically revised the manuscript and approved the final version of the paper.

Tinnitus in Normal-Hearing Participants after Exposure to Intense Low-Frequency Sound and in Ménière's Disease Patients

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Tinnitus is one of the three classical symptoms of Ménière's disease (MD), an inner ear disease that is often accompanied by endolymphatic hydrops. Previous studies indicate that tinnitus in MD patients is dominated by low frequencies, whereas tinnitus in non-hydropic pathologies is typically higher in frequency. Tinnitus of rather low-frequency (LF) quality was also reported to occur for about 90 s in normal-hearing participants after presentation of intense, LF sound (120 dB SPL, 30 Hz, 90 s). LF sound has been demonstrated to also cause temporary endolymphatic hydrops in animal models. Here, we quantify tinnitus in two study groups with chronic (MD patients) and presumably transient endolymphatic hydrops (normal-hearing participants after LF exposure) with a psychophysical procedure. Participants matched their tinnitus either with a pure tone of adjustable frequency and level or with a noise of adjustable spectral shape and level. Sensation levels of matching stimuli were lower for MD patients (mean: 8 dB SL) than for normal-hearing participants (mean: 15 dB SL). Transient tinnitus after LF-exposure occurred in all normal-hearing participants (N = 28). About half of the normal-hearing participants matched noise to their tinnitus, the other half chose a pure tone with frequencies below 2 kHz. MD patients matched their tinnitus with either high-frequency pure tones, mainly above 3 kHz, or with a noise. Despite a significant proportion of MD patients matching low-pass (roaring) noises to their tinnitus, the range of matched stimuli was more heterogeneous than previous data suggested. We propose that in those participants with noise-like tinnitus, the percept is probably generated by increased spontaneous activity of auditory nerve fibers with a broad range of characteristic frequencies, due to an impaired ion balance in the cochlea. For tonal tinnitus, additional mechanisms are conceivable: focal hair cell loss can result in decreased auditory nerve firing and a central auditory overcompensation. Also, normal-hearing participants after LF-exposure experience alterations in spontaneous otoacoustic emissions, which may contribute to a transient tonal tinnitus.

Keywords: tinnitus, low-frequency, Ménière's disease, bounce phenomenon, endolymphatic hydrops

Abbreviations: BP, bounce phenomenon; DPOAE, distortion product otoacoustic emission; ICC, intra-class correlation coefficients; IHCs, inner hair cells; LF, low-frequency; MD, Ménière's disease; NH, normal-hearing; OAE, otoacoustic emission; OHCs, outer hair cells; SOAE, spontaneous otoacoustic emission.

INTRODUCTION

Tinnitus is defined as the perception of sound in the absence of external acoustic stimulation and can take various forms from pure tones to more atonal percepts (1, 2).

Tinnitus can be distinguished into two main classes: objective tinnitus and subjective tinnitus. Objective tinnitus is caused by sounds originating from internal sources, e.g., from the patient's inner ear such as prominent spontaneous otoacoustic emissions (SOAEs) (3, 4). Subjective tinnitus is characterized by an abnormal spontaneous activity within the auditory periphery or the central auditory pathway in the absence of any acoustic stimulation, which is interpreted by the brain as sound (5, 6). A psychophysical characterization of tinnitus serves to quantify loudness and pitch/timbre, which can, in the second step, contribute to the understanding of the underlying pathology (7). For the sake of simplicity, in the following, the term pitch will be applied to pure tones and noises, although the term pitch refers to pure tones only and the equivalent for noises would be timbre. Pitch and loudness of a tinnitus sensation are typically characterized by matching a synthesized sound to the tinnitus percept [see Ref. (8, 9) for an overview]. Pitch matches of tinnitus patients are usually in the rather high-frequency region above 3 kHz and only rarely below 1 kHz (10).

However, patients with Ménière's disease (MD) seem to be an exception regarding tinnitus pitch. MD is classically characterized by a triad of symptoms: fluctuating hearing loss, episodes of vertigo, and tinnitus (11). In early stages of the disease, tinnitus seems to be related to episodes of vertigo, being more intense before and during attacks. With progression of the disease, which is typically associated with increasing hearing loss and ceasing of vertigo, tinnitus stays and its intensity increases (12–14). Descriptions of tinnitus in MD patients range from roaring over buzzing to ringing (13, 15); except for few MD patients presumably in later stages of the disease, who describe their tinnitus as tonal and containing high-pitched components (12, 13). Overall, studies concluded that tinnitus in the majority of MD patients was confined to lower-frequency ranges below 1 kHz and in particular between 125 and 250 Hz (16–21).

An excess of endolymph volume, termed endolymphatic hydrops, is often suggested to cause MD and specifically symptoms such as tinnitus. A transient endolymphatic hydrops was experimentally induced in rodents by an intense low-frequency (LF) sound (22).

In human participants, transient endolymphatic hydrops induced by an intense LF sound has not been shown directly. However, a post-LF-exposure phenomenon called "Bounce Phenomenon" (BP), lasting only a few minutes, was found in humans. The BP includes a transient tinnitus percept, fluctuating hearing thresholds with transient improvement and subsequent worsening (23–26), and biphasic amplitude level changes in otoacoustic emissions (OAEs) (27–31). As OAEs are sounds emitted by the cochlea due to active amplification processes by outer hair cells (OHCs) (32), the origin of the BP is thought to be cochlear (33). Increased endocochlear potentials in the cochlea have been recorded after presentation of LF sound (22) and were suggested to lead to an increase in the spontaneous firing rate of auditory nerve fibers, resulting in a "rate tinnitus" (34), presumably in the absence of any structural cochlear impairment.

In BP studies, human participants reported a transient "roaring" tinnitus immediately after LF sound exposure (23, 26, 27). The time course and relative loudness of the transient tinnitus percept were characterized with psychophysical studies (26, 30), but tinnitus pitch has not been systematically described with psychophysical measures yet. This study employed a tinnitus-matching procedure to quantify and compare tinnitus percepts in MD patients and normal-hearing (NH) participants after exposure to intense LF sound. Both study groups presumably presented with impaired cochlear ion balance that might manifest in endolymphatic hydrops in MD patients and shows indirectly as BP after LF sound in NH participants.

MATERIALS AND METHODS

Subjects

The study population consisted of two groups: a group of NH participants and a group of patients with MD. In the following, members of both groups will be referred to as participants. NH participants comprised 18 females and 10 males (age range 20–29, mean age 23.7) with no reported hearing problems, no ear surgery, no recent ear infections, and no tinnitus. All NH participants had hearing thresholds of less than 25 dB HL between 0.25 and 8 kHz, tested with a Matlab-based automated procedure [Automatic Pure Tone Audiometry APTA-HF 2012 V2.28 (HörTech, Oldenburg, Germany)]. One ear was pseudo-randomly chosen (14 left ears, 14 right ears) and exposed to a LF sound. The transient tinnitus was lateralized and perceived on the exposed side only.

The group of MD patients consisted of nine females and nine males (age range 26–74, mean age 53.1) diagnosed with definite MD according to the criteria recently formulated by the Bárány Society joint with several national and international organizations (14). Additionally, endolymphatic hydrops had been detected with magnetic resonance imaging after intra-tympanic gadolinium injection (35) before study participation in 15 of 18 patients.

Ménière's disease patients were included when they were unilaterally affected only (10 left ears, 8 right ears) and when they reported tinnitus localized to the affected ear.

Patients with middle ear disorders, pathologies of the auditory nerve or recent ear infections were excluded from the study. Patients with obvious noise-related hearing damage (i.e., notched audiograms) as well as patients with a history of noise exposure were not eligible either. All MD patients were required to be in a stage of the disease with fluctuating symptoms, with at least one vertigo attack during the 6 months preceding the experiment. In the contralateral, non-affected ear patients were required to have hearing thresholds better than 40 dB HL below 2 kHz, and better than 70 dB HL at higher frequencies.

This study was approved by the ethics committee of the University Hospital of the Ludwig-Maximilians-Universität Munich, Germany, in agreement with the Code of Ethics of the World Medical Association for experiments involving humans

(Declaration of Helsinki) and all participants (MD and NH) gave their written informed consent.

Experiments with NH participants were conducted in a double-walled, sound-attenuated booth at the Department of Biology, Ludwig-Maximilians-Universität Munich, Martinsried. Experiments with MD patients were carried out in a sound-attenuated booth at the ENT Department at the University Hospital of the Ludwig-Maximilians-Universität Munich, Germany.

Signal Generation and Data Acquisition

Signal generation and data acquisition was implemented with scripts written in MATLAB 7.5 (MathWorks, Natick, MA, USA). Sound generation and acquisition was done with an RME Fireface UC 24-bit external sound card (RME, Audio AG, Haimhausen, Germany). The sampling rate was 44.1 kHz. For sound stimulation, SoundMexPro (HörTech GmbH, Oldenburg, Germany) was employed, which enables low-latency multi-channel Audio Stream Input/Output and interactive changes of stimuli properties within the MATLAB environment.

SOAEs Recording

In NH participants, SOAEs were recorded with the ER-10C distortion product otoacoustic emission (DPOAE) probe microphone (Etymotic Research Inc., Elk Grove Village, IL, USA). The recorded signal was amplified 30 dB by the preamplifier of the external sound card. Level and frequency of SOAEs were recorded in the control trial for 120 s (measured in 21 of 28 NH participants) and after LF stimulation for 240 s (measured in 15 of 28 NH participants). In an artificial ear (B&K 4157, Brüel & Kjaer Sound and Vibration Measurement A/S, Denmark), no artifacts exceeding the noise floor of the system could be detected during recording. A probe-fit-check procedure preceded and concluded each measurement by presenting a band-stop noise consisting of a low- and a high-frequency band and analyzing the ear response using a Fourier transform analysis. If the probe-fit-check procedure at the end of a trial indicated that the probe position had changed, the trial was rejected and repeated.

Tinnitus-Matching Procedure

In experiments with MD patients, the output of the sound card was sent to HDA 200 headphones (Sennheiser, Wedemark-Wennebostel, Germany). In NH participants, two different sound systems were used for the two ears: an ER4 insert ear phone (Etymotic Research Inc., Elk Grove Village, IL, USA) was used to present the matching stimuli to one ear. An ER-10C DPOAE probe system with an additional tube coupled to an external transducer was used to present LF sounds (30 Hz sine wave, 120 dB SPL, 90 s, including 0.1 s raised-cosine ramps) to the other ear in order to elicit a tinnitus sensation.

The external transducer was a small broadband unit (NSW1-205-8A, Aura Sound Inc., Santa Fe Springs, CA, USA) driven by a RB-960BX power amplifier (Rotel, Worthing, UK). This transducer was connected to a 50-cm long polyethylene tube (inner diameter 1 mm), the tip of which was fed through the foam ear tip of the ER-10C DPOAE probe. The ER-10C includes a microphone for recording sound pressure in the ear canal enabling the examiners to calibrate the LF sound. The amplitude response of the DPOAE probe microphone was compared with the amplitude response in an artificial ear (B&K 4157, Brüel & Kjaer Sound and Vibration Measurement A/S, Denmark) and corrected for deviations. The level of the first harmonic of the LF sound was at least 50 dB lower than the level of the desired LF frequency.

Participants (NH and MD) were given standardized, written and illustrated instructions for the tinnitus-matching procedure. In NH participants, each trial was started with LF-sound exposure to one ear, thereby inducing a transient tinnitus percept in the exposed ear (see **Figure 1A**). Since the transient tinnitus lasted only for about 90 s after LF exposure (30), NH participants were allowed to restart the LF stimulus playback if necessary. As tinnitus was a unilateral symptom in both groups, matching stimuli were presented to the contralateral, unaffected ear (see **Figures 1A,B**). Participants were able to interactively adjust matching stimuli regarding loudness and pitch with a gamepad (Bigben Interactive GmbH, Bergheim, Germany). Matching stimuli were continuous and generated in real time by a SoundMexPro plug-in.

The matching procedure was split into two stages. The first stage served to determine whether the participants' tinnitus was matched best either with a pure tone or with a noise. Therefore, 10 different synthesized stimuli were presented. The stimuli comprised pure tones of five different frequencies, equally spaced on a logarithmic frequency axis between 0.25 and 8 kHz (0.25, 0.595, 1.414, 3.364, and 8.0 kHz) and five noises derived from Gaussian noise with different filter slopes (see **Figure 1C**). In the following, those slopes are referred to as spectral tilts with units of decibel per octave. They ranged from negative values with dominant LF components (−12 and −6 dB/octave) over white Gaussian noise (0 dB/octave) to positive values with dominant high-frequency components (+6 and +12 dB/octave). Stimuli were filtered such that linear phase and frequency distortions of the transducers (NH participants: ER4, Etymotic Research; MD patients: HDA 200, Sennheiser) were exactly compensated for. Participants started the playback of the individual stimuli successively by choosing from a graphical representation of the stimuli on a user interface. No information regarding the physical properties of the stimuli was available from the graphical representation. The 10 stimuli were identical but reordered on the user interface for each trial.

After having listened to all stimuli, participants were asked to select the stimulus best matching their tinnitus. After stimulus selection, participants had to adjust the stimulus level, such that the selected tone matched their tinnitus as well as possible. Selecting 1 out of the 10 stimuli and adjusting its loudness was counted as one trial in the first stage of the matching procedure. In NH participants, trials were repeated until the same stimulus class was selected in two successive trials. In MD patients, due to time constraints, only two trials were carried out regardless of whether participants selected the same stimulus or different stimuli in those two trials.

In the second stage, participants carried out fine adjustments of level and pitch of either a pure tone or a noise. Matching stimuli were presented with a starting frequency or starting spectral tilt corresponding to the selected stimulus of the first stage, respectively. In MD patients selecting two different stimuli in the first stage, the matching stimulus was chosen based on the patient's statement of which of the two selected stimuli was the better fit.

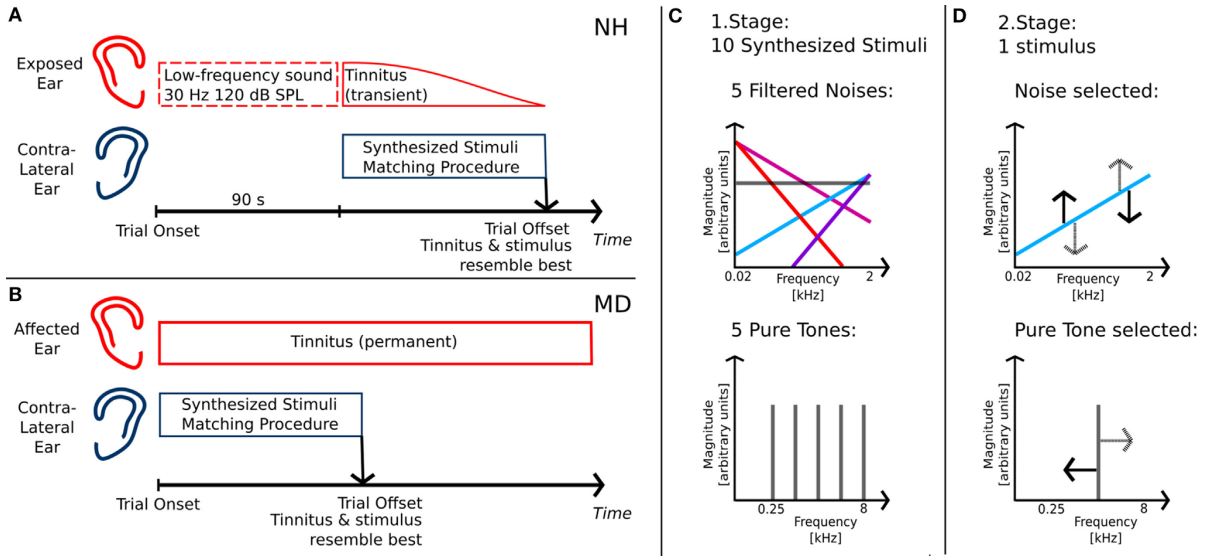

FIGURE 1 | **Tinnitus-matching procedure**. **(A)** Matching procedure for normal-hearing participants with intense low-frequency sound exposure prior to matching; **(B)** matching procedure for Ménière's disease patients with unilateral disease; **(C)** first stage of matching procedure: determining stimulus class of tinnitus percept; **(D)** second stage of matching procedure: adjusting stimulus regarding pitch/timbre and loudness of tinnitus.

The starting sound level was the mean sound level derived from the last two trials of the first stage.

During presentation of the matching stimulus, participants could continuously adjust loudness and frequency or spectral tilt (see **Figure 1D**). When participants found an adjustment for the matching stimulus that resembled their tinnitus, they stopped the adjustment procedure. Subsequently, the adjusted matching stimulus was again presented continuously and participants were required to adjust its sound level such that it was just audible. Similar to a Békésy tracking procedure, participants decreased the level of their adjusted matching stimulus until they did not perceive it anymore and then increased its intensity until they heard the stimulus again. This was done to estimate the sensation level of the matching stimulus. Adjusting the matching stimulus pitch and level and tracking the corresponding hearing threshold was considered as one trial in the second stage of the matching procedure.

In this second matching stage, NH participants always ran five trials and MD patients two to five trials depending on how many they were capable of doing due to time constraints and cognitive load.

After each trial, NH participants and MD patients were asked to describe their transient tinnitus in their own words. In NH participants, measurements were taken on two to four different days and lasted between 25 and 60 min each. In MD patients, testing was embedded in their clinical routine and carried out on 1 or 2 days. Measurements lasted 40–60 min.

All data analysis and statistics were carried out with scripts written in MATLAB 7.5 (MathWorks, Natick, MA, USA). Visualizations were done either with MATLAB 7.5 or Inkscape 0.91 (The Inkscape Team, http://www.inkscape.org).

RESULTS

Estimates of Level and Pitch of Tinnitus in NH Participants after LF Sound Exposure

All 28 NH participants exposed to the LF sound experienced a transient tinnitus percept and were able to match its pitch and level with a matching stimulus presented to the contralateral ear. Matching stimuli were adjusted after offset of the LF exposure, on average within 68.7 ± 32.5 s (mean ± SD, $N = 28$). This suggests that most NH participants concluded the matching while still hearing the transient tinnitus, which lasts about 90 s on average (30).

Conclusions drawn from questioning the NH participants after the software-based adjustment procedure are summarized as follows: 7 of the 28 NH participants described their tinnitus as tonal, 8 NH participants as a noise. The remaining 13 NH participants reported a hybrid percept consisting of noise and one or more tones. During the matching procedure, 8 out of those 13 NH participants experiencing a hybrid percept selected a matching tone and only 5 selected a matching noise. Altogether, 15 NH participants selected and adjusted a tone and 13 NH participants selected a noise (**Figure 2A**). Subjective descriptions of tinnitus perceived by NH participants after LF exposure are summarized in **Table 1**.

The loudness of the transient tinnitus percept was reported to be faint up to clearly audible with the tinnitus starting to fade out after a minute. Some participants also described qualitative changes in their tinnitus percept over time. Most of those participants reported a hybrid percept of pure tone(s) and noise. In those cases, the relative contribution of noise and tones to the tinnitus percept shifted over time. The tonal components seemed

to fade out over time, while the noise serving as a background noise at the beginning of the BP got more prominent at later time points. Participants did not complain about the percept and its loudness. At most participants stated the tinnitus to be irritating, especially in combination with a feeling of aural fullness.

Tonal Tinnitus Percepts in NH Participants after LF Exposure

Fifteen NH participants selected pure tones to characterize their tonal tinnitus. The pure tone frequency was determined by averaging all adjustments per subject. The selected matching tone frequencies (see **Figures 3A,B**) show an accumulation between 0.1 and 2 kHz, where 14 out of 15 participants selected matching tones below 2 kHz.

The mean frequency of the 15 averaged pure tones chosen was 0.96 ± 0.89 kHz (mean ± SD, $N = 15$). As the SD across adjustments in hertz is only of limited value, frequency deviations were also expressed logarithmically as fraction of an octave in cent, where 100 cents equal a semitone, so that 1,200 cents correspond to an octave.

In 13 of 15 NH participants, SDs for single participants comprised less than 1,200 cents, and for some participants, the SD was as low as 30 cents. The inter-subject mean of the sound pressure level of the adjusted matching tone was around 49.5 ± 15.9 dB SPL (mean ± SD, $N = 15$).

Sound levels for matching tones decreased with increasing frequency from 90 dB SPL at 70 Hz to 30–35 dB SPL at 2–4 kHz. Matched pure tone levels followed equal-loudness contours for human hearing within a loudness level range of 25–60 phons (see **Figure 3A**). Thus, for NH participants, matching tones at lower frequencies were not generally perceived louder than at higher frequencies, despite the difference in sound pressure levels. For an interpretation of how loud participants (NH and MD) perceived their tinnitus, sensation levels were calculated (see **Figure 4A**). The sensation level is the difference (in decibel) between the presented sound level and the absolute hearing threshold for the same sound. The mean sensation level for tonal tinnitus matches of NH participants was 15.2 ± 6.7 dB SL (mean ± SD, $N = 15$).

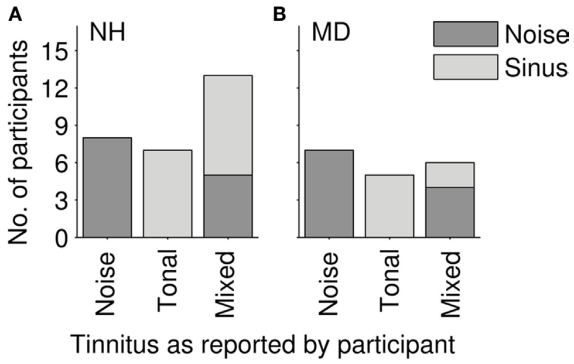

FIGURE 2 | Distribution of reported tinnitus quality. (A) Normal-hearing participants ($N = 28$). **(B)** Ménière's disease patients ($N = 18$); gray tones code for the stimulus class that participants selected in the tinnitus-matching procedure (light gray—pure tone; dark gray—noise).

TABLE 1 | Qualitative description of transient tinnitus after intense low-frequency sound exposure (30 Hz, 120 dB SPL, 90 s) by normal-hearing participants ($N = 28$).

	N
Noise-like tinnitus	**8**
Noise—exactly like matching noise	5
Low-Pitched Noise (Roaring like a fan)	2
Noise but tonal character	1
Tonal tinnitus	**7**
Pure Tone—exactly like matching tone	2
Two Tones—alternating/simultaneously (1 tone faint + 1 tone more intense) (Ringing like church bell, Chinese meditation balls)	5
Hybrid tinnitus percept	**13**
Low-Pitched Noise (Roaring) + tone	5
High-Frequency Tone(s) + machine-like sound (Rattling, jackhammer, sewing machine)	5
Other: noise with low-frequency (LF) modulation LF tone with tinny ring Sound of pressure valve	3

Noise-Like Tinnitus Percepts in NH Participants after LF Exposure

Thirteen NH participants chose noises to match their LF-induced transient tinnitus. Adjusted spectral tilts showed a bimodal distribution slightly shifted toward negative tilts with an average adjusted spectral tilt of −1.8 ± 7.9 dB/octave (mean ± SD, $N = 13$) (see **Figures 5A,B**). Except for one participant choosing white noise (mean tilt: ~0 dB/octave) to match the tinnitus percept, all NH participants selected either noises with a clearly positive tilt (mean ± SD = 6.7 ± 3.3 dB/octave, $n = 5$) or with a clearly negative tilt (mean ± SD = −8.1 ± 3.1 dB/octave, $n = 7$).

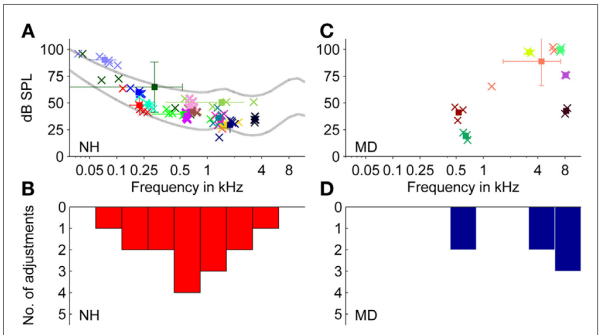

FIGURE 3 | Adjustments of level and frequency of externally presented pure tones to match a tonal tinnitus percept. (A,B) Normal-hearing participants ($N = 15$). **(C,D)** Ménière's disease patients ($N = 7$). **(A,C)** Plots show adjusted pure tones of individual participants. Data from individual participants are represented by different colors. Square symbols represent the mean of the two to five individual adjustments of one participant (indicated by cross symbols). Error bars represent the SD regarding sound pressure level (dB SPL) (vertically) and frequency (Hz) (horizontally). Lines in **(A)** represent equal-loudness contours for loudness levels at 25 and 60 phons. **(B,D)** Distribution of the selected matching tone frequencies of data shown in **(A,C)**, respectively (bin width = 1,249 cents).

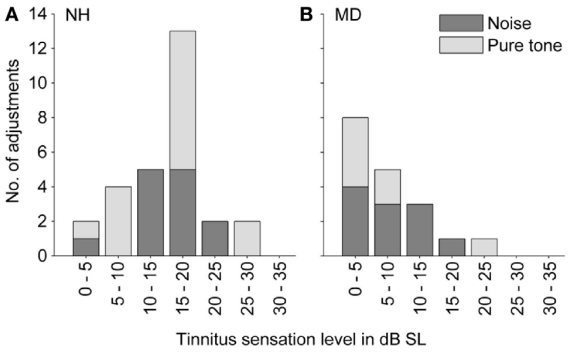

FIGURE 4 | Distribution of tinnitus loudness (expressed as sensation level). (A) Normal-hearing participants ($N = 28$). **(B)** Ménière's disease patients ($N = 18$). Colors code for the stimulus class that participants selected in the tinnitus-matching procedure (light gray – pure tone; dark gray – noise).

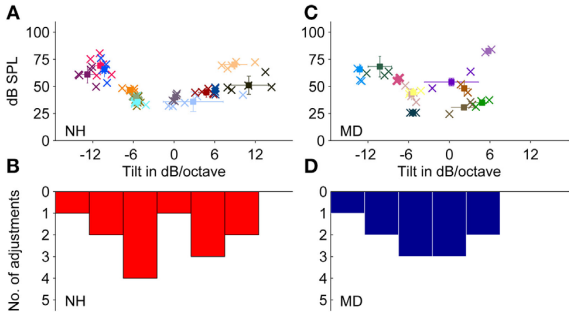

FIGURE 5 | Adjustments of level and frequency content of externally presented noises to match a noise-like tinnitus percept. (A,B) Normal-hearing participants ($N = 13$). **(C,D)** Ménière's disease patients ($N = 11$). **(A,C)** Plots show adjusted noises of individual participants. Error bars represent the SD regarding sound pressure level (dB SPL) (vertically) and frequency content (dB/octave) (horizontally). **(B,D)** Distribution of the matching noise tilts of the data shown in **(A,C)**, respectively, within the range of −17.5 to +17.5 dB/octave (bin width = 5 dB/octave). Color coding and symbols as in **Figure 3**.

Within-participant variability between adjustments was quite small (SD between 0.02 and 4.4 dB/octave).

Sound levels of the matching noises were within the range of 36–70 dB SPL with a mean of around 49.5 ± 12.9 dB SPL (mean ± SD, $N = 13$). Matching noises with highest sound pressure levels were noises with the steepest spectral tilts, either negative or positive. The mean sensation levels of matching noises were 15.3 ± 5.6 dB SL (mean ± SD, $N = 13$).

Sensation levels of both selected matching tones and matching noises in NH participants were summarized (see **Figure 4A**), and the mean was calculated as 15.2 ± 6.1 dB SL (mean ± SD, $N = 28$).

Estimates of Level and Pitch of Tinnitus in MD Patients

In all 18 MD patients, the tinnitus was a permanent sensation, sometimes varying in loudness over time. In eight patients, the loudness of the tinnitus was apparently correlated to the vertigo attacks increasing right before and/or during an attack. Three patients reported not only a loudness change correlated to the attacks but also an increase of tinnitus pitch. In this case, patients were asked to match their current tinnitus percept. Seven patients reported their tinnitus to be a noise, and five patients reported it to be a pure tone. The other six patients reported to hear a hybrid percept of pure tone(s) and noise. In the tinnitus-matching procedure, two of those patients perceiving a hybrid tinnitus matched their tinnitus with a pure tone and four of them with a noise (see **Figure 2B**). Qualitative tinnitus descriptions by the patients are summarized in **Table 2**. Descriptions included low-pitched noise, modulated noise, and high-frequency whistling.

Tonal Tinnitus in MD Patients

Seven MD patients selected pure tones in the matching procedure. Thereby, data did not show the previously suggested correlation that older patients or patients in later stages of the disease were more likely to experience tonal tinnitus of high-frequency quality instead of noise-like tinnitus (linear correlation coefficients, age: $r = -0.12$, p-value = 0.64, stage of disease $r = 0.08$, p-value = 0.76).

TABLE 2 | Qualitative description of tinnitus by Ménière's disease patients ($N = 18$) with unilateral disease and one-sided, permanent tinnitus.

	N
Noise-like tinnitus	**7**
Low-pitched noise (roaring, buzzing)	4
Noise with modulation/pulsatile (rushing blood)	2
Other: humming	1
Tonal tinnitus	**5**
Pure Tone—exactly like matching tone	2
High-frequency whistling, swishing	3
Hybrid tinnitus percept	**6**
High-frequency tone(s) + *low-pitched noise* (angle sander, whistling, and dull roaring)	4
Other: rushing blood	2
Swarm of bees	

Selected matching tone frequencies showed a bimodal distribution with peaks around 500 Hz and around 5 kHz (see **Figures 3C,D**). The mean frequency was 4.6 ± 3.3 kHz (mean ± SD, $N = 13$).

Matched sound levels increased with increasing frequency from 20 dB SPL at 0.6 kHz to 80–100 dB SPL at 7–8 kHz. Sensation levels of matching tones, however, were small (mean ± SD = 7.0 ± 7.8 dB SL, $N = 7$).

Noise-Like Tinnitus Percepts in MD Patients

Eleven of 18 MD patients selected noises to describe their tinnitus percept. The matching noise properties were averaged across the two to three trials for each patient.

Across patients, the average spectral tilt of matched noises was −2.9 ± 6.3 dB/octave (mean ± SD, $N = 11$). Matching noises showed adjusted tilts between −13 and +4 dB/octave and resulted

in an almost normal distribution with a shift toward negative tilts (see **Figures 5C,D**).

On average, the noise level adjusted by MD patients was 50.4 ± 17.3 dB SPL (mean ± SD, $N = 11$), which was comparable to noise levels of noises matched by NH participants with transient tinnitus. Due to hearing loss in the majority of MD patients, the sensation levels of the matching tones were with 8.5 ± 4.9 dB SL (mean ± SD, $N = 11$) significantly lower than in NH participants (Mann–Whitney test, p-value = 0.0002).

Sensation levels of both matched tones and matched noises in MD patients were summarized (see **Figure 4B**), and the mean was 7.9 ± 6.0 dB SL (mean ± SD, $N = 18$).

Comparison of Tinnitus Percepts in MD Patients and in NH Participants after LF-Sound Exposure

Tonal Tinnitus Percepts

Pure tones matched by MD patients and matched by NH participants differed in their distribution (see **Figure 3**) (two-sample Kolmogorov–Smirnov test, p-value = 0.019). While the majority of MD patients chose pure tones above 3 kHz, which is comparable to most tinnitus percepts of tinnitus patients (10, 16), the majority of NH participants selected pure tones below 2 kHz.

Noise-Like Tinnitus Percepts

Comparing the two subgroups of MD patients selecting noises and NH participants with BP-induced tinnitus selecting noises did not show pronounced differences. Although the distribution appeared bimodal for NH participants and rather normally distributed in MD patients, the null hypothesis stating that the two subgroups were from equal distributions could not be rejected (two-sample Kolmogorov–Smirnov test; p-value = 0.86). Both subgroups had a tendency to select noises with a negative spectral tilt, which corresponds to previous reports from the literature (16–18, 23, 30).

Test–Retest Reliability

In general, the pitch and loudness matches obtained with the current two-stage procedure showed good test–retest reliability. Intra-class correlation coefficients (ICC) were calculated for the five trials of fine matching run in one to three different sessions in NH participants (pitch matching: ICC = 0.92; loudness matching: ICC = 0.85). In MD patients, data were usually collected in one session only, but for the two to three different adjustments ICC indicated very strong test–retest reliability, too (pitch matching: ICC = 0.93; loudness matching: ICC = 0.92).

SOAE Measurements in NH Participants

In 15 of the 28 NH participants, SOAEs were measured in the same ear that was exposed to the LF sound. The first measurement was run before LF exposure to find SOAEs. The second SOAE measurement was carried out immediately after LF exposure during the BP, while NH participants perceived the corresponding transient tinnitus. During the BP, "new" SOAEs could develop and existing SOAEs increased in level. Occasionally, those SOAEs exceeded the hearing threshold determined for the same ear by audiometric testing at the beginning of the experiments. In 10 NH participants, SOAEs could be found. In all of those participants, SOAEs were slightly altered when recorded during the BP compared to baseline before LF exposure. In eight NH participants, SOAE levels exceeded the individual hearing threshold at the SOAE characteristic frequency during the BP. From these eight participants, five matched their tinnitus with a pure tone, three with a noise. **Figure 6** shows SOAEs, hearing threshold and selected tinnitus-matching tones from three participants with "audible" SOAEs. These data suggest that some NH participants might possibly have matched their tinnitus to a transiently audible SOAE.

DISCUSSION

The current study, by applying a comprehensive tinnitus-matching procedure, compared tinnitus in two groups with

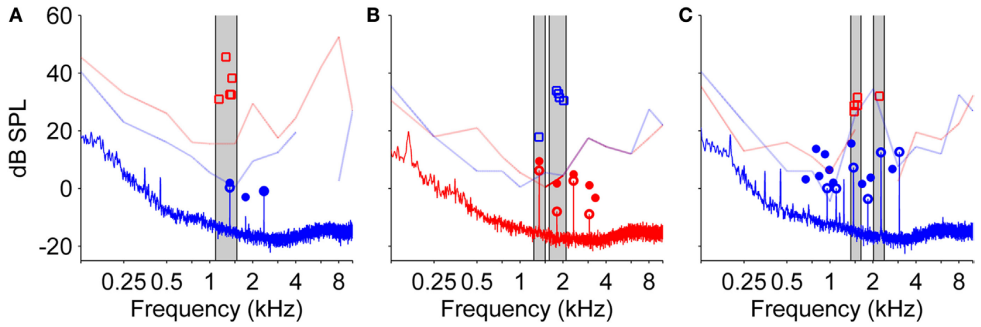

FIGURE 6 | Audiograms from both ears, spontaneous otoacoustic emissions (SOAEs), and level and frequency estimates of the tinnitus percept after low-frequency (LF) sound exposure for three different normal-hearing participants (A–C). SOAEs were recorded in ipsilateral ears before and immediately after exposure to a 30 Hz tone at 120 dB SPL for 90 s. In all three measures, red color refers to the right ear, blue color refers to the left ear. Light lines indicate audiogram thresholds (gaps within light lines are due to lack of valid data points of audiometric testing), open squares represent five adjustments of level and frequency of the matching tone presented to the contralateral ear. Solid lines represent the spectrum of the SOAE recording. Open circles represent SOAEs before LF sound exposure, filled circles represent SOAEs after LF sound exposure. Gray patches highlight SOAEs and corresponding matching tone adjustments.

chronic (MD patients) and transiently (NH participants after LF exposure) challenged cochlear homeostasis presumably resulting in hydropic conditions.

Our results show that sensation levels of tinnitus percepts were on average between 8 dB SL (MD patients) and 15 dB SL (NH participants after LF sound). Tinnitus pitch varied substantially both within and across MD patients and NH participants. While MD patients matched their tinnitus mainly with either high-pitched pure tones or noises with a tendency to low-pitched (roaring) noises, NH participants perceiving a transient tinnitus after LF-exposure matched that tinnitus with high- or low-pitched noises or low-pitched pure tones. In the few studies available in the literature, MD patients were found to perceive mainly LF tinnitus (16–21), albeit reports on MD patients with high-frequency tinnitus exist (12, 13, 36). Tinnitus percepts of MD patients in the current study were more diverse, and this is probably due to the following reasons: first, the matching procedures employed in the available literature differed from our advanced matching paradigm, in that participants could adjust matching stimuli on their own without any interference or bias caused by the examiner. Furthermore, participants could choose between two matching stimulus classes (pure tones and noises). Second, participating MD patients were heterogeneous regarding age (26–74 years). As the onset of MD symptoms generally occurs around 30–50 years (37, 38), younger MD patients without age-related hearing loss are underrepresented in our patient population. Nevertheless, contralateral, unaffected ears of all participating patients were within the normal hearing range when considering the age-corrected pure-tone average at 2, 3, and 4 kHz. This is important as the matching stimulus was delivered to the unaffected ear. All patients, despite their age, were in a stage of the disease with fluctuating symptoms.

In the following, we will dissect tinnitus percepts into tonal and noise-like tinnitus and discuss tinnitus loudness for both kinds of percepts in the end. Underlying mechanisms possibly generating the tinnitus percepts in both MD patients and NH participants after LF exposure will be proposed for tonal and noise-like tinnitus separately.

Tonal Tinnitus Generation

MD patients mainly chose high-frequency matching tones when their tinnitus was predominantly tonal. This is comparable to the tinnitus quality typically related to inner hair cell (IHC) and OHC damage (39). Here, it was suggested that the tinnitus frequency roughly corresponds to characteristic frequency of the impaired hair cells or to the frequency at the boundary between intact and impaired hair cells (40, 41). This tinnitus mechanism might generate the tonal tinnitus percepts MD patients perceive. Audiograms of MD patients often show LF hearing loss but high-frequency hearing loss also occurs, mostly in later stages of the disease (12, 13). The majority of MD patients in this study showed greatly reduced DPOAE levels as judged from measurements acquired during the clinical routine. This indicates some degree of OHC function impairment, but it is unclear if this is age-related or due to the presence of endolymphatic hydrops. OHC loss might not suffice to induce tinnitus. Therefore, damage to IHCs or the auditory nerve, which cannot be detected with DPOAE measurements, might also be required. Given the presence of both IHC and OHC dysfunction, tonal tinnitus could also be the result of endolymphatic hydrops (or its underlying pathology) combined with the pre-existing hair cell dysfunction.

Tonal tinnitus in NH participants after LF exposure showing frequencies below 2 kHz is presumably triggered by transiently challenged cochlear homeostasis. Intense, LF sound causes broad excitation patterns peaking at the apical part of the cochlea. Thus, effects on cochlear homeostasis are not restricted to the characteristic frequency region of LF sound and higher-frequency regions can be affected as well.

Temporary tinnitus in humans is generally thought to be caused by temporary decrease of OHC amplification, which results in increased firing in the central auditory system and tinnitus generation (42). Although this generation mechanism cannot be ruled out, especially for participants showing temporary worsening of hearing threshold after LF exposure, it seems to be unlikely because of concurrent, increasing OAE levels typically following LF exposure due to enhanced OHC amplification (30). Temporary enhanced amplification and increased OAE levels might contribute to a different generation mechanism.

In three participants, it could be shown that frequencies of the selected matching tones roughly corresponded to the recorded SOAE frequencies. Besides the fact that permanent tinnitus percepts originating from an SOAE are rare [between 2 and 4.5% (4, 43)], SOAEs seem to be a plausible explanation in the case of transient tonal tinnitus after LF sound exposure for the following reasons:

Tinnitus frequencies selected by NH participants in the present study correspond to typical SOAE frequencies. SOAEs in human adults are usually in the range between 0.9 and 4 kHz with distribution maxima near 1.5 and 3 kHz (44, 45).

Under normal conditions, SOAEs are relatively stable in both frequency and level (46). After intense LF exposure, however, frequency and level of SOAEs slowly cycle for a time period of about 2 min in a stereotypic manner with a level maximum typically occurring about 50 s after LF sound offset (27, 31). SOAEs presumably buried in the noise floor become detectable during that period. Typically, SOAEs are not perceived by their owner (47). It has been shown, however, that induced frequency or level changes of SOAEs can result in SOAEs becoming transiently audible (47–49), as the inhibition of SOAE perception at higher stages of auditory processing (45) might not be active anymore. Not all NH participants perceiving tonal tinnitus had SOAEs with frequencies similar to the chosen matching tone frequency. NH participants with recordable SOAEs often showed more than one SOAE, which would have resulted in a percept difficult to match with a single pure tone.

In NH participants without recordable SOAEs, SOAE levels might be significantly underestimated by external recordings after OAE back-propagation, compared to sound pressure level of OAEs within the cochlea (47). This is strongly supported by the comparison of DPOAE sound levels measured in the

gerbil meatus and the electrophysiological response to the same DPOAE in the gerbil cochlear nucleus (50). Thus, for SOAEs to be perceived by their owner, it might not be necessary that SOAE levels, as measured in the meatus, exceed the individual hearing thresholds at the SOAE characteristic frequency.

Noise-Like Tinnitus Generation

In both subject groups, a major proportion of tinnitus matches indicated a noise-like tinnitus. In experimental animals, it has been shown that the endocochlear potential increases temporarily with intense LF exposure (22, 51), resulting in a depolarization of IHCs and consequently in an increased spontaneous activity of the auditory nerve (34).

While direct recordings of the endolymphatic potential in MD patients are not feasible, a similar mechanism is nonetheless conceivable. Increased spontaneous activity of the auditory nerve could lead to the perception of a tinnitus, for which Patuzzi (34) coined the term "rate tinnitus." If a large expanse of the cochlea was affected by the above mechanism, a noise-like percept would result.

MD patients perceiving noise-like tinnitus percepts chose mainly noises with negative tilts (low pitches). This is in line with reports that MD patients typically show LF hearing loss at frequencies below 3 kHz (14), suggesting that, for hitherto unknown reasons, the apical part of the cochlea is most affected, resulting in a LF rate tinnitus. But, presumably depending on the duration of the disease, audiograms of MD patients reveal a broad range of affected frequencies (15), which is also reflected in our study population. This might explain why MD patients selected matching noises not necessarily limited to the LF range.

During intense LF stimulation, cochlear excitation is not restricted to the characteristic frequency, but the whole cochlea is excited and individual differences in cochlear excitation patterns might exist. LF stimulation affects the apical end of the cochlea strongest and LF components should be more prominent in noise-like tinnitus after intense LF exposure in NH participants (corresponding to selected noises with negative tilt). Those NH participants that chose high-pitched noises to match their LF-induced tinnitus may have done so as a compromise to represent hybrid tinnitus percepts consisting of both noise and tonal components or cochlear locations other than the apex dominate the tinnitus percept.

Tinnitus Sensation Levels

Sensation levels of tinnitus percepts were estimated with previously selected matching stimuli. In MD patients, hearing thresholds even on the contralateral, unaffected ear were slightly elevated at higher frequencies due to age-related hearing loss. Therefore, for MD patients choosing a high-pitched pure tone to match their tinnitus, loudness matches might have resulted in lower values than loudness matches using frequencies at which hearing was normal (8, 52). Loudness recruitment could also contribute: for participants suffering from cochlear hearing loss, low to moderate sensation levels may be much louder than for NH participants because the lack of cochlear compression decreases the overall dynamic range of loudness perception dramatically (52, 53). Furthermore, low sensation levels of matching tones in MD patients can be due to tinnitus loudness fluctuations. Patients were measured between vertigo attacks when tinnitus loudness was typically lower than immediately before or during attacks.

On the other hand, high loudness values in NH participants could be explained with a shift of attention toward the suddenly occurring tinnitus percept. Tinnitus sensation levels after LF exposure were on average higher than tinnitus sensation levels in MD patients and higher than tinnitus sensation levels in most patients with pathologies other than MD. Studies showed that tinnitus loudness was generally matched with stimuli below 10 dB SL, even in participants referring to their tinnitus as loud (8, 10, 54).

Procedural Limitations

Participants were presented with a limited range of sounds from which they had to select. Although this limited range of sounds was chosen to facilitate the matching procedure, participants complained about not being able to adjust amplitude modulations or about being restricted to one stimulus class when hearing hybrid percepts with both pure tones and noises. Furthermore, for both MD patients and NH participants with an LF-induced transient tinnitus, tinnitus loudness, and sometimes even tinnitus pitch were varying over time. In case of MD patients, these variations can take place over days or weeks depending on vertigo attacks or the current stage of the disease (12, 13). In NH participants, fluctuations appear within the 1–2 min of tinnitus duration. This is inherent to the transient percept after LF exposure. To guarantee consistency, NH participants were able to retrigger the tinnitus percept by turning on the LF stimulation again.

In either case, tinnitus-matching results can only represent approximations of actual tinnitus percepts, constrained by both choice of offered stimuli and experimental procedures.

CONCLUSION

Estimates of tinnitus pitch reported in the literature are heterogeneous and depend heavily on the methods used. Here, we implemented a tinnitus-matching procedure with fewer constraints than in previous, comparable studies, including noises with adjustable spectral shapes. As a consequence, our results revealed a relatively large proportion of participants with noise-like tinnitus, which might have not been detected in previous studies mostly employing sinusoidal matching tones. Contrary to reports in the literature, tinnitus pitch in NH participants after LF sound exposure and in MD patients is not exclusively LF, and hybrid percepts with noise-like and tonal components can occur.

Noise-like tinnitus cannot easily be explained with localized damage to cochlear regions. Rather, a mechanism affecting a broad frequency range is needed. An increase of spontaneous activity of the auditory nerve was suggested to cause the noise-like tinnitus observed here. However, further experiments are now required to identify unusual patterns in auditory nerve spontaneous activity as a potential tinnitus generator in chronic and induced hydropic conditions.

AUTHOR CONTRIBUTIONS

MU contributed to study design, performed data acquisition, statistical analysis and interpretation of results, drafting of the manuscript, revised the manuscript, and approved the final manuscript. LW contributed to study design and data acquisition, critically reviewed and approved the final manuscript. EK contributed to data acquisition, revised and approved the final manuscript. RG contributed to data acquisition, revised and approved the final manuscript. MD conceptualized and designed the study, contributed to data acquisition, interpretation of results, drafting of the manuscript, critically reviewed and approved the final manuscript. All authors are agreeable to be accountable for the content of the work, integrity, and accuracy of the data.

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CHAPTER 3

TRANSCUTANEOUS ELECTRICAL STIMULATION OF THE INNER EAR

The following study was accepted and published under the title "Modulation of Auditory Percepts by Transcutaneous Electrical Stimulation" in the Journal Hearing Research by Margarete Anna Ueberfuhr, Amalia Braun, Lutz Wiegrebe, Benedikt Grothe and Markus Drexl on March 18th 2017 (doi: 10.1016/j.heares.2017.03.008).

3.1 Author Contribution

The author of this thesis contributed to study design, was responsible for data acquisition, statistical analysis and interpretation of data, as well as drafting, revising and approving the manuscript. The other authors contributed as follows:
Markus Drexl conceptualized and designed the study, while Lutz Wiegrebe contributed to study design. Markus Drexl, Lutz Wiegrebe and Benedikt Grothe provided support during data acquisition. Amalia Braun conducted data acquisition and analyzed and interpreted the data as part of her Bachelor thesis. Markus Drexl analyzed data, interpreted the results of the experiments and wrote and edited the manuscript. Markus Drexl, Amalia Braun, Lutz Wiegrebe and Benedikt Grothe critically revised and approved the final manuscript.

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Research Paper

Modulation of auditory percepts by transcutaneous electrical stimulation

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ABSTRACT

Transcutaneous, electrical stimulation with electrodes placed on the mastoid processes represents a specific way to elicit vestibular reflexes in humans without active or passive subject movements, for which the term galvanic vestibular stimulation was coined. It has been suggested that galvanic vestibular stimulation mainly affects the vestibular periphery, but whether vestibular hair cells, vestibular afferents, or a combination of both are excited, is still a matter of debate. Galvanic vestibular stimulation has been in use since the late 18th century, but despite the long-known and well-documented effects on the vestibular system, reports of the effect of electrical stimulation on the adjacent cochlea or the ascending auditory pathway are surprisingly sparse.

The present study examines the effect of transcutaneous, electrical stimulation of the human auditory periphery employing evoked and spontaneous otoacoustic emissions and several psychoacoustic measures. In particular, level growth functions of distortion product otoacoustic emissions were recorded during electrical stimulation with alternating currents (2 Hz, 1–4 mA in 1 mA-steps). In addition, the level and frequency of spontaneous otoacoustic emissions were followed before, during, and after electrical stimulation (2 Hz, 1–4 mA). To explore the effect of electrical stimulation on the retrocochlear level (i.e. on the ascending auditory pathway beyond the cochlea), psychoacoustic experiments were carried out. Specifically, participants indicated whether electrical stimulation (4 Hz, 2 and 3 mA) induced amplitude modulations of the perception of a pure tone, and of auditory illusions after presentation of either an intense, low-frequency sound (Bounce tinnitus) or a faint band-stop noise (Zwicker tone).

These three psychoacoustic measures revealed significant perceived amplitude modulations during electrical stimulation in the majority of participants. However, no significant changes of evoked and spontaneous otoacoustic emissions could be detected during electrical stimulation relative to recordings without electrical stimulation.

The present findings show that cochlear function, as assessed with spontaneous and evoked otoacoustic emissions, is not affected by transcutaneous electrical stimulation, at the currents used in this study. Psychoacoustic measures like pure tone perception, but also auditory illusions, are affected by electrical stimulation. This indicates that activity of the retrocochlear ascending auditory pathway is modulated during transcutaneous electrical stimulation.

1. Introduction

In the late 18th century Alessandro Volta described the effects of electrical stimulation (ES) on the human body for the first time (Volta, 1800). During self-experimentation, Volta placed electrodes, connected to batteries, near his ear. He subsequently collapsed and experienced spinning and the acoustic sensation of an explosion

Abbreviations: AC, alternating current; DC, direct current; DPOAE, distortion product otoacoustic emission; ES, electrical stimulation; EEOAE, electrically evoked otoacoustic emission; GVS, galvanic vestibular stimulation; OAE, otoacoustic emission; OHC, outer hair cell; AM, amplitude modulation; SOAE, spontaneous otoacoustic emission

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inside his head (Volta, 1800). Nowadays, transcutaneous ES with electrodes placed on the mastoid processes, for which the term galvanic vestibular stimulation (GVS) was coined, is frequently used as a tool to probe vestibular function in humans without passive or active movements of the tested participants. GVS with direct current (DC) stimulation activates the vestibular system and causes a sway, which can be interpreted as the summed activity of the otolithic organs and the semicircular canals (Fitzpatrick and Day, 2004). It is still a matter of debate which parts of the vestibular system (vestibular hair cells and/or vestibular afferents) are specifically excited by GVS (Cohen et al., 2011, 2012; Curthoys and Macdougall, 2012; Wardman and Fitzpatrick, 2002).

Despite the long-known and well-documented activation of GVS on the vestibular system, reports on auditory percepts or on effects on the cochlea and the ascending auditory pathway are surprisingly sparse. During GVS, Bucher et al. (1998) showed activity in Heschl's gyrus containing the primary auditory cortex, but none of their subjects reported auditory sensations. High-frequency (0.5–20 kHz) ES of the mastoid in humans can result in auditory percepts, but this is due to an indirect, mechanical effect caused by tissue vibrations near the stimulation side which are consequently transmitted to, and detected by, the cochlea (Flottorp, 1976; Lopponen et al., 1991).

ES in general could affect the auditory system on several levels. This might include electrophonic activation of the basilar membrane, direct changes of hair cell receptor potentials, modulation of associated transmitter release, changes of spiral-ganglion-cell membrane potentials, and modulation of auditory-nerve spontaneous activity (Rubinstein and Tyler, 2004). A single previous study reported no changes of distortion-product otoacoustic emissions (DPOAEs) during ES (Cevette et al., 2012).

From animal experimentation, however, it is known that the cochlea indeed responds directly to ES: Electrically evoked otoacoustic emissions (EEOAEs) are sounds which can be recorded in the ear canal, when an alternating current (AC) stimulus excites the cochlea with electrodes typically placed on the round window membrane (e.g. Drexl et al., 2008; Ren and Nuttall, 1995, 2000; Ren et al., 1996). EEOAEs are thought to be generated by the electromechanical transduction of outer hair cells (OHCs) near the electrode location, driven by the external ES, and travel to the external ear canal with the same frequency as the electrical stimulus (Drexl et al., 2008; Ren and Nuttall, 1995; Ren et al., 1996; Ren et al., 2000; Zou et al., 2003). In addition, Frank and Kossl (1997) demonstrated that low-frequency (5 Hz) ES of the round window altered acoustically evoked otoacoustic emissions (OAEs).

In some human patients, extracochlear ES has been shown to suppress tinnitus, indicating that the activity of the retrocochlear auditory pathway can be modulated by ES (Cazals et al., 1978; Dobie et al., 1986; Kuk et al., 1989; Lyttkens et al., 1986; Portmann et al., 1979; Vernon and Fenwick, 1985).

Auditory illusions have been suggested as tinnitus models (Drexl et al., 2014; Hoke et al., 1996; Hoke et al., 1998; Norena et al., 2000; Patuzzi and Wareing, 2002) and might represent a convenient way to study the effect of ES in the absence of acoustic stimulation. The Zwicker tone is an auditory illusion lasting between 1 and 6 s after the presentation of band-stop noise and is perceived as a pure tone with a frequency within the spectral gap of the band-stop noise (Fastl, 1989; Lummis and Guttman, 1972; Zwicker, 1964). Another illusion, the Bounce tinnitus, is perceived after the offset of intense, rather low-frequency sound (Hirsh and Ward, 1952; Hughes, 1954; Patuzzi and Wareing, 2002). The Bounce tinnitus is accompanied by small hearing threshold fluctuation and stereotypic OAE level changes (Drexl et al., 2014; Kemp, 1986; Kemp and Brill, 2009; Kevanishvili et al., 2006; Kugler et al., 2014; Zwicker and Hesse, 1984). These transient effects are thought to be the result of a temporarily challenged cochlear homeostasis and are summarized under the term Bounce Phenomenon. The Bounce tinnitus, which can be of different tonal quality (Ueberfuhr et al., 2016), lasts for around 1.5 min and vanishes gradually (Drexl et al., 2014; Patuzzi and Wareing, 2002).

The aim of the current study was to dissect the specific effects of ES on different levels of auditory processing: On a peripheral level, the effect of ES on OHCs, due to their electromotile properties and their role in OAE generation, can directly be assessed by OAE recordings. On a retrocochlear level, the effects of ES on the perception of an externally presented pure tone were examined. Additionally, the influence of ES on two auditory illusions, in the absence of external acoustic stimulation, was explored with psychoacoustic measures.

2. Methods

2.1. Participants

Participants were eligible for this study when they had neither hearing problems nor tinnitus, and had not had any ear surgery or recent ear infection. All participants showed hearing thresholds of less than 25 dB HL between 0.25 and 8 kHz, tested with a Matlab-based automated procedure (Automatic Pure Tone Audiometry APTA-HF 2012 V2.28 (HörTech, Oldenburg, Germany)).

This study was approved by the Ethics Committee of the University Hospital Munich, Ludwig-Maximilians-Universität München, Germany, in agreement with the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave their written informed consent. Experiments were conducted in a sound-attenuated booth at the Department of Biology, Ludwig-Maximilians-Universität München, Martinsried, Germany.

2.2. Signal generation and data acquisition

Signal generation and data acquisition was carried out with an RME Fireface UC 24-bit external sound card (RME, Audio AG, Haimhausen, Germany), operated with a sampling rate of 44.1 kHz. Scripts written in MatLab 7.5 (MathWorks, Natick, MA, USA) controlled the external sound card, employing the SoundMexPro sound application (HörTech, Oldenburg, Germany). The sound card forwarded commands to both the current stimulator for ES and to the sound systems for auditory stimulation. For OAE recordings, an ER-10C DPOAE probe system (Etymotic Research Inc., Elk Grove Village, IL) was used in one ear. In psychoacoustic experiments, both ears were exposed to sound and two different sound systems were used, the ER4 insert ear phones (Etymotic Research Inc., Elk Grove Village, IL) for the contralateral ear (here and in the following: the ear where a matching tone was presented) and the ER-10C DPOAE probe system for the ipsilateral ear (here and in the following: the ear where sounds were presented to induce an auditory percept or auditory illusions). To induce the Bounce tinnitus, an additional external loudspeaker (NSW1-205-8A, Aura Sound Inc., Santa Fe Springs, CA) driven by a power amplifier (RB-960BX, Rotel, Worthing, UK) was used to generate low-frequency sounds (30 Hz sine wave, 120 dB SPL, 90 s, including 0.1 s raised-cosine ramps). The loudspeaker was connected to a 50 cm long polyethylene tube (inner diameter 1 mm), the tip of which was fed through the foam ear tip of the ER-10C DPOAE probe. As the ER-10C probe contains a probe microphone for recording in the ear canal, ipsilaterally presented stimuli, including very low-frequency sounds, were calibrated in-situ. The magnitude response of the probe microphone at low frequencies is non-linear and was therefore compensated against the magnitude response of an artificial ear microphone (B&K 4157, Brüel & Kjær, Sound &

Vibration Measurement A/S, see Drexl et al. (2012) for details on the calibration procedures).

2.3. Electrical stimulation

During recordings of OAEs and psychophysical experiments, transcutaneous ES was delivered with a DS 5 bipolar constant current stimulator (Digitimer Ltd., Hertfordshire, UK). Two surface electrodes (tDCS rubber electrodes, 5 cm × 7 cm, neuroConn, Ilmenau, Germany) were covered with electrode gel and placed bilaterally on the mastoid processes behind the ears, where they were held in place with a flexible ribbon. Although slightly different skull shapes of participants might have caused some variation, care was taken to always place the electrodes in the same way on the mastoid processes and to avoid any contact between surface electrodes and pinna. Depending on the experiment, AC currents at a frequency of 2 or 4 Hz within a range of 1–4 mA, in 1 mA-steps, were applied. Before each current increment, participants were asked if they were willing to tolerate stimulation with a higher current and if not, this part of the experiment was concluded.

2.4. Psychoacoustic experiments

In three different psychoacoustic tasks, participants were asked to indicate whether they perceived an amplitude modulation (AM) of externally presented tones or of auditory illusions during ES. They were asked to adjust the AM depth of a contralaterally presented matching tone with a sinusoidal AM accordingly. The matching tone was always presented after the offset of the participants' auditory illusion. Participants could only adjust level and AM depth of the matching tone; the modulation frequency was always equal to the frequency of the ES. The pure tone frequency or spectral content of the matching sound could not be changed, neglecting possible pitch/timbre shifts caused by ES.

Participants were given standardised written and illustrated instructions for the matching procedures. They were seated in a sound-attenuated booth and received visual feedback on a computer screen during the experiments. They were also asked to focus on their auditory perception. Participants were able to interactively adjust matching tones regarding loudness and AM depth via a gamepad (Bigben Interactive GmbH, Bergheim, Germany). The starting point for matching stimuli was always a stimulus with an AM depth of −6 dB (corresponding to a linear modulation depth of 50%).

The AM depth could be adjusted within the range of 0 dB (full modulation) and −20 dB. In previous studies, detection thresholds for sinusoidal AM with a modulation frequency of 4 Hz (carrier frequency: 1 kHz and 4 kHz) were found to be on average around an AM depth of −20 dB (Edwards and Viemeister, 1994; Viemeister et al., 2010). Therefore, in this study, an AM depth of −20 dB was considered the detection threshold and taken as the lower boundary of the AM depth range (i.e. no perceived modulation).

2.4.1. Amplitude modulation detection – pure tone

A faint pure tone (4 kHz, 30 dB SPL) was presented to the ipsilateral ear for 10 s. During sound exposure, participants were stimulated with AC stimuli (4 Hz) at 2 and 3 mA, respectively (see Fig. 1). In the control condition, participants were exposed to acoustic stimulation only. Participants were able to restart concurrent acoustic stimulation and ES at their own discretion.

After the sound exposure, a matching tone (4 kHz, starting level = 42.5 dB SPL, AM = 4 Hz, starting AM depth = −6 dB) was played to the contralateral ear of the participants. Participants were asked to adjust the matching tone regarding loudness and AM depth, so that it best resembled their auditory perception of the pure tone during ES (Fig. 1).

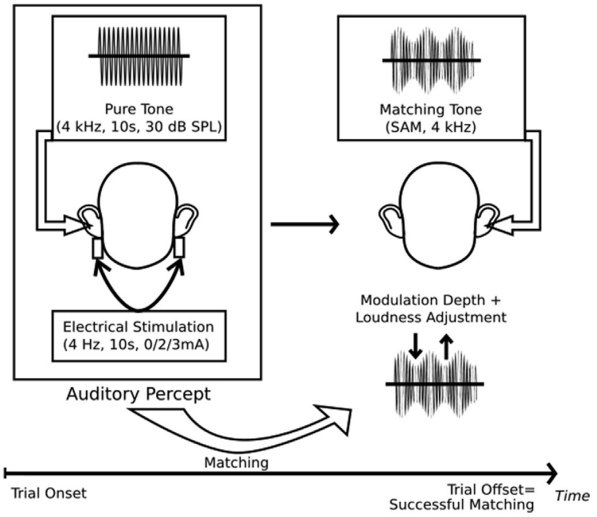

Fig. 1. Detection of electrically induced amplitude modulations of an externally presented pure tone. After the presentation of concurrent transcutaneous electrical stimulation and an externally presented pure tone, participants adjusted the loudness and modulation depth of a contralaterally presented matching tone (sinusoidal amplitude modulation (SAM) of 4 Hz) such that it resembled their auditory percept during electrical stimulation best.

2.4.2. Amplitude modulation detection – Zwicker tone

Participants were exposed to a sequence consisting of seven 3 s-noise intervals (Gaussian noise with a spectral gap between 3.4 and 4.8 kHz, 50/55 dB SPL) alternating with 2 s-pause intervals, presented to the ipsilateral ear. During the 2 s-pauses, right after the presentation of stop-band noise, participants perceived the Zwicker tone. As a consequence of repeated band-stop noise exposures, the Zwicker tone typically increases in amplitude and duration (Lummis and Guttman, 1972). During the 2 s-pauses, in which participants perceived the Zwicker tone, ES was delivered with an AC of 4 Hz at 2 and 3 mA, respectively (unless under control conditions where no ES was applied). After presenting the stimulus sequence of alternating acoustic stimulation and ES, a matching tone (4 kHz, starting level = 42.5 dB SPL and thus frequency and level similar to the Zwicker tone, AM = 4 Hz, starting AM depth = −6 dB) was played to the contralateral ear of the participants (see Fig. 2). Participants were asked to adjust the matching tone regarding loudness and AM depth so that it best resembled their perceived Zwicker tone without ES (control condition) and during ES. Participants were able to restart the stimulus sequence any time, if they needed to.

2.4.3. Amplitude modulation detection – Bounce tinnitus

Participants were exposed to a low-frequency sound (30 Hz, 120 dB SPL, 60s) on the ipsilateral ear, which induced a transient, tinnitus-like percept, lasting on average around 1.5 min after the acoustic stimulation offset (Drexl et al., 2014). As the tinnitus percept after low-frequency sound exposure differs individually (Ueberfuhr et al., 2017), the tinnitus quality was assessed. For this, the presented stimuli comprised of two stimulus classes: pure tones with five different frequencies equally spaced on a logarithmic frequency axis between 0.25 and 8 kHz (0.25, 0.595, 1.414, 3.364, 8.0 kHz) and noises derived from Gaussian noise with five

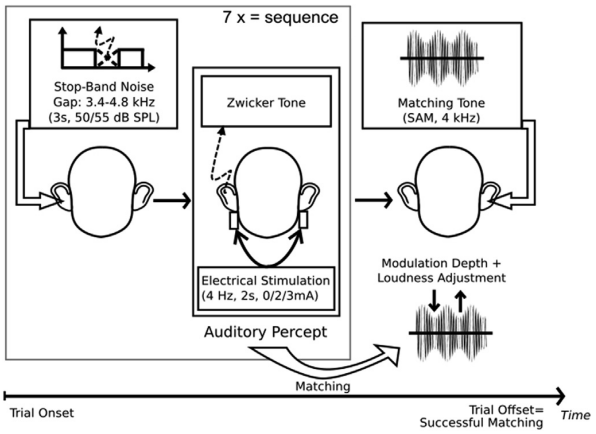

Fig. 2. Detection of electrically induced amplitude modulations in the perception of the Zwicker tone. After presentation of a stop-band noise, participants perceived the Zwicker tone and concurrent electrical stimulation was delivered. This sequence was repeated seven times. After the conclusion of the stimulation sequence, participants adjusted loudness and modulation depth of an externally, contralaterally presented matching tone (sinusoidal amplitude modulation (SAM) of 4 Hz), such that it resembled their auditory percept during electrical stimulation best.

different spectral slopes (dB/octave), ranging from negative values with a dominant low-frequency component (−12 and −6 dB/octave) over white Gaussian noise (0 dB/octave) to positive values with dominant high-frequency components (+6 and +12 dB/octave). The selected and adjusted stimuli (spectral quality and sound pressure level) were used as matching tones in the following procedure (see Fig. 3). During perception of the Bounce tinnitus, participants could enable and disable ES (with an AC of 4 Hz at a current of 2 and 3 mA, respectively) as they wished, with the exception of the control condition where no ES was applied. After the cessation of the Bounce tinnitus, participants could continuously adjust loudness and AM depth of the matching stimulus presented to the contralateral ear (AM = 4 Hz, starting AM depth = −6 dB), so that it best resembled their tinnitus percept during ES.

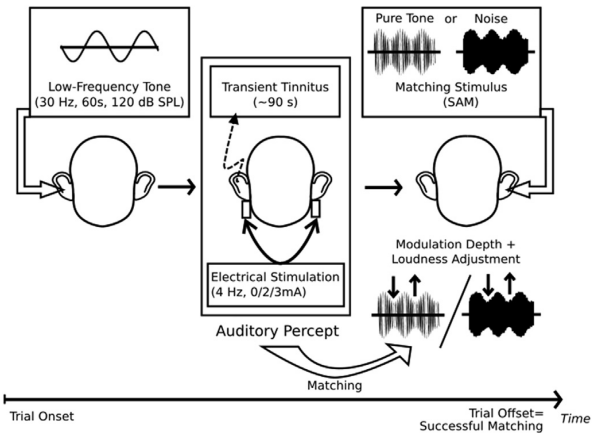

Fig. 3. Detection of electrically induced amplitude modulations in the perception of a transient tinnitus. After presentation of an intense, low-frequency tone, participants perceived a transient tinnitus, and concurrent electrical stimulation was delivered. After cessation of the electrical stimulation, depending on the quality of the perceived tinnitus, participants adjusted loudness and modulation depth of an externally presented matching tone or noise (sinusoidal amplitude modulation (SAM) of 4 Hz) such that it resembled their auditory percept during electrical stimulation best.

2.5. Biophysical experiments

2.5.1. Otoacoustic emissions — DPOAE growth functions

Level growth functions of cubic ($2f_1$-f_2) and quadratic (f_2-f_1) DPOAEs were recorded. Primary tones were presented with a stimulus duration of 2 s. Each 2 s-interval of primary tone presentation was paired with ES (AC, 2 Hz, 1–4 mA) with the exception of control recordings taken before and after recordings with ES (pre- and post-control, respectively). Stimulus presentation was repeated between 8 and 16 times, depending on the number of rejected recordings. Recordings were averaged in the time domain (see Drexl et al., 2012 for details on the recording and noise rejection procedures).

DPOAEs were recorded with starting primary tone levels $l_1 = 40$ dB SPL and $l_2 = 30$ dB SPL (cubic DPOAEs), and $l_1 = l_2 = 50$ dB SPL (quadratic DPOAEs), increasing in 5 dB increments up to $l_1 = 70$ dB SPL, $l_2 = 60$ dB SPL (cubic) and $l_1 = l_2 = 75$ dB SPL (quadratic). For cubic DPOAEs, a fixed f_2/f_1 ratio of 1.22 was used. The individual f_2 frequency within a range of 1 and 8 kHz resulting in the largest cubic DPOAE was chosen for further measurements. For quadratic DPOAEs, the best f_2/f_1 ratio resulting in the largest DPOAE level evoked with $f_2 = 4$ or 5 kHz was used.

2.5.2. Otoacoustic emissions — DPOAE biasing

Cubic and quadratic DPOAEs were recorded continuously during an acquisition period of 20 s. Primary tone levels were set to $l_1 = 60$ dB SPL, $l_2 = 50$ dB SPL for the recording of cubic DPOAEs, and $l_1 = l_2 = 70$ dB SPL for quadratic DPOAEs. During DPOAE recordings, concurrent ES was delivered (AC, 2 Hz, 1–4 mA in 1 mA-increments), with the exception of control recordings without ES taken before and after recordings with ES (pre- and post-control, respectively). As a measure of DPOAE level modulation patterns, coupled to the phase of the ES stimulation, the magnitudes of spectral lines at the DPOAE frequency ±2 Hz (i.e. the upper and lower sidebands) were extracted from the Fourier Transform of the 20 s-recordings.

For each of the experimental conditions, the ratios of the DPOAE spectral magnitude to the mean of the spectral magnitude of the upper and lower sidebands were then calculated in dB. A decrease of this ratio indicates increased side bands and thus AM (at the frequency of the ES).

2.5.3. Otoacoustic emissions — spontaneous otoacoustic emissions (SOAEs) biasing

SOAEs were recorded for 150 s in total. In the initial 90 s, SOAEs were recorded without ES, followed by another 30 s-period with ES, and concluded by another 30 s-period without ES. Level and frequency of the individual SOAEs were analysed in consecutive 5 s time intervals and significant changes along this time series were detected with a change-detection algorithm (Taylor, 2000). For this, the difference between the maximum and the minimum of the cumulative sum time series of SOAE level was calculated. A bootstrap analysis (1000 samples) was used to randomly re-order the SOAE time series and the analysis described before was repeated for each of the re-ordered samples. The confidence level was then determined by calculating the percentage of 1000 bootstrap samples where the difference between the maximum and minimum of the bootstrapped cumulative time series was smaller than in the original time series. We considered changes of SOAE levels and frequencies in the original time series as significant when the confidence level was at least 99%.

3. Results

3.1. Psychoacoustic experiments

Participants listened to an externally presented acoustic stimulus or an auditory illusion during ES. Subsequently, they were asked to adjust a matching tone so that it resembled their auditory percept best. As adjusted sound pressure levels did not differ significantly between control condition and corresponding ES conditions (paired t-tests, $p > 0.05$), neither for pure tone exposure nor for auditory illusions, only AM depth adjustments will be discussed in the following. For comparing test situations without and with 2 mA or 3 mA ES, two-sided Wilcoxon signed rank tests with Bonferroni correction were conducted, of which p-values are reported in brackets in the following paragraphs (unless specified otherwise). In control experiments without ES, participants typically adjusted the AM depth to about −20 dB, the lowest AM value possible in this study.

3.1.1. Amplitude modulation detection – pure tone

23 participants (mean age = 23.3, age range: 20–29, 14 females, 9 males) took part in this subset of experiments.

When matching their percepts during ES at currents of 2 and 3 mA, participants adjusted the AM depth of the matching tones significantly higher than in the control condition without ES ($n = 23$, 2 mA: $p = 0.0037$, 3 mA: $p = 0.0018$, see Fig. 4). Only 30% ($n = 7$) of all participants selected an AM depth of −20 dB (the lowest AM possible) during ES, indicating that no AM was perceived neither at 2 mA nor at 3 mA. In half of the participants who perceived an AM ($n = 8$), adjusted AM depths increased monotonically with increasing current of the ES, whereas in the other half ($n = 8$), adjusted AM depths increased during ES with 2 mA, but AM depth decreased again when the current was increased from 2 to 3 mA.

There was no significant difference between adjusted AM depths during ES with 2 and 3 mA ($n = 23$, $p = 1.8153$, see Fig. 4).

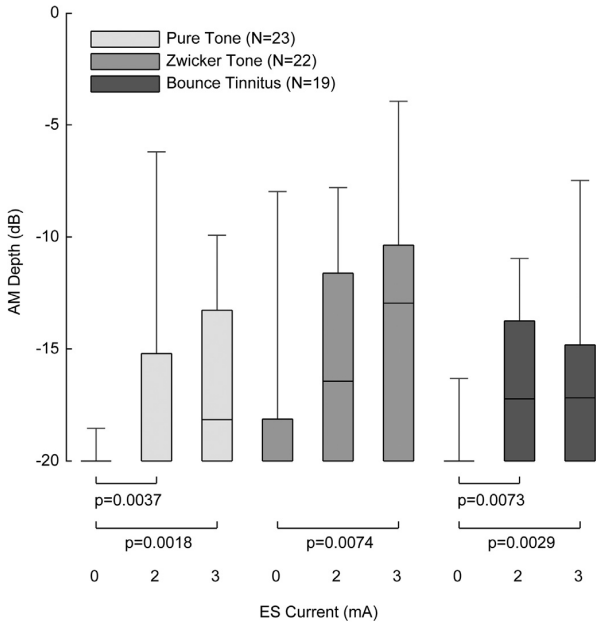

Fig. 4. Electrically induced modulation depths of three different auditory percepts as a function of the current of a simultaneous electrical stimulation (ES). Boxplots represent medians and the lower and upper quartiles. Whiskers represent the 5th and 95th percentile, respectively. Please note that minimum adjustable amplitude modulation (AM) was limited to −20 dB and considered "no modulation". Significant differences between pairs are indicated with brackets and corresponding p-values (paired, two-sided Wilcoxon test, p-values were adjusted for multiple comparisons with the Bonferroni method).

3.1.2. Amplitude modulation detection – Zwicker tone

A stable percept of the Zwicker tone could be evoked in 22 participants (mean age = 22.4, age range: 18–29, 15 females, 7 males). In 12 participants, the evoking stimulus was presented with 55 dB SPL, and in the remaining participants with 50 dB SPL. With 22 out of 42 participants, only 52% of all subjects, compared to 80% in the literature (Fastl, 1989), were able to hear the Zwicker tone. This might be due to the fact that the percept is labile (Lummis and Guttman, 1972) and participants of the current study were not expert listeners, thus having problems to identify the short and faint Zwicker tone. At the group level, Zwicker tones were not significantly modulated during ES at 2 mA ($n = 22$, $p = 0.1483$). But a significant increase in adjusted AM depth was seen with ES at 3 mA ($n = 22$, $p = 0.0074$, see Fig. 4). During ES only five participants adjusted AM depths of −20 dB at both currents, suggesting that they did not perceive any electrically induced modulation of the Zwicker tone. In eleven participants, AM depths of the matching tone correlated positively and monotonically with increasing currents of the ES. In three participants, AM depths showed a maximum at a stimulation current of 2 mA, and decreased again at 3 mA.

3.1.3. Amplitude modulation detection – Bounce tinnitus

In 19 participants (mean age = 23.3, age range: 20–19, 12 females, 7 males), a stable tinnitus percept could be evoked after the presentation of low-frequency sound. 10 participants selected noises (−12 dB/octave to +12 dB/octave), 9 participants selected pure tones (0.25–8 kHz) to match their tinnitus. Five participants adjusted an AM depth of −20 dB indicating no modulation during ES similar to control recordings. In 8 participants, the adjusted AM depths correlated positively and monotonically with increasing ES current. The remaining five participants adjusted maximum AM depths at 2 mA, and showed decreasing AM depths at 3 mA.

Statistically, ES induced a modulation of the tinnitus percept at 2 mA ($n = 19$; $p = 0.0073$) and 3 mA ($n = 19$; $p = 0.0029$, see Fig. 4).

The matching-stimulus class (tones or noises) did not play a role in whether participants perceived a modulated tinnitus during ES or not (Mann-Whitney-Test, 2 mA: $p = 0.2991$, 3 mA: $p = 0.3870$, see Fig. 4).

In summary, about 90% of the 19 participants who were tested for all three conditions (pure tone, Zwicker tone, Bounce tinnitus) perceived an electrically induced AM in at least one of the conditions.

3.2. Biophysical experiments

Biophysical experiments were carried out in 15 participants (mean age = 23.4, age range: 21–29, 10 females, 5 males).

3.2.1. Otoacoustic emissions – DPOAE growth functions

Cubic and quadratic DPOAE level growth functions were recorded with ES at several currents and before and after ES (see Fig. 5). No overall effect of current was found in the pooled data of all participants for both cubic and quadratic DPOAEs at electrical currents between 1 and 4 mA (two-way ANOVA, cubic DPOAEs: $F_{5,540} = 0.98$, $p = 0.43$ and quadratic DPOAEs: $F_{4,275} = 1.14$, $p = 0.34$).

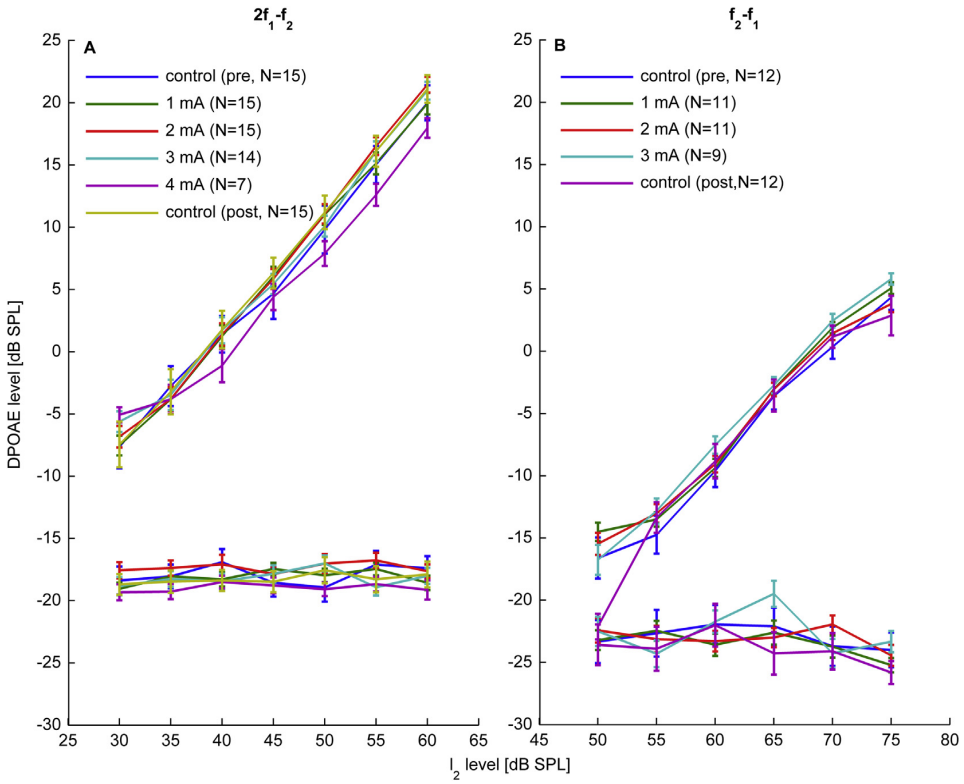

Fig. 5. Cubic (A) and quadratic (B) DPOAE level as a function of primary tone level during delivery of electrical stimulation with currents between 1 and 4 mA and controls before (pre) and after (post) electrical stimulation (colour-coded). DPOAE levels are shown in the upper part of the plots, corresponding noise floors in the lower parts. Mean values are shown; error bars represent the standard error of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2.2. Otoacoustic emissions – DPOAE biasing

Similar to low-frequency biasing of DPOAEs (e.g. Drexl et al., 2012; Drexl et al., 2016), DPOAEs were recorded during ES stimulation in order to detect amplitude changes coupled to the phase of the ES stimulation phase. There was no overall effect of current on the ratio of DPOAE spectral lines and the mean of the modulation side band magnitudes in neither cubic (Kruskal-Wallis test, $H = 1.7832$, 5 d.f., $p = 0.8893$) nor quadratic DPOAEs (Kruskal-Wallis-test, $H = 1.34$, 5 d.f., $p = 0.8592$, see Fig. 6).

3.2.3. Otoacoustic emissions – SOAE biasing

Nine out of 15 tested participants had at least one SOAE, and a total of 36 SOAEs could be recorded. No significant changes (i.e. confidence levels larger than 99%) of SOAE level were seen (see Fig. 7). As testing for SOAEs was the only experimental condition without acoustic stimulation and ES only, all participants were asked after SOAE testing if they perceived auditory sensations during periods of ES. None were reported. However, as the detection of electrically evoked auditory percepts was not a main focus of this study, we did not use a specific protocol to assess this.

Taken together, no significant effect of ES on evoked and spontaneous OAEs could be detected within the ES current range used in this study.

4. Discussion

Here, we have shown that transcutaneous AC stimulation causes AM in the perception of externally presented tones and of two auditory illusions, the Zwicker tone and the Bounce tinnitus. In contrast, spontaneous and evoked OAEs showed no significant changes during ES, suggesting that at least OHCs are not affected by ES. This is consistent with the findings of Cevette et al. (2012) who reported no significant effects of ES with DC on cubic DPOAEs in humans evoked with constant-level primary tones. Irrespective of these findings in humans, there is evidence from animal experiments showing that OAEs can be evoked or altered by extra- and intracochlear ES (e.g. Drexl et al., 2008; Frank and Kossl, 1997; Nuttall and Ren, 1995; Sun et al., 2000; Zou et al., 2003), which indicates that the electromotile properties of OHCs can be driven by external ES. However, the data reported in those publications were obtained with a rather direct stimulation of the cochlea with electrodes placed on, or even in, the cochlea, in contrast to the transcutaneous type of stimulation used here. To electrically evoke OAEs, ES frequencies within the relevant auditory range of the species were used (i.e., several kHz), whereas in this study, stimulation frequencies between 2 and 4 Hz were employed, well below the lower human limit of human hearing (about 20 Hz). It could thus be argued that ES with very low frequencies is not an adequate stimulus to drive OHC electromotility to an extent detectable in OAE recordings. However, in animal experiments, cochlear activity was biased with very low-frequency ES (5 Hz) resulting in significant phase-coupled changes of evoked OAEs (Frank and Kossl, 1997). Since the available current range for ES of human participants is limited due to unpleasant skin sensations at the electrode

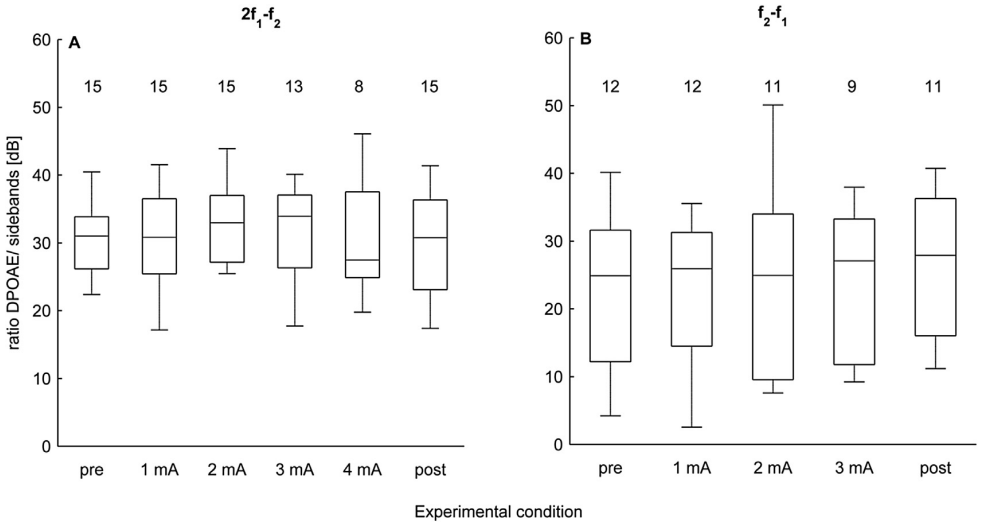

Fig. 6. The magnitude ratio of DPOAE spectral lines and the mean of the modulation side bands (i.e. the upper and lower sidebands) as an indication of cubic (A) and quadratic (B) DPOAE amplitude modulation coupled to the phase of the electrical stimulation during the various experimental conditions (i.e. electrical stimulation with 1–4 mA, and controls before (pre) and after (post) electrical stimulation). There is no effect of the experimental condition on the ratio, indicating that electrical stimulation does not induce a DPOAE amplitude modulation. Sample sizes are given in the top row.

site with increasing current, it is conceivable that the currents we used in this study were too low to affect OAE generation in OHCs. Taken together, we consider it unlikely that the alterations of auditory percepts reported in this study are based on OHC activity changes. Rather, our results point to an inner hair cell or retrocochlear origin. If ES affects signal propagation via the auditory nerve, it can result in auditory percepts during ES. None of our participants perceived auditory sensations during transcutaneous ES without acoustic stimulation, or in the absence of auditory illusions. This suggests that the currents used in this study were not strong enough to evoke activity changes of the auditory nerve sufficient for an auditory percept.

Nevertheless, the results of the current psychoacoustical experiments indicate that transcutaneous ES may modulate driven activity of the auditory nerve, as most (but not all) our participants reported AM of pure tones or auditory illusions during ES. Those participants who did not show any modulation of their auditory percept may either have had methodological difficulties or their thresholds for detecting AM may have been higher than the modulations induced by the ES.

In humans, extracochlear ES has been shown to modulate the perceived loudness of tinnitus (Cazals et al., 1978; Hatton et al., 1960; Portmann et al., 1979), presumably as a result of depressed spontaneous activity of the auditory nerve.

Noreña et al. (2015) showed in an animal model that ES on the cochlear round window with a positive DC current resulted in reduction of spontaneous activity of units in the inferior colliculus a negative DC current resulted accordingly in an increased activity. They suggested that this was a consequence of a modulation of auditory nerve spontaneous activity at the level of the inner hair cell synapse or of a direct effect on the membrane potential of auditory nerve fibres. The Bounce tinnitus, as it was used here, is thought to be caused by an increased activity at the inner hair cell synapse resulting in an increased spontaneous firing rate of the auditory nerve which thus becomes temporarily audible (Patuzzi and Wareing, 2002). Our data show that this tinnitus could be reliably modulated by the AC transcutaneous ES (AM = 4 Hz). It is conceivable that the perceived modulation of the Bounce tinnitus arises from the modulation of the (temporarily audible) spontaneous activity in the auditory nerve.

Transcutaneous ES of the ear canal can activate the middle ear reflex (Klockhoff and Anderson, 1959; Pichler and Bornschein, 1957) and this reflex activation may underlie the perceived AM described here. However, this is unlikely to dominate our results, as activity of the middle ear reflex should also affect OAEs (e.g. Buki et al., 2000; Burns et al., 1993), which is not what we observed. In addition, transcutaneous ES can cause a tingling sensation at the stimulation site, which acts as a somatosensory stimulus and might shift attention. Reports on the interaction between the somatosensory and the auditory system are scarce, and only a few reports are available (e.g. Yau et al., 2010). In those reports, tactile stimulation during auditory tasks appears to act as a distractor and impairs test performance, but no reports of phase-coupled effects of low-frequency tactile stimulation on the loudness of a test tone are available. We consider it therefore unlikely that ES with a frequency of 4 Hz results in a precise modulation of attention with the same frequency, resulting in the exact, periodic AM the majority of our participants perceived.

Vestibular sensations are the most prominent percepts reported during transcutaneous stimulation of the mastoid processes, unambiguously the result of a stimulation of the vestibular system. Vestibular stimulation has been shown to influence auditory perception in different contexts. For instance vestibular manipulations can affect tinnitus (e.g. jaw movements, Chole and Parker, 1992; Lanting et al., 2010), and head tilts and body rotation were found to play a role in sound localization (Lewald and Karnath, 2000, 2001; Van Barneveld et al., 2011). Those cross-modal findings are probably due to a multimodal integration of auditory and vestibular information (Smith, 2012) taking place at different stages of the auditory pathway up to the cortical level (Bense et al., 2001). Although it is still unclear how vestibular information is specifically used within the auditory processing, the dorsal cochlear nucleus is considered to be the relevant site of multimodal integration (Oertel and Young, 2004). There is also clear evidence for a direct

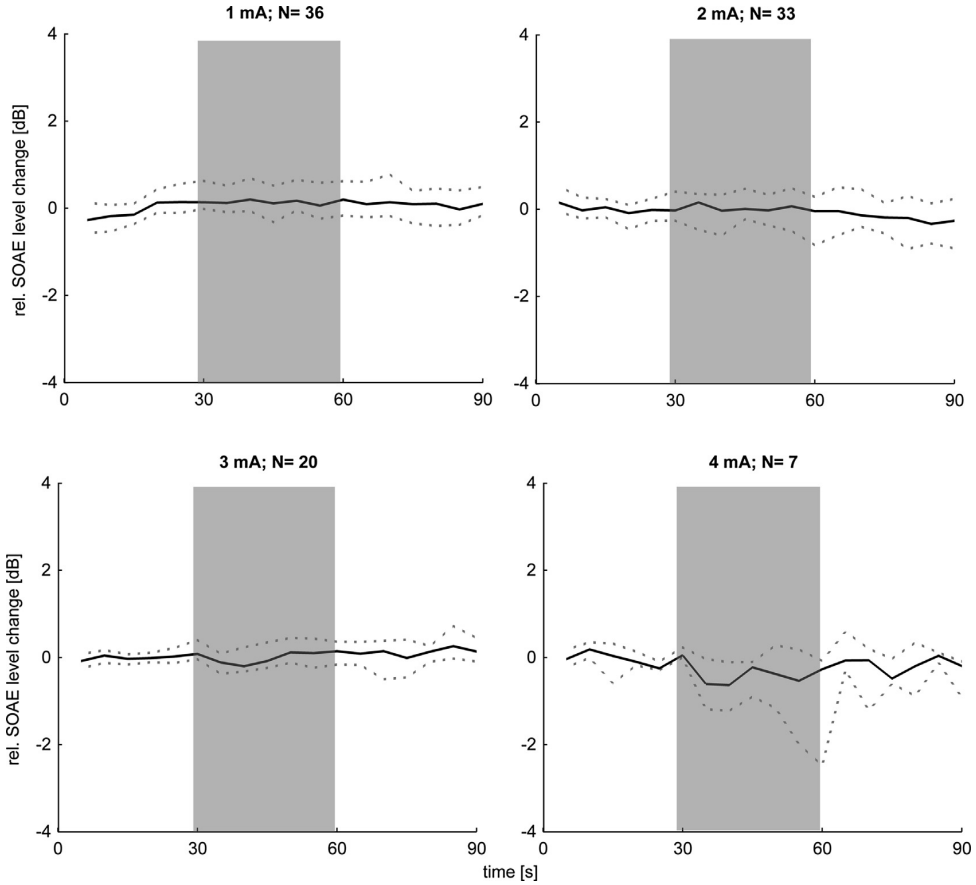

Fig. 7. SOAE level (rel. to the median of the pre-exposure period) as a function of time for electrical stimulation with currents between 1 and 4 mA. The grey bars indicate the period of electrical stimulation (4 Hz, current as indicated). Solid line, median; dotted lines, 25th and 75th percentile, respectively.

projection from the primary vestibular nerve to the cochlear nucleus (review: Newlands and Perachio, 2003) and for a direct projection from the vestibular nucleus to the dorsal cochlear nucleus (Barker et al., 2012; Bukowska, 2002; Wigderson et al., 2016).

Thus, another possible explanation for the current effects of ES on auditory percepts would be that ES exclusively activates the vestibular system and that auditory percepts are modulated through neural vestibular-auditory integration at the level of the cochlear nucleus. This is also supported by our finding that the Zwicker tone is modulated by ES. It has been found that the Zwicker tone is unlikely to originate in the cochlear periphery (Wiegrebe et al., 1996) or in the auditory nerve (Palmer et al., 1995). Rather, it has been suggested that the Zwicker tone could originate in the dorsal cochlear nucleus (Franosch et al., 2003; Wiegrebe et al., 1996), which features strong lateral inhibition (Young and Brownell, 1976), a suggested prerequisite for the Zwicker tone (Franosch et al., 2003). It is thus conceivable that the Zwicker Tone is modulated by ES via vestibular inputs into the dorsal cochlear nucleus.

To isolate the specific effects of ES on the auditory system, experiments will be needed where the electrically induced vestibular percepts are cancelled by kinetic stimuli.

Current ES technology using surface electrodes behind the ears is not a precise tool to specifically target the vestibular system. Due to the spread of ES the auditory system might also be directly or indirectly affected.

Here, we have shown that sinusoidal ES of the mastoid leads to perceivable AMs in auditory percepts. Since this effect is not limited to externally presented tones and is also present in auditory illusions, it might help to shed light on the origins of such after-effects.

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CHAPTER 4

BOUNCE PHENOMENON IN GERBILS

The following study was published under the title "Slow oscillatory changes of DPOAE magnitude and phase after exposure to intense low-frequency sounds" in the Journal of Neurophysiology by Margarete Anna Ueberfuhr and Markus Drexl on the 1st of May 2019.

4.1 Author Contribution

The author of this thesis performed experiments, analyzed and interpreted the data and prepared figures for publication. The corresponding author, Markus Drexl, conceived and designed the study, was of great support during data acquisition and data analysis and interpreted the results of the experiments. Both authors drafted, edited and revised the manuscript. They approved the final version of the manuscript.

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Slow oscillatory changes of DPOAE magnitude and phase after exposure to intense low-frequency sounds

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Ueberfuhr MA, Drexl M. Slow oscillatory changes of DPOAE magnitude and phase after exposure to intense low-frequency sounds. *J Neurophysiol* 122: 118–131, 2019. First published May 1, 2019; doi:10.1152/jn.00204.2019.—Sensitive sound detection within the mammalian cochlea is performed by hair cells surrounded by cochlear fluids. Maintenance of cochlear fluid homeostasis and tight regulation of intracellular conditions in hair cells are crucial for the auditory transduction process but can be impaired by intense sound stimulation. After a short, intense low-frequency sound, the cochlea shows the previously described "bounce phenomenon," which manifests itself as slow oscillatory changes of hearing thresholds and otoacoustic emissions. In this study, distortion product otoacoustic emissions (DPOAEs) were recorded after Mongolian gerbils were exposed to intense low-frequency sounds (200 Hz, 100 dB SPL) with different exposure times up to 1 h. After all sound exposure durations, a certain percentage of recordings (up to 80% after 1.5-min-long exposure) showed oscillatory DPOAE changes, similar to the bounce phenomenon in humans. Changes were quite uniform with respect to size and time course, and they were independent from sound exposure duration. Changes showed states of hypo- and hyperactivity with either state preceding the other. The direction of changes was suggested to depend on the static position of the cochlear operating point. As assessed with DPOAEs, no indication for a permanent damage after several or long exposure times was detected. We propose that sensitivity changes occur due to alterations of the mechanoelectrical transduction process of outer hair cells. Those alterations could be induced by different challenged homeostatic processes with slow electromotility of outer hair cells being the most plausible source of the bounce phenomenon.

NEW & NOTEWORTHY Low-frequency, high-intensity sound can cause slowly cycling activity changes in the mammalian cochlea. We examined the effect of low-frequency sound duration on the degree of these alterations. We found that cochlear changes showed a stereotypical biphasic pattern independent of sound exposure duration, but the probability that significant changes occurred decreased with increasing sound duration. Despite exposure durations of up to 1 h, no permanent or transient impairments of the cochlea were detected.

bounce phenomenon; Ca^{2+} oscillations; endocochlear potential; low-frequency sound; mechanoelectrical transfer function

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INTRODUCTION

Detecting faint acoustic pressure waves with amplitudes of a few micropascals is a demanding task, performed with exquisite sensitivity by the mammalian cochlea. To ensure sensitivity and integrity, the cochlea relies on a variety of homeostatic mechanisms. These mechanisms regulate intracellular conditions of the cochlear primary receptors, the hair cells, and sustain the extracellular ion balance within surrounding cochlear fluids.

Incoming sound waves cause pressure oscillations within the cochlear fluids. Hair cells, characterized by stereocilia bundles protruding from the apical surface of the cell bodies, detect those pressure oscillations. The stereocilia are deflected in response to pressure oscillations, and as a result, cation-selective mechanoelectrical transduction (MET) channels at the tips of the stereocilia change their opening probability.

Two populations of hair cells can be distinguished on the basis of their morphology and function. The inner hair cells serve to inform the ascending auditory pathway. The outer hair cells (OHCs) are electromotile and translate their receptor potential into mechanical energy, serving as receptors and effectors at the same time. Electromotility has a fast component (Frank et al. 1999), mainly serving to amplify cochlear pressure waves, but can also be slow and induced by oscillations of intracellular calcium ion concentration ($[Ca^{2+}]$) (Dulon et al. 1990; Patuzzi 2011). Thereby, calcium ions (Ca^{2+}) affect the cochlear amplification process, and regulation of $[Ca^{2+}]$ is consequently relevant to control hearing sensitivity.

Both populations of hair cells plus supporting cells are incorporated in the organ of Corti, which rests on the basilar membrane within the so-called scala media. The basilar membrane is displaced by pressure waves and shows topographically organized peak displacements along the membrane length depending on the sound frequency, where high-frequency sounds localize near the cochlear base and low-frequency (LF) sounds travel all the way to the apex. The upper surface of the organ of Corti forms an ion-tight boundary between two inner ear fluids: potassium-rich endolymph in the scala media and perilymph, a fluid low in potassium ions (K^+), in the enclosing structures. As a result, hair cells are surrounded by two environments with very different potassium ion concentration ($[K^+]$), with their apical surfaces exposed to the endolymph and their basolateral surfaces to the perilymph.

The high $[K^+]$ in the endolymph is due to the interaction of several ion transporters in a highly vascularized structure of the cochlear lateral wall, the stria vascularis, and results in an endocochlear potential (EP) of scala media, which can be as large as 120 mV in mammals (for review, see Köppl et al. 2018). The large EP is essential, because it yields a voltage difference compared with the negative membrane potential of hair cells (−70 mV) and thereby serves as the main driving force for the transduction current, chiefly carried by K^+. Because the K^+ influx via MET channels into the hair cells causes a depolarization of their membrane potential, a high EP, and as the underlying cause, a high $[K^+]$, is an essential requirement for sensitive hearing. This becomes obvious when the EP is transiently or permanently reduced and sensitive hearing is lost due to anoxia (Konishi et al. 1961), loop diuretics (Kusakari et al. 1978; Sewell 1984), or acoustic overstimulation of the cochlea (e.g., Hirose and Liberman 2003; Ide and Morimitsu 1990; Ikeda et al. 1988; Konishi et al. 1979; Salt and Konishi 1979; Wang et al. 2002). Therefore, processes providing a tight regulation of $[K^+]$ should be in place. Besides $[K^+]$ and $[Ca^{2+}]$, other components modulate cochlear sensitivity. The signaling molecule adenosine triphosphate (ATP) is involved in the homeostasis of the EP (Housley et al. 1999; Marcus et al. 1998; Muñoz et al. 2001) and must be regulated. Endolymphatic ATP can activate purinergic receptors and induce K^+ currents (Housley et al. 1999, 2002, 2013) in cells within the stria vascularis or in supporting cells and hair cells of the organ of Corti (Liu et al. 1995; Nakagawa et al. 1990; Nilles et al. 1994).

To study cochlear sensitivity changes and their underlying homeostatic mechanisms, a previously established paradigm, the induction of the bounce phenomenon (BP), was applied in the current study. The BP is a transient phenomenon that was shown to be elicited by a relatively short (up to 5 min) LF stimulation and takes the form of cochlear activity changes assessed with psychophysical or biophysical methods:

Human behavioral hearing thresholds oscillated 2–4 min after exposure to intense LF tones between states of hyper- und hyposensitivity (Hirsh and Ward 1952; Hughes 1954; Noffsinger and Olsen 1970; Noffsinger and Tillman 1970; Zwicker and Hesse 1984). With respect to biophysical methods, otoacoustic emissions (OAEs) of different types [transient OAEs (Kemp 1986; Kemp and Brill 2009), distortion product otoacoustic emissions (DPOAEs) (Drexl et al. 2014), and spontaneous otoacoustic emissions (SOAEs) (Jeanson et al. 2017; Kemp 1986; Kugler et al. 2014)] were recorded after short LF sound stimulation. OAEs are a by-product of active cochlear amplification processes attributed to the OHCs (i.e., the cochlear amplifier) and can represent a surrogate for cochlear activity. Whereas SOAEs occur without sound presentation, DPOAEs can be elicited by presenting two so-called primary tones. OAEs showed slow magnitude, frequency (SOAEs only), and phase changes after exposure to LF sound with a time course remarkably resembling those of BP-associated hearing threshold changes.

In the present study, the BP was tested in Mongolian gerbils. DPOAEs were recorded after exposure to intense LF sounds with stimulus durations ranging from a few seconds up to 1 h. The purpose of this study was to understand the time course of adjustments made by cochlear homeostatic processes challenged by a cochlear insult (LF sound exposure), how these adjustments change as a function of insult duration, and whether they eventually evolve into cochlear damage with very long exposure durations.

METHODS

Animals

Eighteen Mongolian gerbils (*Meriones unguiculatus*) of the in-house breeding colony at the Department Biology II of the Ludwig-Maximilians Universität Munich were investigated. Gerbils were housed in groups of three to five individuals with a 12:12-h light-dark cycle and with food and water ad libitum. All animals used in this study weighed between 55 and 80 g, were 2–5 mo old, and were of both sexes (11 females and 7 males). They had clean ear canals and did not show signs of middle ear infection. The animals were anesthetized with midazolam (0.15 mg/kg body wt), medetomidin (7.5 mg/kg body wt), and fentanyl (0.03 mg/kg body wt; MMF). MMF was injected subcutaneously under the skin of the neck region. To maintain anesthesia levels during recording sessions, one-third of the initial dosage was injected every 60 min, or earlier when the regularly checked pedal withdrawal reflex indicated a drop of anesthesia levels. At the end of the experiments, MMF was antagonized with a one-time injection of atipamezol (4 mg/kg body wt), flumazenil (0.4 mg/kg body wt), and naloxone (0.5 mg/kg body wt). Body temperature was monitored and maintained by a heating pad (38°C ± 0.5°C) and blanket, which covered the animal during the experiments. Each animal was used in three sessions including several experimental trials. Experimental protocols were approved by the District Government of Upper Bavaria ("Regierung von Oberbayern"; Ref. No. ROB-55.2-2532.Vet_02-17-17).

Signal Generation and Data Acquisition

DPOAEs were recorded in a sound-attenuated chamber with a custom-made acoustic coupler (based on the Eaton-Peabody-Laboratory Acoustic System), featuring a coaxial design of acoustically separate microphone and loudspeaker channels. The coupler was inserted into the ear canal of the anesthetized animal under visual control such that it was at a distance of ~1–2 mm from the tympanic membrane. The acoustic coupler contained a condenser microphone (FG-23329-P07; Knowles, Itasca, IL) and two micro dynamic speakers (CDMG15008-03A; CUI, Tualatin, OR).

Signals were generated and recorded with an external sound card (RME Fireface UC 24-bit; Audio, Haimhausen, Germany) controlled by scripts written in MATLAB (The MathWorks, Natick, MA). The SoundMexPro sound application (HörTech, Oldenburg, Germany) was used for audio stream input/output-based interfacing in the MATLAB environment. The sampling rate was 44.1 kHz, and the digital word length was 24 bits. The magnitude and phase response of the probe microphone were compared with the response of a reference microphone (type 4135; Brüel & Kjær Sound & Vibration Measurement, Nærum, Denmark) and corrected accordingly.

The two DPOAE primary tones were generated by the coupler loudspeakers. These speakers were calibrated in situ before each trial to guarantee constant sound pressure at the probe microphone. For the calibration, the ear canal was stimulated with bandpass-filtered white noise (0.2–20 kHz), and the frequency and phase responses of the loudspeakers were calculated and compared with a flat frequency and phase response. For each loudspeaker, a compensational impulse response was derived. All acoustic stimuli were convolved with the respective compensational impulse response before delivery. The problem of sound pressure level deviations between tympanum and probe microphone due to quarter-wavelength standing waves (Siegel 2007) can be neglected because frequencies used in the present studies did not exceed 8 kHz, for which only small or no deviations in sound pressure level were shown in the gerbil ear (Ravicz et al. 2007).

The LF tone of 200 Hz at 100 dB SPL was presented by a separate loudspeaker (NSW1-205-8A; Aura Sound, Santa Fe Springs, CA) and amplified by an RB-960BX power amplifier (Rotel, Worthing, UK). This woofer was connected to an 82.5-cm-long polyethylene tube (inner diameter 1 mm) and fed and sealed into the loudspeaker channel of the coupler. Because the LF sound for evoking the BP consisted of one fixed frequency (200 Hz), calibration comprised determining the sound pressure level of stimuli with known digital amplitudes in the ear canal and checking that higher harmonics were sufficiently low (magnitudes at least 30 dB lower than the magnitude of the fundamental frequency).

DPOAE Recordings

Because of the interaction of the two primary tones with two different frequencies, f_1 and f_2, DPOAEs are generated that show frequencies derived from f_1 and f_2. In the following, magnitudes and phases of quadratic DPOAEs (QDP; $f_2 - f_1$) and cubic DPOAEs (CDP; $2f_1 - f_2$) were extracted from the same recording and followed over time.

To evoke DPOAEs, two primary tones f_1 and f_2 ($f_2/f_1 = 1.25$), with f_1 level (l_1) and f_2 level (l_2) set to 65/55 (l_1/l_2) and 65/65 dB SPL were presented repetitively (repetition rate = 0.6 Hz) with a stimulus duration of 250 ms. Presentation of primary tones and DPOAE recordings were sample-synchronized and repeated a minimum of 16 times, but up to 32 times to end up with 16 valid repetitions that resulted in one data point (see Fig. 1*A*). Repeated recordings were rejected if the signal-to-noise ratio of the DPOAE spectral line was <6 dB. Depending on the number of rejections, recording time for each data point varied but was typically around 26 s. This resulted in a slightly uneven time spacing of longitudinal measurements, with the benefit that the number of averaged waveforms contributing to one data point was constant.

Optimization of the Primary Tone Frequency f_2

While the ratios f_2/f_1 (1.25) and l_1/l_2 (65/55 or 65/65 dB SPL) were kept constant across animals and ears, the primary frequencies were optimized in each ear before the measurements were performed. A so-called f_2/f_1 run was conducted, in which QDPs were measured with variable f_2 frequencies, starting from 4 kHz and increasing in 0.5-kHz increments up to 8.5 kHz. Of all those frequencies, the f_2 frequency resulting in the largest QDP magnitude was chosen for subsequent measurements, with the restriction that the f_2 frequency was not allowed to coincide with any harmonic of the LF tone (see Fig. 1*A*). Furthermore, the f_2 frequency resulting in the largest QDP magnitude was chosen. If the f_2/f_1 run resulted in several maxima, f_2 with the lowest frequency was chosen for the experimental trials, because the impact of the LF sound was shown to be larger when the difference between the characteristic frequency site of the OAE and the LF sound was small (Bian et al. 2002; Jeanson et al. 2017). f_2 was mostly in the range of 4.5–5.5 kHz.

Measurements

After f_2 frequency was optimized, frequencies and levels of the primary tones were kept constant in all trials of the experimental and control condition (see Fig. 1*A*). One trial consisted of 10 measured DPOAE data points (spaced by ~26 s; see *DPOAE recordings*) before the start of the LF sound and 20 measured DPOAE data points after the end of the LF sound. Trials varied in their overall duration, because LF sound was presented with 12 different durations (in min): 0.17 (=10 s), 0.5, 1, 1.5, 2, 5, 10, 15, 20, 25, 30, and 60. In control trials, the LF sound stimulation was replaced by a period of silence with the same duration as the LF sound presentation. In every animal, recordings were conducted in both ears; however, not all durations were recorded in each ear, and in total, there were only between 5 and 10 control recordings measured per LF duration. In some trials, DPOAEs could also be measured during LF sound presentation. In those cases, as many data points as could be gathered during the exposure duration were recorded (spaced by ~26–50 s).

Data Analysis

Magnitude and phase of QDPs and CDPs were extracted from the ear canal recordings after time-domain averaging with a Fourier transform (44,100 points). Analysis for control trials and for experimental trials was identical, so when LF sound exposure or pre- and postexposure are mentioned in the following, it also refers to intervals during, before, and after silence in control trials.

Control trials and experimental trials were excluded from analysis in cases where one of the three exclusion criteria was fulfilled:

1) Trials were excluded if the levels (l_1 and l_2) of the primary tones were not stable over the time course of the recording, thereby altering the baseline for DPOAEs. This means, in detail, that trials were not included if magnitudes of QDPs and primary levels changed (>1 SD) in recordings with sound pressure level changes of >5 dB.

2) Trials were excluded if the magnitude of the QDP was not stable during the preexposure period. To detect changes, a change-point analysis was applied (Taylor 2000). For this, the mean of time series data points was subtracted from the individual data points during preexposure, and the cumulative sum was calculated. The difference between the minimum and maximum of this sum was then determined. A bootstrap analysis was conducted to randomly reorder measured preexposure data points. The percentage of the 1,000 bootstrap samples that show smaller differences between the minimum and maximum of their cumulative sums resulted in a confidence interval. A change in the preexposure QDP magnitude was considered significant when the confidence interval was ≥95%.

3) Trials were excluded if during the postexposure period, 8 or more of the 20 data points were not considered valid due to a low signal-to-noise ratio (<6 dB).

The noise floor was calculated by averaging the magnitudes of six spectral lines below and six above the respective DPOAE frequency.

To detect changes following the LF sound exposure, four preparatory steps were taken:

1) Magnitudes of QDPs and CDPs were expressed relative to the mean magnitude or mean phase of the preexposure period.

2) To compensate for slow DPOAE magnitude drifts that occurred mainly at longer recording durations, probably due to changes of anesthesia level, the best least-squares fit representing the linear magnitude drift was removed from recorded DPOAEs.

3) When postexposure recordings did not return to preexposure levels but showed a baseline shift at the end of the recording (6–10 min postexposure), mean displacements were subtracted from relative values and analyzed separately from the oscillatory behavior. This ensured that oscillatory changes could be pooled over several measurements.

4) In addition, the number of data points was increased by linear interpolation to facilitate the main analysis.

The main analysis comprised two principal steps (see Fig. 1*B*):

1) A change-detection analysis analogous to the analysis for the preexposure period (see *exclusion criterion 2*) was conducted to evaluate whether a significant magnitude or phase change occurred after LF sound exposure. The maximal difference of the cumulative sums of DPOAE magnitude and phase, from which the means were previously subtracted, were compared with those of respective bootstrap samples (1,000 samples). When the confidence interval resulted in a value >95%, the recording was considered to change significantly.

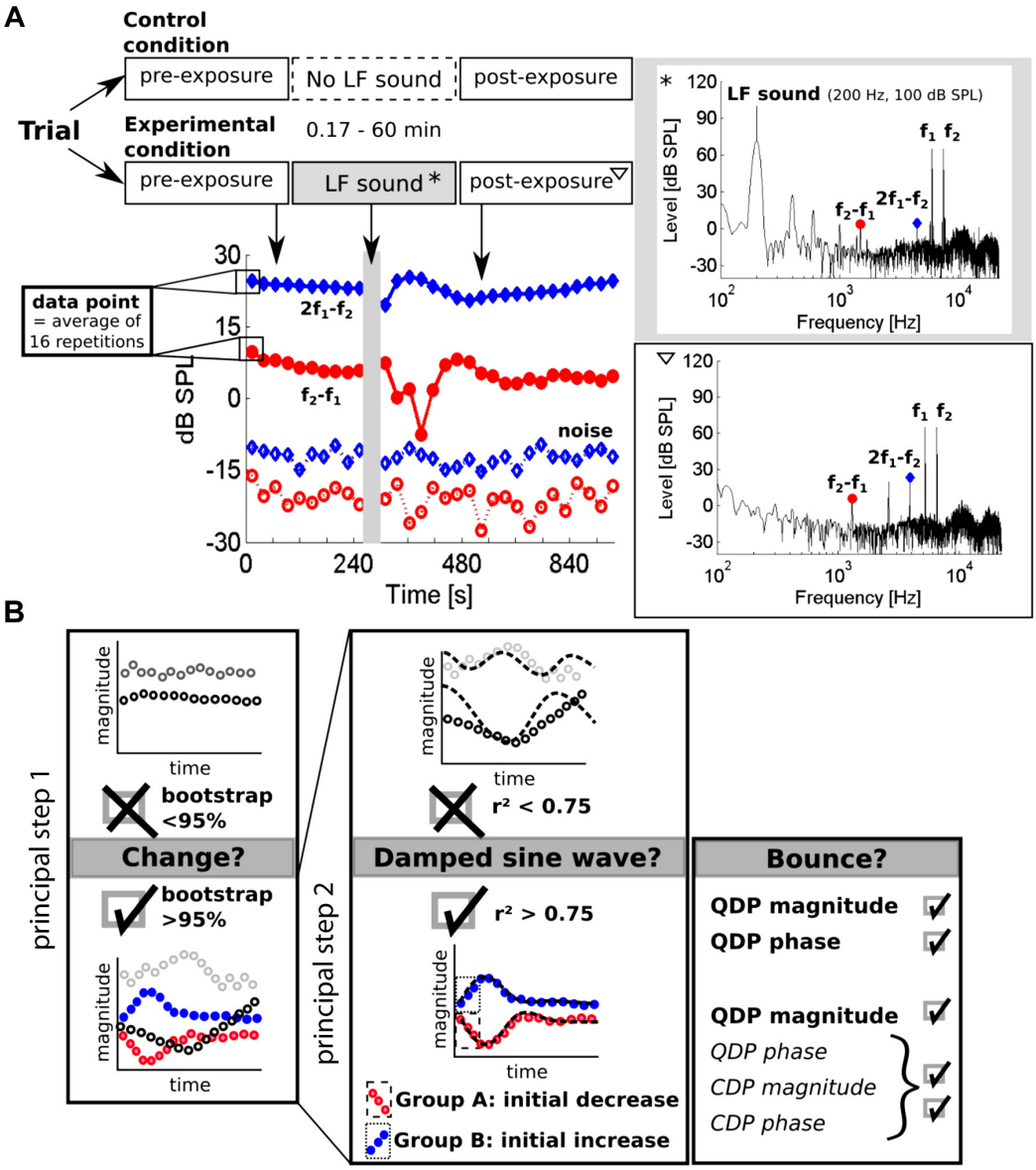

Fig. 1. *A*: schematic explanation of time course and terminology of trials, and data from an exemplary trial. Asterisk indicates the frequency spectrum in the ear canal during sound exposure with low-frequency (LF) sound at 200 Hz, 100 dB SPL, and concurrent stimulation with primaries at 6 kHz (f_1) and 7.5 kHz (f_2) at 65 dB SPL. The spectral lines of distortion product otoacoustic emissions (DPOAEs) are marked, with the cubic DPOAES (CDP; $2f_1 - f_2$) in blue and the quadratic DPOAEs (QDP; $f_2 - f_1$) in red. *B*: protocol applied for data analysis. Time series data of postexposure periods had to fulfill 2 principal steps and were then assigned to *group A* with an initial decrease or to *group B* with an initial increase of the oscillatory change. To classify a trial as a trial with bounce phenomenon (BP), conservative criteria were applied with at least 2 of the 4 measures (QDP magnitude and phase, CDP magnitude and phase) required to show a BP-associated change.

2) To test whether this change showed a typical bounce-like slow oscillatory behavior, postexposure values were fitted with an underdamped sinusoidal oscillation: if r^2 reached values ≥0.75, the DPOAE was thought to show a bounce-like behavior.

Change-detection analysis and fitting procedures were run for QDPs (magnitude and phase) and CDPs (magnitude and phase). Finally, to determine whether a BP took place, the QDP magnitude had to fulfill *principal steps 1* and *2*: a significant change of magnitude or phase with a sinusoidal time course. In addition, the QDP phase or at least two of the three measures (QDP: phase; CDP: magnitude and phase) had to show a significant change (*principal step 1*), and one of those measures had to fulfill the fitting criteria (*principal step 2*) with $r^2 \geq 0.75$. The fitted sinusoidal waves were also used for an estimate of decay time constant and frequency of the oscillation.

According to the pattern of the oscillatory behavior, two groups could be distinguished (see Fig. 1*B*). Therefore, slopes between data points were calculated.

Group A showed rather a decrease followed by an increase of the first data points after LF sound exposure. The mean of the first three

slope values was <0, and the oscillation reached its maximum ~90 s after LF sound offset.

Group B, in contrast, was characterized first by an increase, followed by a decrease, of postexposure data points. The average of the first three slope values was positive, suggesting that the oscillation maximum was happening within the first 90 s after LF sound exposure.

Depending on whether the data were normally distributed (one-sample Kolmogorov-Smirnov test, $\alpha = 0.05$) or not, statistical analysis was carried out with parametric or nonparametric tests. In the text, quartiles were indicated in brackets or given as the range between which values were found. The Skillings-Mack test (SMT; Skillings and Mack 1981) for nonparametric data and repeated nonsymmetric design was applied to establish whether bounce indicators differed significantly between LF sound exposure durations.

RESULTS

Measurements from 28 ears of 17 animals were included in the analysis. In six ears, measurements were not attempted as the tympanic membrane or the ear canal were obstructed by cerumen. In 25 of those 28 ears, a bounce-like oscillatory behavior could be found in at least one of the experimental trials (see criteria explained in Fig. 1*B*). No bounce was found in the control trials except in 3 of the 53 trials with a silent period of 10 min, where recorded QDP magnitudes could be fitted with a damped sine wave. However, the changes were small: the median showed a value below 1.5 dB for peak-to-peak difference. This was significantly lower than all medians in the experimental trials (starting at around 4 dB). Of the 226 experimental trials included in the analysis, 146 trials were found to show a bounce with postexposure changes in QDP magnitude and at least one other measure (see criteria explained in Fig. 1*B*). The QDP magnitude was the most sensitive measure for the bounce with oscillatory changes occurring with a higher probability than in the other measures (see Fig. 6*A*). This was consistent with previous findings in humans (Drexl et al. 2014).

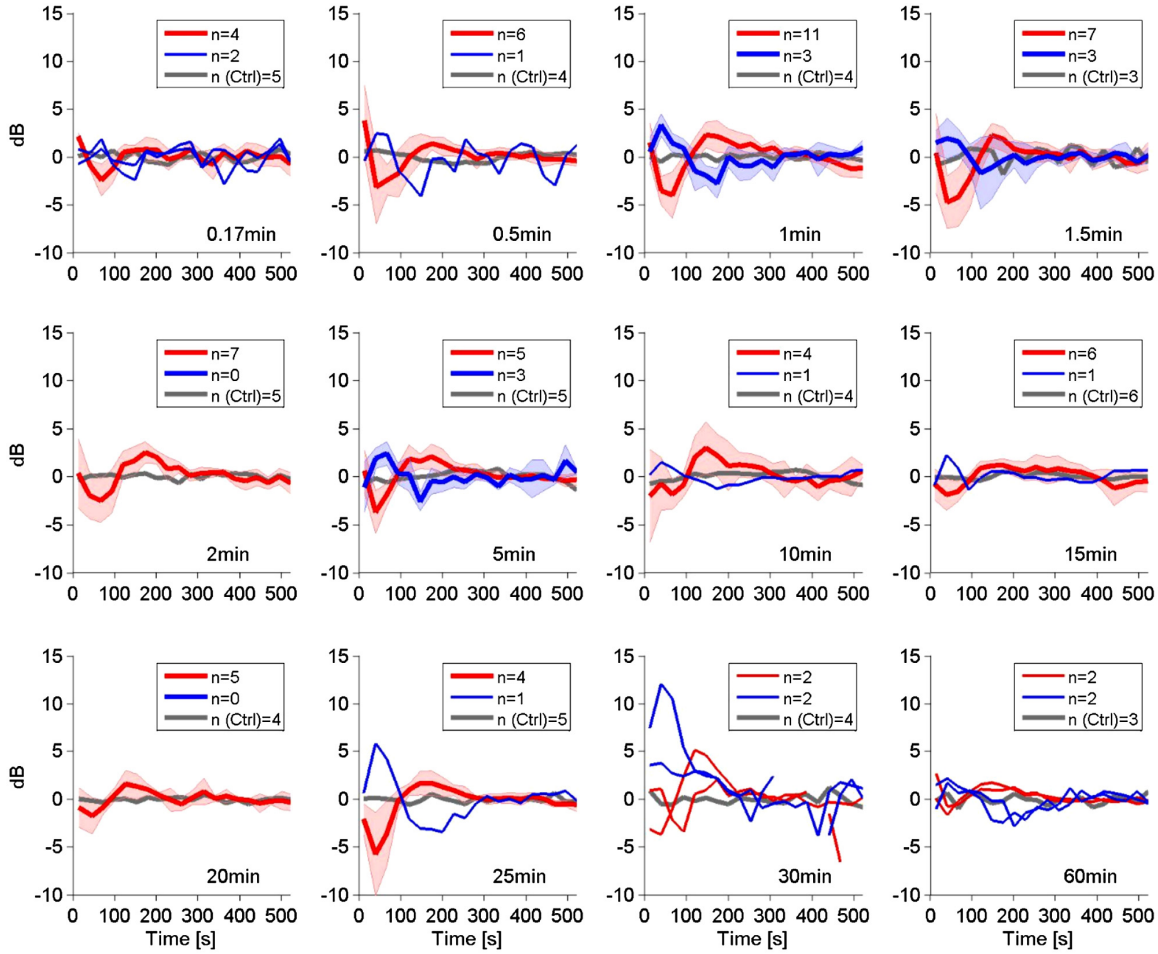

Fig. 2. Quadratic distortion product otoacoustic emissions (QDP; $f_{2-} - f_1$) showing a bounce phenomenon after low-frequency (LF) sound exposure at different exposure times. Plots show average changes of QDP magnitude (lines) with corresponding SD (shaded areas) relative to the average magnitude of the preexposure magnitude as a function of postexposure time (indicated in each panel; LF sound offset = 0 s). Gray lines represent the mean of data from control (Ctrl) trials with a period of silence instead of LF sound presentation. LF sound was presented with a frequency of 200 Hz at 100 dB SPL. Recordings were split into 2 groups: *group A*, experimental trials showing changes with initial decrease (red); *group B*, oscillatory changes with initial increase (blue). Number of ears (n) is indicated in each panel. For $n < 3$, single recording traces relative to average magnitude of the preexposure magnitude are shown instead of means ± SD.

Baseline Shifts

Bounce phenomena could be recorded at all LF sound durations from 0.17 min (10 s) up to 60 min (Figs. 2 and 3). Baseline shifts, defined as DPOAEs not returning to preexposure levels at the end of the postexposure recording, occurred to a small extent at all exposure durations. Baseline shifts regarding DPOAE magnitudes were in the range between −1.6 and 1.7 dB (QDP magnitude) and between −1.4 and 1.9 dB (CDP magnitude). Regarding DPOAE phases, baselines shifted between 5.3° and 22.2° (QDP phase) and between 0.3° and 13.7° (CDP phase).

Sinusoidal Oscillations Categorized into Two Oscillatory Patterns

Sinusoidal oscillations after LF sound offset showed different signs. Changes were roughly categorized into two groups (*groups A* and *B*; see Fig. 1*B*). In *group A*, values (QDP magnitude and phase, CDP magnitude and phase) decreased, resulting in a minimum as the first extreme value, and then increased to the second extreme value, a maximum, before returning to baseline. In *group B*, the inverted oscillatory behavior was observed. The first extreme value was a maximum and the second extreme value, a minimum. For *groups A* and *B*, according to the underlying categorization, the 1st extreme value was found between 38 and 67 s, the 2nd extreme value at 120–180 s, and the return to baseline occurred around 200–280 s. Interestingly, the two bounce patterns of *groups A* and *B* could be detected in the very same ear in different experimental trials. In 13 ears, oscillatory behaviors varied under different experimental conditions, e.g., different LF sound exposure durations; in 2 ears, inverted oscillatory behavior could also be observed under the same experimental conditions. The majority of QDP magnitude changes began with a decrease followed by an increase (see Fig. 2). Only 30% (median = 30%; [18%,

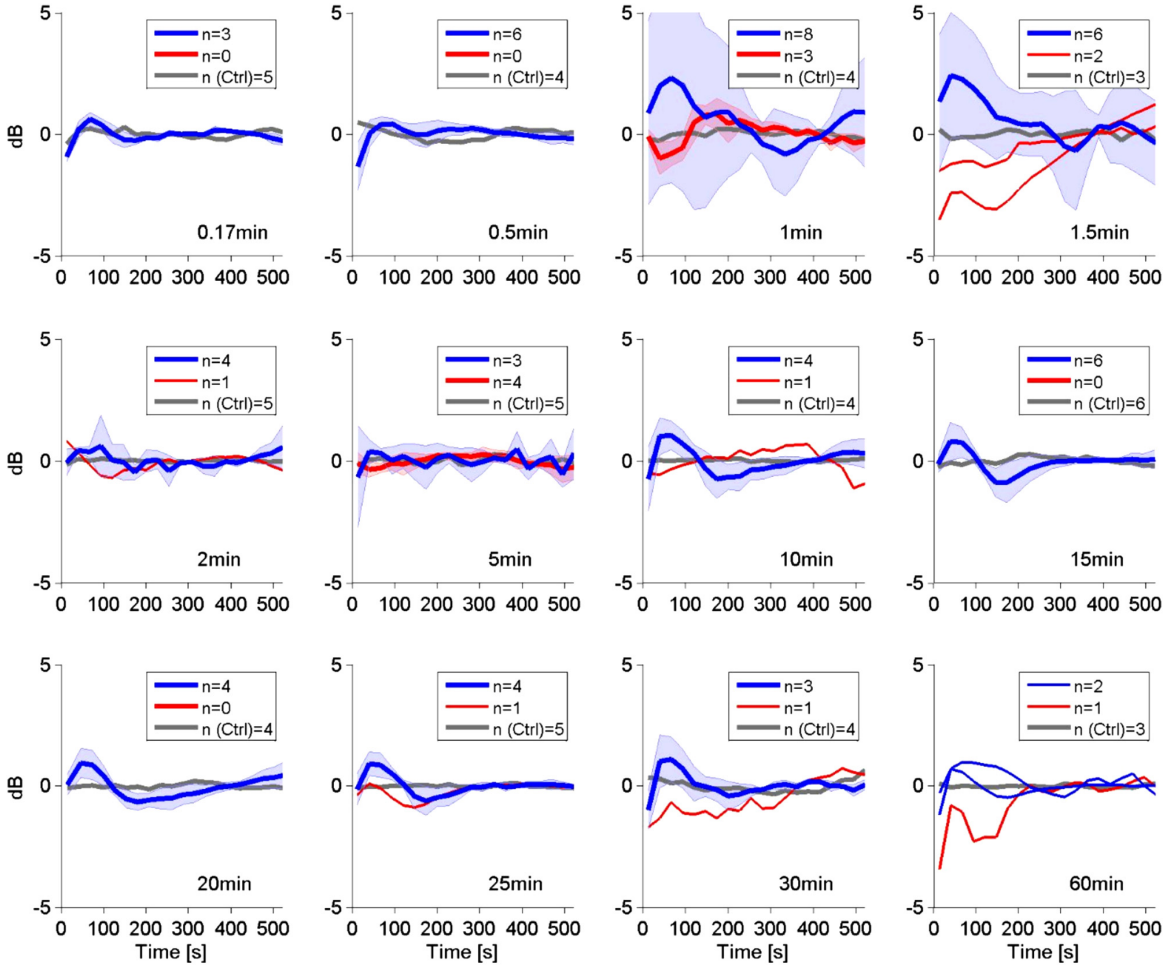

Fig. 3. Cubic distortion product otoacoustic emissions (CDP; $2f_1 - f_2$) showing a bounce phenomenon after low-frequency (LF) sound exposure at different exposure times. Plots show average changes of CDP magnitude (lines) with corresponding SD (shaded areas) relative to the average magnitude of the preexposure magnitude as a function of postexposure time (indicated in each panel; LF sound offset = 0 s). Gray lines represent the mean of data from control (Ctrl) trials with a period of silence instead of LF sound presentation. LF sound was presented with a frequency of 200 Hz at 100 dB SPL. Recordings were split into 2 groups: *group A*, experimental trials showing changes with initial decrease (red); *group B*, oscillatory changes with initial increase (blue). Number of ears (n) is indicated in each panel. For $n < 3$, single recording traces relative to average magnitude of the preexposure magnitude are shown instead of means ± SD.

38%]) of experimental trials showed an initial increase (*group B*) for the QDP magnitude. In contrast, QDP phase changes started off with a phase increase followed by a decrease far more often than the other way round (median = 75%; [63%, 86%]). This suggested that oscillation patterns were not parallel with regard to all four measures (QDP magnitude and phase, CDP magnitude and phase).

In experimental trials in which a bounce was found, either CDP and QDP magnitude oscillated concurrently in parallel (19.7%) or the two magnitudes showed an inverted effect regarding their time courses with a probability of 59.1%. However, CDP magnitude changes were smaller compared with QDP magnitude changes (SMT: $t = 48.4$, df = 1, $P = 0.34 \times 10^{-12}$; see Fig. 3) and even nonexistent in the remaining 21.2% of experimental trials with a significant bounce.

Characterizing the Peak-to-Peak Differences of Magnitude and Phase Changes

To further characterize the bounce, the differences between the maximum and the minimum of the oscillatory change (QDP magnitude and phase, CDP magnitude and phase) were measured and termed peak-to-peak difference (see Fig. 4, *A* and *D*). Peak-to-peak-differences of changes did not differ significantly between varying LF sound exposure durations (QDP magnitude: $t = 9.5995$, df = 11, $P = 0.5667$; QDP phase: $t = 3.6224$, df = 11, $P = 0.9797$; CDP magnitude: $t = 1.7697$, df = 11, $P = 0.9992$; CDP phase: $t = 5.5047$, df = 11, $P = 0.9043$). No significant differences between single exposure times were found when post hoc pairwise comparisons were run with Benjamini-Hochberg corrections.

Across all sound exposure durations, QDP peak-to-peak differences showed medians within the interval 4 and 7.3 dB for the magnitude (see Fig. 4*B*) and between 22.3° and 45.6° for the phase (see Fig. 4*E*). In comparison, CDP peak-to-peak differences were smaller with changes between 1.2 and 2.1 dB for the magnitude (see Fig. 4*C*) and between 5.9° and 12.4° for the phase (see Fig. 4*F*).

DPOAEs were elicited by different primary tone frequencies with f_2 between 4 and 8 kHz. Changes in DPOAE magnitude and phase were evoked by an intense 200-Hz tone. This implies that the intense LF tone traveling to the cochlear apex modulated cochlear sensitivity not only at its characteristic frequency but also at higher frequencies. In the present study, higher frequencies in the range from 4 to 8 kHz seemed to be influenced by the intense LF sound to a similar degree, because no statistically significant effect was found between the QDP magnitude change and the corresponding f_2 frequency (SMT: $t = 3.8025$, df = 7, $P = 0.8022$) regarding the size of the peak-to-peak difference.

Characterizing the Temporal Pattern of Magnitude and Phase Changes

Decay time constants and frequencies of the slow-magnitude oscillations (see Fig. 5, *A* and *C*) were determined for all recordings showing a significant bounce. To determine time constant and frequency, sinusoidal waves fitted to the QDP magnitude were used, which showed r^2 values of 0.96 [0.87, 0.99]. No significant differences with regard to decay time constant (QDP magnitude: $t = 2.5617$, df = 11, $P = 0.9953$) and frequency (QDP magnitude: $t = 5.9178$, df = 11, $P = 0.8788$) were shown. Post hoc pairwise comparison with Benjamini-Hochberg correction did not show any significance between single exposure times either.

Some of the bounce recordings (14.4%) did not show a damping strong enough to induce a 50% decrease within 10 min of postexposure tracking. In cases where damping took place, the time constants showed medians between 52.3 and 97 s for sine waves fitted to QDPs (see Fig. 5*D*). The frequency of the time course of the QDP magnitude was characterized with medians within the range of 0.29 and 0.32 cycles/min (see Fig. 5*B*).

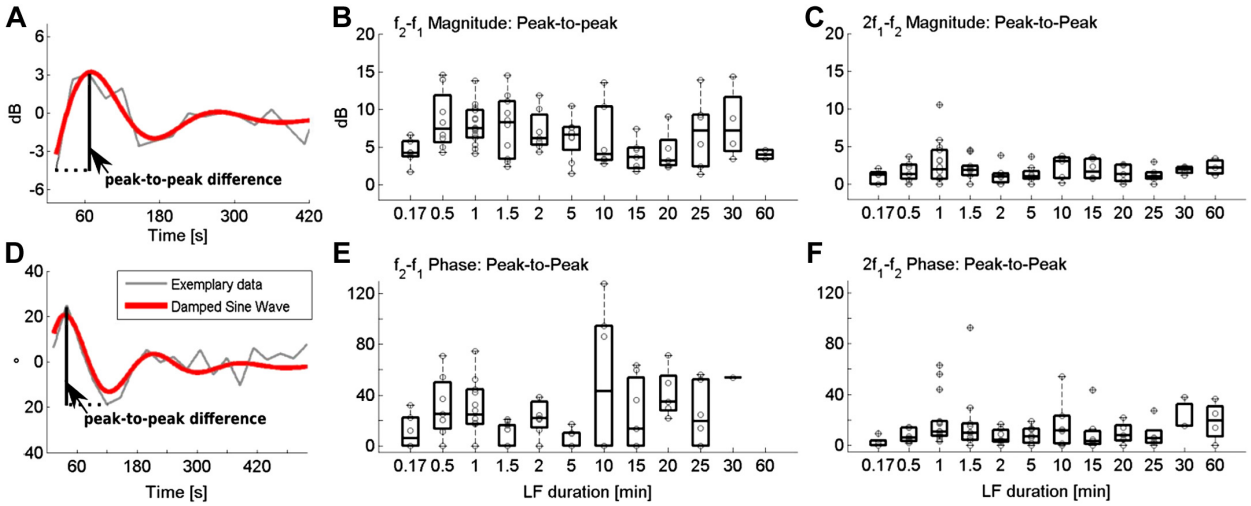

Fig. 4. Comparison of the size of oscillatory changes of transient distortion product otoacoustic emissions (DPOAEs) showing a bounce phenomenon as a function of exposure duration with low-frequency (LF) sound. *A* and *D*: exemplary illustration of bounce parameters assessed with DPOAE magnitude (*A*) and phase changes (*D*). *B* and *E*: quadratic DPOAE ($f_2 - f_1$) magnitude and phase changes, respectively. *C* and *F*: cubic DPOAE ($2f_1 - f_2$) magnitude and phase changes, respectively. Peak-to-peak is the difference between minimum and maximum of the oscillatory change after LF offset. Box plot borders indicate 25%, 50% (median), and 75% quartiles; whiskers indicate the most extreme values not considered as outliers (+).

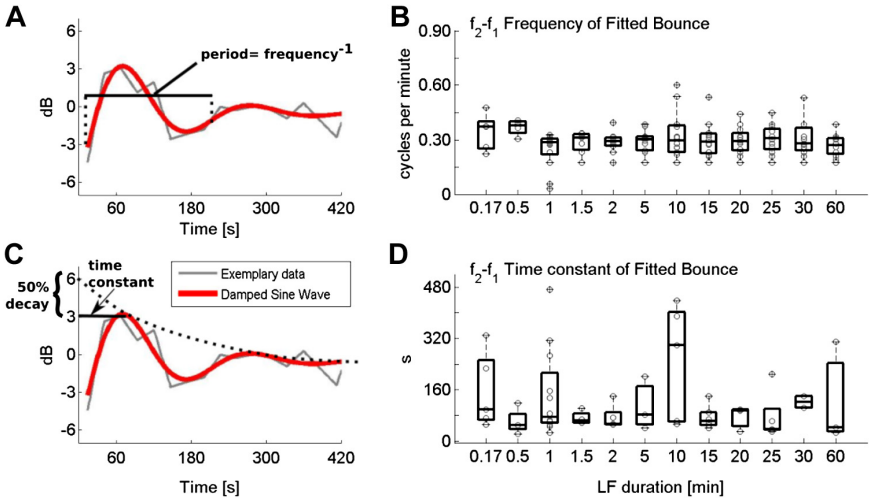

Fig. 5. Comparison of the time-related parameters drawn from fitted underdamped sine waves to describe oscillatory changes of distortion product otoacoustic emissions (DPOAEs) with bounce phenomenon (BP) as a function of exposure duration with low-frequency sound. *A* and *C*: exemplary illustration of bounce parameters period (*A*) and time constant (*C*). *B* and *D*: quadratic DPOAE ($f_2 - f_1$) frequency of BP (expressed as cycles/min; *B*) and time constant of BP (*D*). Box plot borders indicate 25%, 50% (median), and 75% quartiles; whiskers indicate the most extreme values not considered as outliers (+).

Uniform Bounce Pattern?

Peak-to-peak-differences, frequencies, and time constants of the slow oscillations suggest that the BP shows a stereotypical time course and that bounce changes are independent from LF sound exposure times. The BP is not a phenomenon restricted to a particular mammalian species; it has now been shown in guinea pigs (Kirk et al. 1997; Kirk and Patuzzi 1997), gerbils (current findings), and humans (e.g., Drexl et al. 2014; Kemp 1986; Kemp and Brill 2009), and it is conceivable that it might be a feature of the mammalian cochlea in general. Despite the different DPOAE-generating mechanisms (Whitehead 1998) in humans (e.g., Mauermann et al. 1999a, 1999b) and rodents (e.g., Lukashkin and Russell 2005), the bounce shares several characteristics across species.

Table 1 summarizes the BP characteristics in gerbils and compares it with the BP in humans. Indicators were averaged over all valid experimental trials with BP, assuming that bounce characteristics are constant and independent from LF sound exposure duration.

Probability for Bounce Changes with LF Sound Exposure Duration

The percentage of experimental trials with a bounce is illustrated in Fig. 6*A* as a function of LF sound exposure duration. Independent of the duration, bounce-associated changes were found in 61.3% [55%, 65.5%] of cases. With sound exposures of 30 and 60 min, the bounce probability fell to <40%. QDP phase changes occurred less frequently (median = 22.3%, [10.9%, 31%]), and so did changes in the CDP magnitude and phase (median = 28.4%, [22.3%, 39.2%]; and median = 27.3%, [22.3%, 35.9%], respectively). For QDP magnitude and QDP phase changes, the probabilities to evoke the BP after a sound exposure of 60 min were smallest, suggesting that very long sound exposure durations were less likely to elicit a bounce.

Recordings During LF Sound Exposure

QDP and CDP (magnitudes and phases) were recorded while the LF sound was presented. In the following exemplary recording, the QDP magnitude was suppressed by 2–10 dB and CDP magnitude by 14–22 dB during LF sound exposure relative to the preexposure baseline. The largest suppression occurred right after the LF sound onset and decreased over the course of the LF sound presentation. Apart from the suppression, slow oscillatory magnitude changes (QDP and CDP) with a period of around 200–210 s were visible. Those oscillations were more prominent in the CDP magnitudes than in the QDP magnitudes and decreased in amplitude with longer exposure

Table 1. *Comparison of bounce parameters of otoacoustic emissions in gerbils and humans*

	BP Indicators in Gerbils	BP Indicators in Humans
Peak-to-peak difference: magnitude	6.4 ± 3.5 dB	QDP: 5.8 dB (median) (Drexl et al. 2014)
Peak-to-peak difference: phase	37.5 ± 30°	QDP: 39.7° (median) (Drexl et al. 2014)
Time constant of damped sinusoids	120 ± 108 s	SOAEs: 120 s (median) (Kugler et al. 2014)
Frequency of damped sinusoids	0.3 ± 0.09 cycles/min	QDP: 0.28 cycles/min (median) (Drexl et al. 2014)
		SOAEs: 0.23 cycles/min (0.204 cycles/min, 0.297 cycles/min) (Kugler et al. 2014)
		TEOAEs: 0.3 cycles/min (Kemp and Brill 2009)

Table summarizes values determined in the current study for bounce phenomenon (BP) indicators in gerbils (n = 146 trials, means ± SD) and values reported in literature for BP indicators in humans. All BP indicators in gerbils refer to quadratic distortion product otoacoustic emissions (QDPs). SOAEs, spontaneous otoacoustic emissions; TEOAEs, transient evoked otoacoustic emissions.

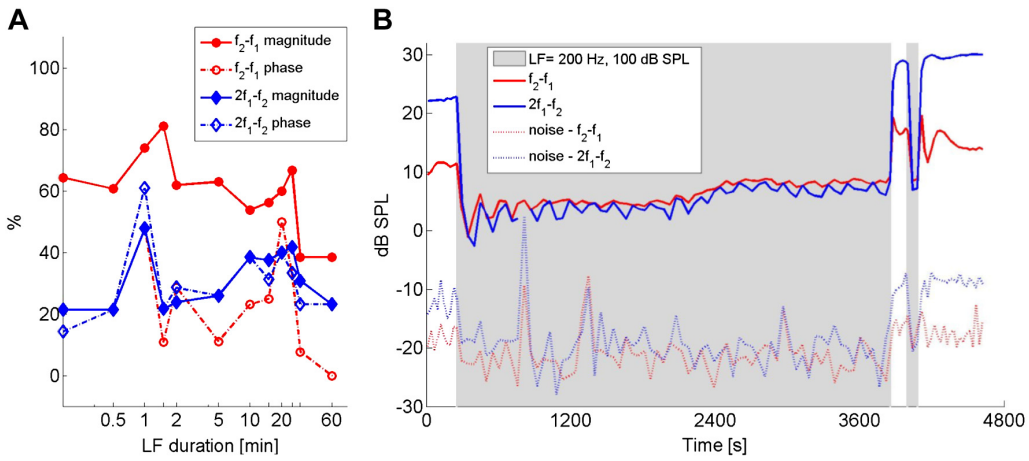

Fig. 6. *A*: percentage of experimental trials showing a bounce phenomenon (BP) in distortion product otoacoustic emissions (DPOAEs) as a function of low-frequency (LF) sound exposure time. $f_2 - f_1$, quadratic DPOAEs (QDPs; red); $2f_1 - f_2$, cubic DPOAEs (CDPs; blue). Solid lines are QDP and CDP magnitude, and dashed lines are QDP and CDP phase. *B*: representative example of data from an experimental trial before, during, and after LF sound exposure. Gray areas indicate LF sound exposure of 60 min and 1 min, respectively; LF sound was presented at 200 Hz, 100 dB SPL. Colored lines show magnitude of QDPs (red solid line) and corresponding noise floor (red dashed line), and magnitude of CDPs (blue solid line) and corresponding noise (blue dashed line). Beginning of trial = 0 s. DPOAE magnitudes are suppressed during LF sound exposure but show oscillations with a period similar to BP oscillations after LF sound offset. The BP occurs after a 60-min-long exposure and can be re-elicited by a 1-min-long exposure immediately after the 60-min-long exposure.

times. Interestingly, a stereotypical BP occurred at the offset of the 60-min-long LF sound exposure and could also be re-elicited after a short break, with a short 1-min-long LF sound. This second bounce also displayed stereotypical characteristics of a BP, but with a larger peak-to-peak difference than the first bounce shown in this example. Therefore, it can be proposed that an intense sound exposure of 60 min does not impair the underlying mechanism of the BP.

DISCUSSION

Summary of Results

Magnitude and/or phase of DPOAEs (QDPs and CDPs) oscillated for ~3–4 min after LF sound offset around the preexposure levels with periods of sensitization and desensitization. This shows that cochlear sensitivity in gerbils is significantly affected by LF sound stimulation. Tonotopic locations basal to the LF sound characteristic frequency place are affected when LF sound waves travel from base to apex. Primary tone frequencies in the present study were chosen such that they should be represented in cochlear regions where excitation patterns evoked by intense LF sound resulted in sizable basilar membrane displacements.

The two most significant results found in this study are the following:

1) Transient oscillations of DPOAE magnitude and phase occurred after LF sound exposure times of up to 60 min without transitioning into a permanent cochlear damage, as far as assessed with OAEs.

2) The BP observed in this study was independent from LF sound exposure duration. However, the probability of a BP with significant changes decreased with longer exposure times. Up to the highest exposure durations, the BP showed constant size and duration of oscillatory changes and presence of two separate, inverted oscillation patterns.

Noise trauma? Our results suggest that intense LF sound exposures, even with fairly long durations, did not cause OHC damage in the tonotopic region between 0.8 and 8 kHz (where primary tone and distortion product frequencies were represented). In this study, OHCs appeared to have stayed intact, because the delivered sound pressure level of 100 dB SPL at 200 Hz might not evoke BM displacements large enough to damage components of the organ of Corti. To induce a temporary or permanent noise trauma in gerbils, the animals are typically exposed to narrowband and broadband noises centered around frequencies above 1 kHz at intensities higher than 100 dB SPL (Nowotny et al. 2011).

Ryan and Bone (1978) exposed gerbils to a band noise of 1.4–5.7 kHz at a level of 100 dB SPL for 60 min, and the behavioral hearing thresholds increased temporarily for 24 h. In the current study, with a 200-Hz tone presented at 100 dB SPL for up to 60 min, neither a permanent nor a temporary threshold shift, based on DPOAE recordings, could be detected: after LF sound offset, DPOAEs could be recorded with magnitudes similar to the preexposure period regardless of how long the sound was presented. Furthermore, the same animals were used over a period of 3–4 wk, and no between-sessions decrease of DPOAE magnitudes could be observed over this period. Therefore, it is unlikely that OHC loss, which is considered to be a sensitive indicator for noise trauma (Saunders et al. 1985; Slepecky 1986), occurred as a consequence of the LF sound exposure in this study.

Naturally, DPOAE recordings reflect OHC integrity and are not appropriate to exclude loss of inner hair cells or synaptic damage presenting as hidden hearing loss (Kujawa and Liberman 2009). However, hidden hearing loss was considered to be an unlikely outcome of this study, because it was associated with longer lasting, very prominent temporary cochlear sensitivity changes exceeding the typical time course and amplitude of the BP (Kujawa and Liberman 2009).

Bounce phenomenon pattern. The BP had a quite uniform pattern regarding duration and size of the changes, suggesting a robust underlying mechanism independent from sound exposure duration. However, previous BP studies in humans tested different exposure times and came to a different conclusion: Kemp and Brill (2009) recorded click-evoked OAEs in human ears and studied transient magnitude changes after sound exposure [150 Hz, 105 dB(A)] at several relatively short durations between 7.5 and 120 s. They concluded that the peak-to-peak difference of OAE level modulation increased with prolonging the exposure duration. They hypothesized that exposure times up to 2 min were short enough so that oscillations of cochlear sensitivity caused by sound onset and offset interfered. Interference patterns could change as a function of LF sound duration. Thus, the BP would emerge after LF sound offset, showing a sinusoidal wave but with different temporal shifts depending on the sound duration. In the present study, mostly two distinct BP patterns were observed, independent from the LF sound exposure duration. Patterns with an initial QDP magnitude decrease prevailed. This makes it unlikely that different BP patterns are the result of a systematic and LF sound exposure duration-dependent interference of cochlear sensitivity oscillations triggered separately at LF sound onset and offset.

Why Does Cochlear Sensitivity Oscillate?

The time courses of sensitivity changes in the cochlea observed in the present study are unusual, because they are slow and of oscillatory character. Such behavior could be caused by a single process showing an underdamped behavior. Alternatively, it could be the result of several processes with differing time constants. In the following, potential processes are identified, and their impact on hair cell physiology is discussed.

Endocochlear potential changes. EP changes can elicit DPOAE changes. Auditory sensitivity is impaired significantly by even small EP changes. A 1-mV furosemide-induced decrease of EP elevates auditory nerve fiber thresholds by 1 dB in cats (Sewell 1984) and to a similar extent in gerbils (Schmiedt et al. 2002). With a fairly large EP reduction by ~70 mV (Mills et al. 1993), DPOAE magnitudes in adult gerbils are decreased by up to 40 dB (CDP). Oscillatory changes of the EP could be due to LF sound-induced decrease and increase of cochlear blood flow (Konishi et al. 1961). However, the impact of intense sound on cochlear blood flow has not yet been fully resolved, and the results in the literature are inconclusive: intense noise exposure (sound pressure level >90 dB SPL) did not cause any changes in cochlear blood flow (Perlman and Kimura 1962) or reduced (Thorne and Nuttall 1987) or increased the blood flow (Prazma et al. 1983), sometimes even within the same cochlea (Scheibe et al. 1993).

During intense LF sound exposure (5 Hz, 120 dB SPL), the resting EP in guinea pigs oscillated around baseline at 5 Hz with peak-to-peak differences of ~19 mV (~20% of the initial value) (Salt et al. 2013). After high-level LF sounds, only small EP changes on the order of a few millivolts (Kirk and Patuzzi 1997; Salt 2004) were recorded. Kirk and Patuzzi (1997), for instance, measured an EP elevation of ~4.2 mV with concurrent increase of QDP amplitudes after a 3-min-long stimulation with a 200-Hz sound at 95 dB SPL.

Alterations of endolymphatic K^+. The EP mainly relies on active transport of K^+ (Köppl et al. 2018), and consequently, EP changes are often linked to $[K^+]$ changes in endolymph. How could intense LF sound alter $[K^+]$ in the cochlea?

K^+ is secreted via the stria vascularis, suggesting that direct mechanical impact of the LF sound onto this epithelial structure could cause reduction of K^+ secretion into scala media. Acoustic overstimulation can indeed result in lesions of the stria vascularis due to mechanical energy of the noise (>145 dB SPL) or oxidative stress responses (Shi and Nuttall 2003; Úlehlová 1983). Sound pressure levels and frequencies used in the present study render it unlikely that mechanical changes to the stria vascularis occurred. Furthermore, mechanical changes are difficult to harmonize with our observation of hypersensitive periods.

A change of the standing current through MET channels can affect endolymphatic $[K^+]$ (Kirk and Patuzzi 1997; Patuzzi and Rajan 1990). If asymmetric MET opening probability increases and decreases during LF sound exposure, the EP might drop due to K^+ "drainage" caused by simultaneous opening probability changes of many MET channels. A decreased K^+ outward current might result in an EP increase.

Such an EP increase could also be caused by an LF sound-induced buildup of endolymph volume within the scala media, termed endolymphatic hydrops (Flock and Flock 2000; Salt 2004). Additionally, the EP can decrease as a result of increasing endolymphatic ATP levels. ATP is secreted from cochlear supporting cells (Zhao et al. 2005) in case of acoustic trauma (Housley et al. 2013). Elevated ATP levels increase the open probability of ATP-gated cation channels of supporting and hair cells and consequently facilitate a K^+ outward current. This might reduce the elevated EP after sound exposure to values below baseline. Once the ATP level returns to normal, the EP stabilizes around baseline. This may cause an oscillatory behavior. According to Salt (2004), K^+ changes within the endolymph after LF sound presentation are, however, negligible. A decrease of only 0.6% in $[K^+]$ was detected. The author concluded that modulation of K^+ currents at the hair cells or stria vascularis cannot account for EP changes. Furthermore, Kirk and Patuzzi (1997) suggested that EP changes arise as a secondary phenomenon and are only a consequence of changes at the level of the OHCs and the transduction process via the MET channels.

Ca^{2+} oscillations in OHCs. It has been suggested that LF sound can cause slow Ca^{2+} oscillations in OHCs. This is thought to be the result of large OHC receptor potentials evoked with LF sound, because they are less attenuated by the low-pass properties of the OHC membrane (Patuzzi 2011). As a result, a net influx of Ca^{2+} into OHCs occurs. Within OHCs, Ca^{2+} oscillations can now arise due to intracellular release and uptake of Ca^{2+} with different time constants, respectively. Alternatively, these oscillations can be caused by a rise in endolymphatic ATP. Increased ATP levels in response to sound stimulation also directly induce intracellular $[Ca^{2+}]$ increase in hair cells and supporting cells (Ashmore and Ohmori 1990; Dulon et al. 1993). A short increase in ATP, and ADP as a first decomposition product, was shown to induce oscillating $[Ca^{2+}]$ over a time course of ~4 min in pigment cells within the retina (Reigada et al. 2005). It is feasible that OHCs might undergo the same oscillatory changes, responding to ATP increase similarly to retinal cells (Ashmore and Ohmori

1990). In OHCs of gerbil cochleae, Chan and Rouse (2016) reported variations of intracellular [Ca^{2+}] in response to a 1-kHz sound for 3–4 min. Results of the present study showed that the BP was always elicited with a certain probability, and not all animals showed the BP with significant changes. This might be explained by the fact that ATP-induced changes of intracellular [Ca^{2+}] were subject to a threshold that had to be reached (Chan and Rouse 2016). Ca^{2+} is an important regulator for OHC slow motility, which changes OHC length. Stereociliary angles change when OHCs elongate and contract, and result in altered mechanical feedback and hence cochlear sensitivity, and as a result, cause changes of DPOAE magnitude and phase. The stereotypical size of DPOAE changes independent of LF sound exposure duration could be explained by a limited amount of Ca^{2+} that could be released and sequestered intracellularly. Of all previously mentioned mechanisms, Ca^{2+} oscillations induced by ATP are considered the most probable mechanism for BP generation.

What Might Be the Consequences of Ca^{2+}-Driven OHC Length Changes?

Slow motility of OHCs affects the forward transduction process via the MET channels of the OHCs (Patuzzi et al. 1989; Santos-Sacchi 1993), which is one of the many nonlinear processes within the cochlea (Verpy et al. 2008). The transduction process can be described by an input-output function, the MET transfer function, with a mechanical input, e.g., stereocilia deflection, and an electrical output, e.g., inward current through MET channels (see Fig. 7, *C, F*, and *I*). The current state of this system can be described by a point on the transfer function called the operating point (OP).

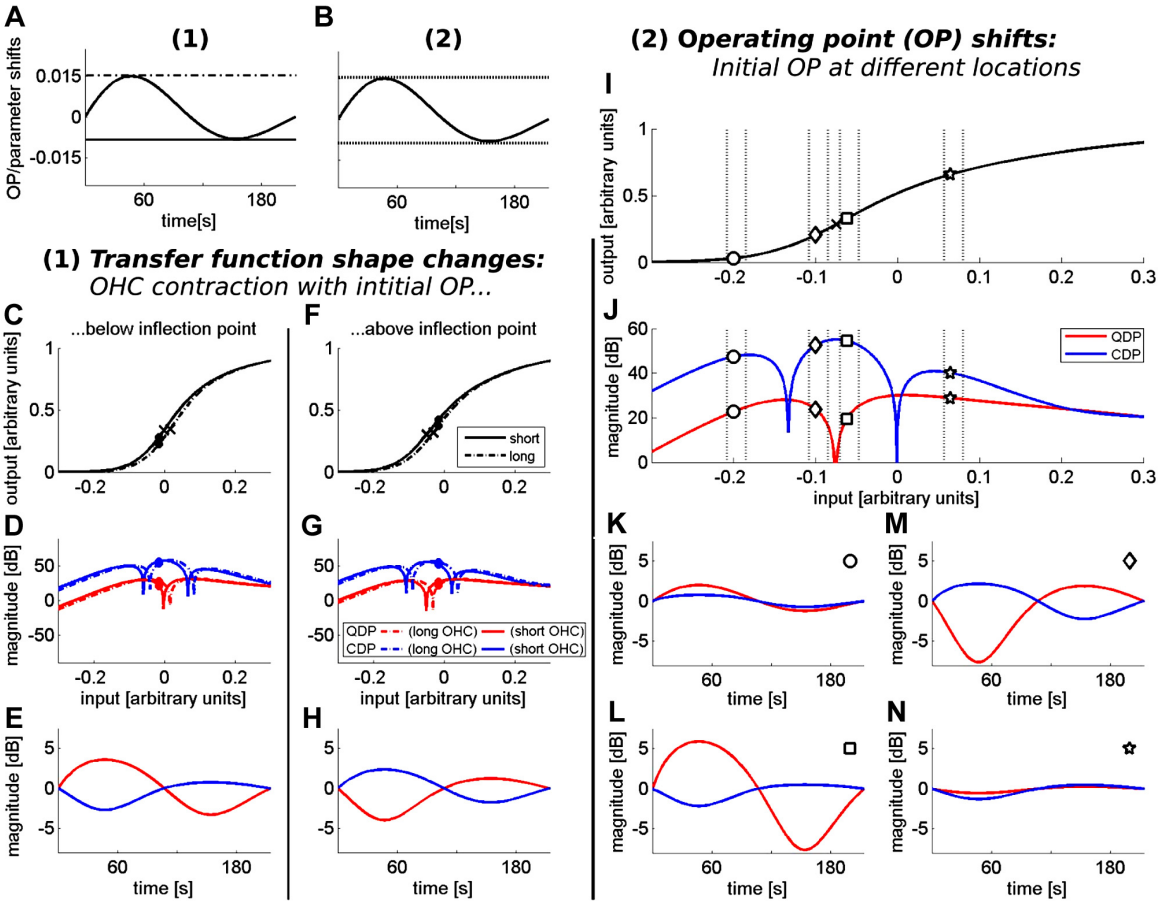

Fig. 7. Simulation of magnitude changes of quadratic (QDP; red) and cubic distortion product otoacoustic emission (CDP; blue) that are to be expected if the parameters (*C–H*) of a single, saturating nonlinearity, the Boltzmann function, or its initial operating point (OP; *I–N*) are altered in an oscillatory manner. Oscillatory changes of transfer function parameter (*A*) and of initial OP (*B*) are applied. *C*, *F*, and *I*: transfer function simulated with 2-exponential Boltzmann function (equation and parameters slightly modified from Abel et al. 2009) with inflection point (×). *C* and *F* show transfer function shape changes due to outer hair cell (OHC) contraction with stable OP (●) below (*C*) and above inflection point (*F*). *I* shows transfer function with OP shifts (dashed lines), with various initial OPs coded with different markers. *D*, *G*, and *J*: absolute value of 2nd ($f_2 - f_1$; red) and 3rd ($2f_1 - f_2$; blue) derivatives of Boltzmann function illustrating DPOAE magnitudes. *D* and *G* show DPOAE magnitudes depending on parameter shift; derivatives with minimum parameter (solid line) and maximum parameter value (dashed line) are illustrated for OP below (*D*) and above inflection point (*G*). *J* shows DPOAE magnitude depending on oscillatory OP shifts. *E*, *H*, and *K–N*: magnitude changes (relative to preexposure level) of QDP (red) and CDP (blue) as a function of time with oscillating contraction and stable OP [OP below (*E*) and above inflection point (*H*)] or oscillating OP shifts (*K–N*). Symbols at *top right* corner of *K–N* refer to OPs indicated on transfer function in *I* and absolute values of derivatives of transfer functions in *J*.

Theoretical studies have demonstrated that the MET transfer function can be approximated with a two-exponential Boltzmann function (Crawford et al. 1989; Frank and Kössl 1996, 1997; Lukashkin and Russell 2005, 1998). The absolute values of the second and third derivatives of the Boltzmann function can be used to predict the magnitude of QDP and CDP, respectively, as a function of the OP location (Abel et al. 2009; Frank and Kössl 1996). Based on this theoretical concept, oscillatory behaviors of DPOAE magnitude and phase can be explained either due to changes affecting the shape of the underlying transfer function or due to OP shifts along the transfer function.

Transfer function changes. The MET transfer function can alter its shape, specifically its slope, in response to contraction and elongation of OHCs (Bian and Chertoff 1998; Patuzzi and Rajan 1990). A steeper slope of the transfer function corresponds to a higher sensitivity and a more efficient OHC-driven amplification. Figure 7, *C* and *F* simulates transfer function changes, with one of the slope parameters oscillating before returning to baseline. Slope changes applied to the second and third derivatives (see Fig. 7, *D* and *G*) could account for oscillatory changes in QDPs and CDPs with both BP patterns (see Fig. 7, *E* and *H*) observed in the present data. In the example, the OP is kept in its resting position, despite the fact that it can be shifted by somatic length changes of OHCs, as well.

Furthermore, DPOAEs are elicited by primary tones that, like every sound, shift the OP slightly. Therefore, a transfer function change without any OP shift is rather unlikely. Changes of the transfer function and OP shifts possibly occur at the same time, but OP shifts are sufficient to explain the present data.

Operating point shifts. Resting OPs can be shifted along the transfer function when stereocilia bundles are deflected as a consequence of somatic length changes of OHCs. With the assumption that the BP-generating mechanisms are the same in all mammals, it is not surprising that QDP changes in the gerbil also showed the same duration as the BP observed in the QDPs of guinea pigs after intense LF sound exposure (Kirk and Patuzzi 1997).

DPOAE magnitude and phase changes in gerbils were also comparable to the BP described in human OAEs regarding temporal parameters and the size of changes (see Table 1). Nevertheless, whereas oscillations of human DPOAEs were mostly restricted to QDPs (Drexl et al. 2014), magnitude and phase changes in gerbils could also be detected in CDPs, albeit with a lower probability and smaller changes than those found in QDPs. QDP and CDP magnitudes depend on the OP resting position (see Fig. 7, *I–N*). Under the assumption that the mechanistic origin in rodents and humans is the same (Lukashkin and Russell 2005), the resting OP in humans is situated close to the inflection point of the transfer function, whereas in rodents it might be located more distant to the inflection point (Lukashkin and Russell 1999). The different position of the resting OP can account for the difference in BP patterns in gerbils and humans, explaining why CDPs in gerbils are more likely to be altered by the LF sound compared with CDPs in humans. However, resting OPs in gerbils are still within a range in which QDPs are more prone to OP changes than CDPs.

Considering the fact that resting OPs are neither stable between individuals of the same species nor necessarily stable in a single individual over time, the resting OP can also explain why in some experimental trials QDP and CDP can change in parallel, whereas they show an inverted behavior in other trials (see Fig. 2, Fig. 3, and Fig. 7, *E*, *H, K, N*).

Conclusion

In the present study, the BP could be observed in DPOAEs of gerbils without any indications of noise trauma. OP shifts and transfer function changes of the mechanoelectrical transduction in OHCs seem to explain the underlying causes for the BP quite well. OP shifts were previously employed to explain hearing threshold and DPOAE changes related to the BP in humans. Different oscillation patterns between gerbils and humans are presumably due to different locations of the resting OP. Rather than EP alterations, ATP-induced and Ca^{2+}-dependent slow electromotility of OHCs was suggested to cause sinusoidal oscillations of QDP and CDP.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.D. conceived and designed research; M.A.U. performed experiments; M.A.U. analyzed data; M.A.U. and M.D. interpreted results of experiments; M.A.U. prepared figures; M.A.U. and M.D. drafted manuscript; M.A.U. and M.D. edited and revised manuscript; M.A.U. and M.D. approved final version of manuscript.

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CHAPTER 5

GENERAL DISCUSSION

In this thesis, three studies were presented addressing transient or permanent impairment of inner ear function, mainly cochlear function with a focus on disturbances of cochlear homeostasis.

Different origins for tinnitus percepts present in Menière's disease and during bounce phenomenon. The first study (*tinnitus study*, chapter 2), a psychophysical study with human participants, focused on tinnitus, a common and prominent symptom with regards to inner ear conditions. Two groups of participants were asked to match their tinnitus percept with tones or noises which they adjusted with regards to spectral content and sound level. Participants of the first group were patients suffering from Menière's disease (MD) with chronic tinnitus and fluctuating symptoms like vertigo and hearing loss. Participants of the second group were normal-hearing participants in which a transient tinnitus was reliably elicited by an intense, but harmless, low-frequency (LF) sound as part of the bounce phenomenon (BP). The hypothesis was tested whether those two study groups showed comparable tinnitus sensations hinting at a common underlying mechanism. Results showed that within and between the two study groups, tinnitus sensations differed. Unlike in previous studies, tinnitus percepts reported by MD patients were heterogeneous and mainly matched pure tones above 3 kHz or noises of various spectral shapes. In comparison, normal-hearing participants primarily matched their tinnitus to pure tones below 2 kHz or to noise. Building on this finding, I proposed various origins for the tinnitus matched by the study participants. Noise-like tinnitus in both groups could be generated by altered cochlear homeostasis, presumably increasing auditory nerve firing.

Retrocochlear impact of transcutaneous electrical stimulation on early auditory processing. In the second study (*GVS study*, chapter 3), I looked into the possible influence of electrical vestibular stimulation (galvanic vestibular stimulation = GVS) on the auditory periphery, specifically the cochlea. Transcutaneous, electrical stimulation with alternating currents of very LFs were applied while recording OAEs and conducting psychophysical experiments in human participants. No effect of the electrical stimulation could be found on the cochlear level. Neither DPOAEs nor SOAEs were modulated or altered by the applied stimulation. However, the majority of human participants perceived amplitude modulations of externally presented tones and of auditory illusions generated by either a faint band-stop noise (Zwicker tone) or an intense LF sound (BP tinnitus). This is suggestive of a retrocochlear mechanism, which is altered during GVS, instead of the cochlear function.

Uniform oscillatory sensitivity changes with increasing low-frequency sound exposure in gerbils. The third study (*BP study*, chapter 4) addresses the relevance of stable cochlear homeostasis including extra- and intracellular ion concentrations in an animal model. Gerbils exposed to intense LF sound showed a post-exposure BP assessed via DPOAE recordings. The probability of eliciting a BP decreased with increasing exposure time. Whenever BP-typical oscillatory changes could be observed, the same uniform time course and size, independent of exposure duration, were found. No indication for permanent cochlear damage could be found suggesting that the BP is the homeostatic reaction of an intact cochlea in case of intense acoustic stimulation.

The results obtained in these studies contribute substantially to the research field of cochlear mechanics and inner ear homeostasis. In the following discussion, I will put my results into a broader context and address three major topics:
1. Tinnitus caused by peripheral processes in the auditory pathway
2. Electrical stimulation of the inner ear
3. Intense LF sound exposure with special focus on the BP

5.1 Tinnitus caused by Peripheral Processes

Different mechanisms are considered to initiate changes within the nervous system which cause many forms of tinnitus (for review see Møller, 2003, 2007). In many cases, cochlear dysfunction might be a prerequisite for tinnitus development (Nuttall et al., 2004; Eggermont, 2003). Furthermore, the central nervous system plays a prominent role in tinnitus generation, but only gets involved later, when neural activity originating from the periphery is altered due to cochlear dysfunction (Nuttall et al., 2004). In our tinnitus study (chapter 2), both study

groups, MD patients and normal-hearing participants with transient tinnitus perceived a tinnitus which presumably developed in response to cochlear disturbances. The first group, MD patients, suffered from an inner ear disorder associated with endolymphatic hydrops (ELH) as assessed with magnetic resonance imaging. The latter group showed the transient BP, which was proposed to be of cochlear origin. The BP might cause a hydropic condition similar to the ELH in MD patients. In the following, various possibly tinnitus-generating processes are discussed.

5.1.1 Tinnitus Pitch

Tinnitus quality, e.g. tinnitus pitch, can give insights in the mechanisms underlying tinnitus generation. However, interpretation is relatively complex. On the one hand, the relationship between tinnitus pitch and audiometric data is unclear (Pan et al., 2009). On the other hand, most tinnitus percepts are not correlated to a physical stimulus, and subjective tinnitus matching procedures might be prone to error (Henry and Meikle, 2000).
Two forms of tinnitus can be differentiated: Transient and chronic tinnitus. While transient tinnitus is an auditory phantom sound that almost everyone experiences from time to time and that vanishes within seconds, minutes or days (Degeest et al., 2014), chronic tinnitus is a persistent percept that lasts from months to years and occurs in around 10-15% of adults (Heller, 2003; Hoffman and Reed, 2004). In the tinnitus study (chapter 2), MD patients showed a chronic tinnitus and normal-hearing participants undergoing sound exposure reported a transient tinnitus. Transient and chronic tinnitus are usually characterized as tonal tinnitus (reviewed Degeest et al., 2014) with frequencies above 3 kHz (Meikle and Taylor-Walsh, 1984; Han et al., 2009). Reports of noise-like tinnitus are less frequent in the literature. This might either be due to previous matching procedures neglecting noise-like percepts or due to lower incidence of noise-like tinnitus (around 16%) versus tonal tinnitus (over 55%) amongst tinnitus patients (Pan et al., 2009; Betz et al., 2017). Nevertheless, in both study groups of the present tinnitus study, noise-like tinnitus was as prevalent as tonal tinnitus.
In patients with known hearing loss, the pitch of chronic tinnitus determined in tinnitus matching studies correlates with the frequency region, in which hearing threshold is significantly elevated (Norena et al., 2002; Schecklmann et al., 2012). It can also be confined to so called edge frequencies in the transition region between dysfunctional and intact hair cells (Moore, 2010; König et al., 2006). Some of the MD patients in the present tinnitus study, with concurrent hearing loss affecting high frequencies and mainly low frequencies, showed tonal high pitch tinnitus rather than low pitch tinnitus.

Transient tinnitus arises frequently together with temporal threshold shifts induced by an intense sound stimulation. In accordance with this, temporary hearing threshold changes and transient tinnitus simultaneously occur after the intense LF sound inducing the BP (Hirsh and Ward, 1952; Kemp, 1986; Drexl et al., 2014). In previous studies, the term "transient tinnitus"

was a more heterogeneous concept that included tinnitus sensations of variable durations ranging from seconds to a few hours (e.g. Atherley et al., 1968; Gilles et al., 2013). In contrast, the transient tinnitus percepts of the BP were highly uniform with tinnitus durations around 90 s. Pitch of noise-induced tinnitus is correlated to, but does not coincide with either the frequency of the highest temporal threshold shift or the frequency of the inducing stimulus (Loeb and Smith, 1967). This is consistent with the proposed mechanism for transient tinnitus known as reduced off-frequency inhibition (Eggermont, 2003). In our tinnitus matching procedure (chapter 2), the frequencies chosen by normal-hearing participants with BP did not resemble the exposure tone of 30 Hz. The chosen frequencies were slightly higher, but mostly of LF character presumably indicative of a correlation with the exposure tone, like reduced inhibition.
The most parsimonious explanation would be that the same physiologic mechanisms are responsible for tinnitus generation and temporary or permanent hearing loss, but this is not necessarily true (Nuttall et al., 2004). Tinnitus can be present in the absence of hearing loss, assessed with audiometric data, (Langers et al., 2012) and the other way round (König et al., 2006; Langguth et al., 2013). Even in cases with concurrent hearing loss and tinnitus, tinnitus pitch and hearing loss frequency might not be associated (Flores et al., 2016). One indicator for the same underlying mechanism of tinnitus and hearing threshold change could be a similar time course. In BP, the transient tinnitus sensation lasts around 90s (Drexl et al., 2014) and threshold changes are around twice that long. If threshold changes oscillate, the tinnitus coincides with the first half period of those oscillatory changes, which, depending on the individual pattern, consist of either desensitization or sensitization. This might point to a common cochlear mechanism in case of BP inducing hearing threshold changes and tinnitus. Nevertheless, different peripheral mechanisms may trigger tinnitus, for instance in MD patients with chronic tinnitus and almost normal hearing thresholds between episodes of vertigo.

5.1.2 Modified Auditory Nerve Activity

The tinnitus mechanism previously suggested for the BP is the so called "rate tinnitus" (Patuzzi, 2002) which is caused by an increased synchronization of nerve fiber responses (Eggermont, 1984; Cazals et al., 1998). Evidence for rate tinnitus is sparse (Searchfield et al., 2002) with no direct proof showing synchronized activity in adjacent nerve fibers. On the contrary, measurements across the fibers innervating a single IHC showed random and independent activity (Johnson and Kiang, 1976). An overactivity of auditory nerve fibers was proposed to account for tinnitus in many tinnitus models (Evans et al., 1981). However, a decrease of spontaneous activity due to transient or permanent cochlear impairment (e.g. hair cell loss, synaptopathy, deafferentiation etc.) was in fact observed in the majority of studies. Comparably to increased spontaneous activity, decreases in auditory nerve activity might deviate from the mean firing rate perceptionally interpreted as silence. As a result, decreased activity could indicate presence of sound and lead to perceptions of tinnitus. Additionally, the central gain hypothesis (Jastreboff, 1990) suggests that decreased

firing deprives higher brain areas from input. This results in hyperactivity or lack of inhibition in these brain areas and consequently tinnitus perception (Kaltenbach et al., 2004; Wang et al., 2011; Schaette and Kempter, 2006; Chrostowski et al., 2011; Norena, 2011; Auerbach et al., 2014; Yang et al., 2011). In case of chronic tinnitus, decreased firing goes hand-in-hand with permanent loss of OHCs and IHCs due to inner ear pathologies like late-stage MD (see MD patient group in tinnitus study) or noise trauma. Tinnitus can manifest within the CNS over a longer time period. Hyperactivity and plastic changes can be caused by reduction of inhibition (Brozoski et al., 2007; Middleton et al., 2011) or upregulation of excitatory synaptic transmission (Whiting et al., 2009). Plasticity changes related to tinnitus were observed along the whole auditory pathway from the dorsal cochlear nucleus (Zhang and Kaltenbach, 1998; Zhang et al., 2006; Kaltenbach, 2011; Vogler et al., 2011) over the inferior colliculus (Bauer et al., 2008; Melcher et al., 2009; Manzoor et al., 2012; Robertson and Mulders, 2012) up to the auditory cortex (Eggermont and Roberts, 2004; Llano et al., 2012). This plasticity can take various forms such as an elevated spontaneous firing rate (Zhang and Kaltenbach, 1998; Norena and Eggermont, 2003; Vogler et al., 2011) or increased bursting and synchrony (Norena and Eggermont, 2003).

5.1.3 Cochlear Involvement in Tinnitus Generation

In early stages of MD, tinnitus usually accompanies vertigo attacks and has a transient character, similar to percepts following intense LF sound exposure. Aside from hair cell loss, various processes are conceivable that might explain such transient tinnitus percepts.

5.1.3.1 Endocochlear Potential Changes

The extraordinarily high endocochlear potential (EP) is critical for the transduction in hair cells (HCs) and has to be sustained by several coordinated processes in the stria vascularis (SV) and the organ of Corti. The EP could play a critical role in tinnitus generation increasing or decreasing the spontaneous activity of the fibers (see 5.1.2).
A reduced EP level decreases the standing current through the IHCs and will decrease the spontaneous rate of afferent type I fibers (Sewell, 1984). Although experimental animals, in which MD was simulated with introduction of an ELH, showed reduction of EP (Cohen and Morizono, 1984), no EP measurements have been conducted in MD patients. Thus, EP changes have not been linked to tinnitus in MD patients.
An EP increase could be caused by altered opening probability of transduction channels in OHCs. Under hydropic conditions, often found in MD patients, the BM is displaced downwards and transduction channels at the stereocilia tips of OHCs close. The EP would consequently increase. IHCs would depolarize and cause neurotransmitter release onto the dendrites of the auditory nerve fibers increasing the spontaneous firing rate of these fibers. A temporary increase of EP of 4-5 mV (Kirk and Patuzzi, 1997) was observed accompanying the BP.

5.1.3.2 Increased Cochlear Amplification

If length changes of OHCs result in MET channel closure as suggested under hydropic conditions, cochlear amplification would be reduced. A reduction of OHC amplification is considered unlikely during the BP, because human experiments showed elevated OAE levels coinciding with the tinnitus sensation (see chapter 2). Instead of reduced activity an excessive activity of the OHC-based cochlear amplifier might explain the BP. Enhanced activity of the cochlear amplifier could amplify IHC responses to thermal noise. Thermal noise can either be prominent in a certain frequency region of the BM or occur along the whole BM evenly. This could result in tinnitus with tonal or noise-like character, respectively (Nuttall et al., 1997, 2004).
Enhanced OHC amplification might deliver another explanation for tonal tinnitus. In BP experiments in humans, SOAE magnitudes show an initial magnitude increase and new "transient" SOAEs appear (Kugler et al., 2014). In the present tinnitus study, a few participants experiencing the BP matched their tinnitus percept with a tone of a frequency very comparable to their SOAEs. It was therefore suggested that participants perceived their SOAEs as transient tinnitus percepts. SOAEs became audible when SOAEs increased in amplitude or temporarily appeared (Raveh et al., 1998; Long, 1998). This resulted in a tinnitus percept with a physical explanation for the perceived sound.

In conclusion, it can be stated that tinnitus is a complex and unique phenomenon. Tinnitus can manifest itself in various perceptual forms even under the same experimental condition like in the present tinnitus study. In our study, the self-developed matching procedure enabled participants to adjust a closer match to their tonal or noise-like percepts than previous tinnitus matching studies. Patients suffering from inner ear disorders and normal hearing individuals can develop transient or chronic tinnitus based on more than one underlying mechanism. This makes it difficult to link tinnitus percepts of a specific pitch reliably to any related peripheral pathophysiology.

5.2 Electrical Stimulation of the Auditory and the Vestibular System

5.2.1 Galvanic Vestibular Stimulation - Mode of Action

Galvanic vestibular stimulation (GVS) is a variant of transcutaneous electrical stimulation influencing brain activity and neurophysiology (Utz et al., 2010) via exposure to a low-intensity current that spreads along the skin and skull. For GVS, electrodes are attached to the temporal bones right behind the pinnae (mastoid bones) in order to stimulate the inner ear.
The responses triggered by GVS depend on various factors, including the kind of stimulus, the type and size of electrodes used and the experimental context. GVS stimulates the inner ear in a

very crude fashion. Direct current GVS activates input from many end organs of the inner ear (Fitzpatrick and Day, 2004) with the main effect on irregular afferent nerve fibers (Goldberg et al., 1984). GVS activates primary vestibular afferents innervating the otolith organs (Cohen et al., 2012) as well as afferents innervating the semicircular canals (Kim and Curthoys, 2004). The behavioral responses are complex. They comprise perceptual, ocular motor and postural responses, associated with activation of semicircular canals and otolith organs(Cohen et al., 2012; Curthoys and MacDougall, 2012). Semicircular canals trigger neck and eye muscle activity. Neck muscles can stabilize the head during body movement (e.g. Denise et al., 1987; Xiang et al., 2008), while eye muscles can stabilize the gaze during head movements (e.g. Suzuki et al., 1964). Vestibular-induced eye muscle activity can manifest in nystagmus with both horizontal and torsional components (MacDougall et al., 2003, 2005). In contrast, the otolith system is more involved in orientation in space and in postural stabilization. GVS stimulates the otolith organs and elicits a body sway to the side, on which the anode is placed (Coats, 1973; Scinicariello et al., 2002).
Sinusoidal GVS, as used within our GVS study (chapter 3), does not induce a sense of rotation or vertigo, but induces an illusion of “rocking in a boat” or “swinging from side to side" in human participants (Lobel et al., 1998; Bent et al., 2006). In the present GVS study, the behavioral responses like eye muscle activity and illusions of side-to-side motion were present, however, they were not the focus of the study. Instead, our study focused on the effect of GVS on the auditory system. GVS activation had been detected in the auditory cortex before (Bucher et al., 1998). Nonetheless, if and in which state GVS altered auditory sensation was unclear. There are two possible scenarios. GVS can influence auditory perception...
1) indirectly via cross-modal interation between the vestibular and the auditory system
2) directly via stimulation of the auditory periphery

5.2.1.1 Indirect Influence on Auditory Perception

Activation of the vestibular system in response to adequate vestibular stimuli like body movement and head tilts can influence auditory perception. Vestibular processes might shape auditory percepts in different contexts. For instance, vestibular input might ease tasks of sound localization (Wigderson et al., 2016; Wu and Shore, 2018; Genzel et al., 2018) and influence perception of musical rhythm (Phillips-Silver and Trainor, 2008). When humans move in rhythm to music, the metrical structure of the auditory percept consists of integrated information from vestibular and auditory information (Phillips-Silver and Trainor, 2008). Rhythm perception might be closest to the scenario of our GVS study (chapter 3), as auditory percepts are rhythmically modulated by sinusoidal stimulation of the vestibular system.
Multimodal integration of vestibular and auditory processing was suggested to occur along the retrocochlear pathway up to the cortex. Neurons of the dorsal cochlear nucleus involved in early auditory processing were shown to receive vestibular input via projection from the primary

vestibular nerve (for review see Newlands and Perachio, 2003; Burian and Gstoettner, 1988) and from the vestibular nucleus (Barker et al., 2012; Bukowska, 2002). The neural underpinnings of how the vestibular and auditory systems might integrate information is still not understood in detail.

5.2.1.2 Direct Stimulation of the Auditory Periphery

A possible candidate to be impacted by GVS is the cochlea which is directly connected to the vestibular endorgans and is exposed to the same electrical field. However, results of our GVS study (chapter 3) showed that GVS with sinusoidal stimulation below audible frequencies did not affect OHCs assessed with SOAEs and DPOAEs. Therefore, it was suggested that auditory perception could be influenced at other sites, possibly on a retrocochlear level.
Our findings were confirmed by a recent study applying transcranial stimulation with alternating current (4 Hz) to two different areas above the auditory-motor cortex. Results of this recent study did not show any effects on DPOAEs either (van Bree et al., 2019). At the same time, transcranial stimulation applied either with direct or with alternating current over the auditory cortex was shown to alter auditory processing and change auditory perception like auditory temporal resolution (Baltus et al., 2018; Heimrath et al., 2014).

5.2.2 Electrical Stimulation as a Treatment Option

Although it is not well understood how electrical stimulation influences neurophysiological mechanisms, transcutaneous and direct electrical stimulation are used as a treatment option in several patient groups. The anatomical site of the stimulation and electrical stimulus parameters such as intensity and frequency determine how efficient the treatment is (e.g. Engelberg and Bauer, 1985). Electrical stimulation might restore normal neural activity in the auditory system of patients with tinnitus and in the vestibular system of MD patients.

5.2.2.1 Tinnitus Suppression

One group of patients eligible for electrical stimulation treatment are patients suffering from tinnitus. Tinnitus can be suppressed by electrical stimulation mainly during stimulation and for a short period afterwards (Rubinstein et al., 2003; Fregni et al., 2006).

Tinnitus can originate from plastic changes and hyperactive, hypersynchronous activity in higher brain areas of the auditory pathway (Mühlnickel et al., 1998; Weisz et al., 2005). Therefore, transcranial electrical stimulation can target cortical auditory and non-auditory areas associated with tinnitus pathogenesis (Mirz, 2000; Schlee et al., 2009; Song et al., 2012). Stimulation over those areas revealed tinnitus suppression in some patients (Fregni et al., 2006; Garin et al., 2011; Shekhawat et al., 2015; Joos et al., 2014), in other patients, however, no significant improvement

or even worsening of tinnitus loudness and annoyance were reported (Vanneste et al., 2010; Garin et al., 2011; Pal et al., 2015).
Tinnitus frequently arises with different inner ear disorders, for instance MD (see chapter 2). GVS and transcutaneous stimulation around the auricle was successfully used to treat tinnitus in the past (Althaus, 1886; Chouard et al., 1981; Engelberg and Bauer, 1985). When electrodes were placed closer to the cochlea, either touching (Kuk et al., 1989) or perforating the tympanic membrane (Ito and Sakakihara, 1994; Portmann et al., 1983; Cazals et al., 1978; Konopka et al., 2001; Rubinstein et al., 2003), tinnitus was suppressed in at least 50% of patients.
Furthermore, an invasive electrode placement like in the case of cochlear implantation can suppress tinnitus. A cochlear implant restores neural activity in the auditory nerve to improve hearing performance and as a secondary effect suppresses tinnitus (Miyamoto et al., 1997; Rubinstein et al., 2003; Kleinjung et al., 2009; Van de Heyning et al., 2008).

5.2.2.2 Treatment for Vestibular Dysfunction

Electrical stimulation can also be applied to treat vestibular deficits. GVS using a white noise stimulus (noisy GVS) induces stochastic resonance. Noisy GVS was shown to improve balance control and vestibular motion perception in healthy subjects (Keywan et al., 2018) and to diminish walking instability in patients with bilateral vestibulopathy (Wuehr et al., 2016). Transcutaneous stochastic stimulation over the mastoids yields a promising therapeutic use for patients with balance problems (Wuehr et al., 2017; Agada and John, 2018).
Direct electrical stimulation of the vestibular endorgans can be used to treat vestibular problems as well. With cochlear implants being successfully applied on a large scale, vestibular prostheses with the same underlying concept are on the rise (Golub et al., 2014). Vestibular implants consisting of one to three electrodes were inserted into the semicircular canals to provide controlled input to the vestibular nerve afferents (Perez Fornos et al., 2014; Guyot et al., 2011; Guinand et al., 2015; Van De Berg et al., 2012; Wall et al., 2007). MD patients were equipped with vestibular implants in order to control repeated vertigo attacks associated with alterations in vestibular firing. The implants were supposed to serve as vestibular "pacemaker", reducing disabling symptoms during an attack by restoring the absent signal (Golub et al., 2014). Unlike in animal studies which showed that vestibular implants were technically feasible (Rubinstein et al., 2012), the first vestibular implants in MD patients did not work properly. During implantation residual hearing and vestibular function were lost (Golub et al., 2014; Phillips et al., 2015). Nevertheless, a device that could halt vertigo attacks and at the same time preserve auditory and vestibular function would be very valuable for future MD treatment.

5.3 Sound-Induced Cochlear Sensitivity Changes

A major challenge with regards to auditory physiology is to understand, how the auditory periphery reacts to acoustic overstimulation and to shed light onto the intrinsic, presumably otoprotective, mechanisms that enable the cochlea to accommodate loud sounds.
On a pre-cochlear level, two reflexes are known to be elicited during intense sound presentation: the acoustic middle ear reflex, which reduces the transmission of vibrational energy to the cochlea, and the medial olivocochlear reflex, which decreases cochlear sensitivity and alters OAEs by inhibitory efferent innervation of OHCs.
On a cochlear level, otoprotective mechanisms still have to be further investigated. In this thesis, a special focus lies on the BP, which consists of oscillatory sensitivity changes with hypo- and hypersensitivity. As mentioned briefly in chapter 4, the BP offers an opportunity to examine homeostatic and possibly otoprotective processes within the cochlea.
In the following, I will discuss the role of the BP and its underlying processes.

5.3.1 The Relevance of the Bounce Phenomenon

In all three studies presented in this thesis, the BP was experimentally induced to address different research questions. In the tinnitus and GVS studies, the transient tinnitus percept associated with the BP was used. In the BP study, the oscillatory behavior of OAEs was in the focus of observation.
Beyond its use within these studies, the BP might naturally occur and could be of relevance for future research focusing on the mammalian inner ear. Relevant aspects about the BP can be summarized as follows:
1) Lack of classical noise trauma
When introducing the BP, intense sound stimulation does not result in the typical transient or permanent noise-induced impairment of the auditory system. No vestibular dysfunction is observed during the time course of the BP either.
2) Model for MD
The BP shares similarities with the symptoms occurring in MD which points to a common underlying mechanism.
3) Readout for homeostatic regulation
Studying the BP might help to gain a better understanding regarding the homeostatic processes in the cochlea under challenged but otherwise intact conditions.

5.3.2 Lack of Classical Noise Trauma

Stimuli used to induce the BP in chapters 2-4 are intense but, at least for the exposure times used here, presumably innocuous. Whether sounds are hazardous depends on the frequency spectrum, level and duration of the noise (Kryter et al., 1966).

5.3.2.1 Bounce Phenomenon inducing Stimuli

BP-inducing sounds are characterized by high intensities and by their prominent LF components. Sounds with those characteristics are omnipresent in the environment and are either generated by natural or by man-made sources like machinery and transportation. Some example sources are shown in figure 5.1.

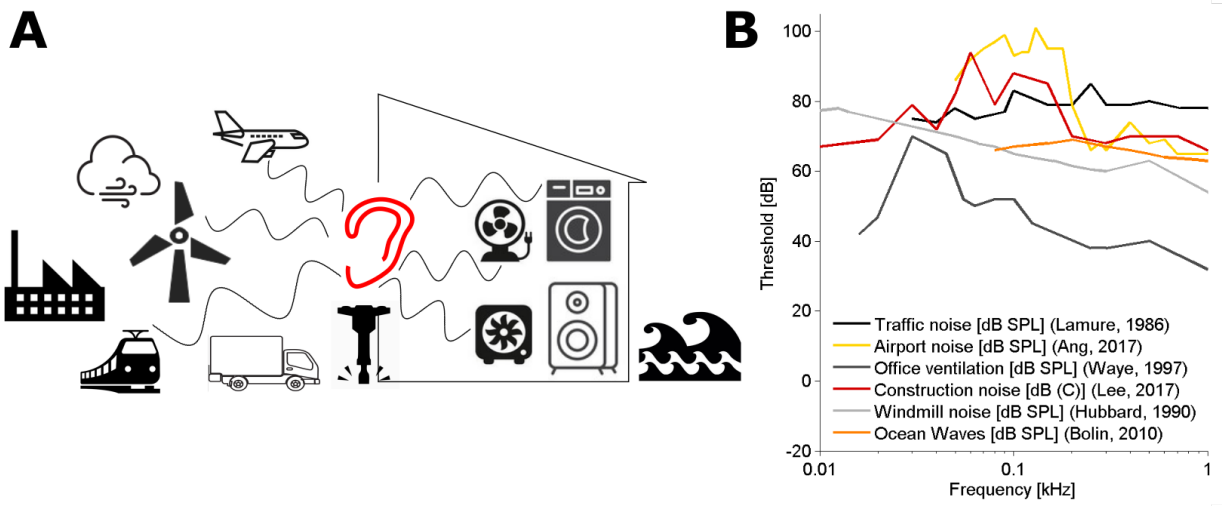

FIGURE 5.1. Low-Frequency Sound Sources. Exemplary sound sources (A) and frequency spectra for low-frequency (LF) sound (B) in our environment (Lamure, 1986; Ang et al., 2017; Waye et al., 1997; Lee et al., 2017; Hubbard and Shepherd, 1990; Bolin and Åbom, 2010). For more frequency spectra of low-frequency sounds see Berglund et al. (1996). *Own work; (A) re-drawn from and inspired by Baliatsas et al. (2016), graphical abstract*

Compared to other sounds, LF sounds have some particularities:
1) Caused by physical properties, LF sound waves travel long distances with only little attenuation.
2) LF sound waves are not well attenuated by ear protection or walls of buildings.
3) LF sound is perceived as highly annoying (Tempest, 1973; Waye and Rylander, 2001) and with increasing sound pressure levels the perceived loudness increases fast (Whittle et al., 1972; Møller and Andresen, 1984; Bellmann et al., 1999; ISO 226, 2003).
The combination of these three characteristics leads to problems. In certain neighborhoods, residents often complain about LF noise which they describe as a constant, deep and rumbling sound. In these cases, LF noise is often caused by traffic noise or wind turbines. However, sometimes acoustic sources of the LF noise cannot be identified and the respective LF noise is termed hum (Deming, 2004). A number of studies suggest an association between LF noise and vari-

ous physiological conditions (headache (Leventhall et al., 2003), stress (Waye et al., 2002) as well as psychological reactions, such as declining performance (Pawlaczyk-Łuszczyńska et al., 2005; Bengtsson et al., 2004; Kaczmarska and Łuczak, 2007)). The World Health Organization highlighted the potential impact of LF noise as environmental pollutant (Berglund et al., 1999). Consequently, research investigating impact of LF sound on the auditory system and the whole organism is highly relevant.

5.3.2.2 Differences between Bounce Phenomenon and Blast Trauma

Impulsive noises like blast noise contain a great proportion of LF components (Berglund et al., 1996; Madshus et al., 2005). However, blast noise generates massive pressure waves and sound pressure levels of over 120 dB SPL, thereby causing central (reviewed by Gallun et al., 2012) and peripheral damage (Hickman et al., 2018) to the auditory system. The auditory periphery might be subject to conductive damage, like rupture of tympanic membrane (Berger et al., 1997) or ossicular dislocation (Chandler and Edmond, 1997; Greene et al., 2017), and subject to sensorineural damage, like damage of the cochlear sensory cells (Patterson Jr and Hamernik, 1997) or synaptopathy (Hickman et al., 2018). These defects can manifest in pain, tinnitus, hyperacusis, permanent or transient hearing loss (Fausti et al., 2009; Mrena et al., 2004).
Comparable to findings after less intense LF stimulation (e.g. in BP studies), the development of an ELH was observed after blast exposure pointing to a disruption of cochlear fluid homeostasis (Kim et al., 2018). Hydrops was accompanied by synaptic damage which could be partially treated by reducing endolymphatic volume (Kim et al., 2018).
In contrast to the BP, blast exposures did also show an effect on the vestibular system (Fausti et al., 2009; Akin et al., 2017; Maxwell et al., 2017; Scherer et al., 2011) with signs such as dizziness, imbalance and vertigo (Hoffer et al., 2010; Akin et al., 2017). Damage to vestibular inner ear structures are also one potential cause for these vestibular symptoms (Akin et al., 2017). The blast possibly damages the vestibular endorgans mechanically the same way as the cochlea. Intralabyrinthine pressure waves which are transmitted from the cochlea still carry sufficient energy to cause injury of the sensory cells in the semicircular canals and otolith organs (Maxwell et al., 2017). As the BP-inducing stimuli with lower sound pressure levels have less acoustic energy, the vestibular system might be spared from impact of LF sound exposure. This could explain, why human participants undergoing BP changes (as in chapters 2 and 3) did not report any vestibular symptoms.

5.3.3 Transient Model for Menière's Disease

Even without any vestibular symptoms, the BP was previously suggested as a transient model for MD (Drexl et al., 2014), specifically for MD in earlier stages with solely transient symptoms. The idea of the transient model came up as both phenomena were proposed to share similarities:
1) BP and MD seem to mainly impact the cochlear apex.
2) Hydropic conditions might be present in MD patients and transiently arise after intense LF sound stimulation.
3) The tinnitus quality described by previous studies in MD patients and in human subjects experiencing the BP seems to be similar.
4) In case of MD and BP, OHC motility seems to be affected and motility changes can be explained by operating point shifts on the OHC MET transfer function.

5.3.3.1 Focus on Low-Frequency Sound

LF tones are processed in the apex of the cochlea. In both conditions, during BP and MD, LF sounds play a prominent role, hinting to the fact that the cochlear apex is mainly affected.
Initially, MD patients often suffer from hearing loss in the LF region (e.g. Belinchon et al., 2011), hinting that the so far unknown pathophysiology of MD specifically impacts LFs. In all previous studies, including the studies of chapter 2-4, the BP is evoked with LF sound below 1 kHz (e.g. Kemp, 1986; Kirk et al., 1997; Drexl et al., 2014). Whether the BP is limited to LF stimuli is not entirely clear. However, with increasing sound frequencies, the BP-related changes assessed by SOAEs slightly decrease in amplitude (Jeanson et al., 2017). Furthermore, the underlying mechanism generating the BP, as suggested by Patuzzi (2011), relies on a cellular low-pass component (see below 5.3.4).

5.3.3.2 Endolymphatic Hydrops

MD patients and healthy participants exposed to intense LF noise are proposed to show hydropic conditions, at least for a transient period of time.
ELH is seen as the anatomical correlate of MD. Therefore, MD is nowadays diagnosed and evaluated in patients by imaging an increased endolymphatic space (Baráth et al., 2014; Hornibrook et al., 2015; Carfrae et al., 2008; Nakashima et al., 2007; Gürkov et al., 2011). In animal models, a transient ELH was experimentally induced by an intense LF sound exposure in isolated temporal bones (Flock and Flock, 2000) and in anesthetized rodents (Salt, 2004), but was so far not shown to occur in healthy humans with BP.

5.3.3.3 Unique Tinnitus Character

Based on previous findings, the following hypothesis could be formulated: Tinnitus in human participants, transiently showing the BP, is presumably similar to the tinnitus in MD patients. The tinnitus associated with MD was summarized in the majority of studies as a LF tinnitus of roaring character (Douek and Reid, 1968; Vernon et al., 1980; Han et al., 2009). The qualitative assessment of tinnitus after intense LF exposure in BP studies indicated a rather low-pitched noise (Patuzzi and Wareing, 2002; Drexl et al., 2014). The present tinnitus study (chapter 2) used a quantitative approach to match tinnitus quality and could not confirm that tinnitus percepts of the BP and in MD patients resembled each other. There was a heterogeneity in both groups, with only a small overlap of tinnitus character between the two groups.

5.3.3.4 Differential Changes of Otoacoustic Emissions

The amplification process of OHCs can be assessed in MD patients and in healthy participants, who experience the BP, via OAE recordings. OAEs are altered which indicates that the operating point (OP) of the OHC MET transfer function might be shifted.
OAE recordings in gerbils which undergo the typical BP behavior (chapter 4) showed differential changes of quadratic and cubic DPOAEs. OP shifts can explain these changes including inverted amplitude changes of quadratic and cubic DPOAEs within the same recording. In MD patients, presumably due to the presence of the ELH, the OP is shifted in the affected ear (Hirschfelder et al., 2005; Brown and Gibson, 2011). ELH results in a displacement of the BM towards the scala tympani and thereby induces OHC elongation and closure of MET channels. As a result, the resting OP, which lies in mammalian OHCs normally in a region of maximum sensitivity of their symmetric transfer function (Russell et al., 1986), is moved away from its most sensitive point, the inflection point. OP shifts away from the inflection point lead to quadratic DPOAE increases and cubic DPOAE decreases. Drexl et al. (2018) could show a similar differential behavior with decreased cubic DPOAEs and concurrent stable quadratic DPOAEs in MD affected ears. The expected increase of quadratic DPOAE amplitudes due to OP shifts was probably counteracted by MD related OHC impairment decreasing all DPOAEs.

5.3.4 Suggestive Mechanisms underlying the Bounce Phenomenon

As mentioned briefly in chapter 4, the BP offers an opportunity to examine homeostatic processes without damaging inner ear structures like in a blast trauma (see section 5.3.2.2). Only a few mechanisms are conceivable for BP generation due to the very uniform time course of a few minutes. Moreover, sensitivity changes of the BP show a slow, oscillatory behavior which is indicative for at least two processes with different time constants. Under these conditions, mechanisms regulating either K^+ or Ca^{2+} are worth considering.

5.3.4.1 Possible Candidate: Potassium Ions

Previously, K^+-ions, the most dominant ions within the endolymph (EL) involved in standing and transduction currents (Wangemann and Schacht, 1996), were considered a probable candidate for BP generation. $[K^+]$ was shown to undergo changes in the organ of Corti following intense sound exposure (Johnstone et al., 1989). During sound exposure $[K^+]$ increased and shortly after the cessation of the sound, $[K^+]$ quickly returned to normal. However, this hypothesized mechanism was ruled out by Salt's measurements of endolymphatic $[K^+]$ after intense LF sound stimulation and minimal changes of around 0.6% (Salt, 2004).

5.3.4.2 Possible Candidate: Calcium Ions

The most probable origin of the BP are intracellular Ca^{2+}-oscillations.
Intracellular Ca^{2+} is present in HCs and supporting cells of the organ of Corti, mostly stored within separate cellular compartments.
The $[Ca^{2+}]$ in OHCs increases after acoustic overexposure (Fridberger et al., 1998). This rise in $[Ca^{2+}]$ can be due to Ca^{2+}-influx from the surrounding fluid.

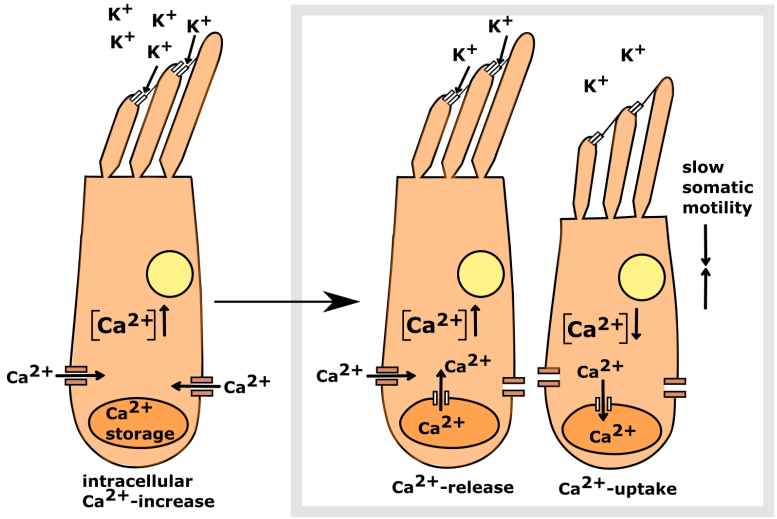

FIGURE 5.2. Sound-Induced Calcium (Ca^{2+}) Oscillations in Outer Hair Cells. Suggestive mechanism of the Bounce Phenomenon based on hypothesized intracellular Ca^{2+}-oscillations. Intense low-frequency (LF) sound exposure or rise in adenosine triphosphate (ATP) levels induces Ca^{2+}-influx at the base of outer hair cells (OHCs). Ca^{2+}-induced Ca^{2+}-release and Ca^{2+}-induced Ca^{2+}-uptake occurs inducing slow motility of OHCs. *Own work re-drawn from and inspired by Purves et al. (2001), Fig. 12.8*

For the BP, Ca^{2+}-influx into OHCs was suggested to be induced by OHC receptor potential changes in response to intense LF sound stimulation (Cody and Russell, 1987; Patuzzi, 2011). Another possibility apart from potential changes would be that Ca^{2+} flows into OHCs when

purinergic receptors are activated by adenosine triphosphate (ATP) (Ashmore and Ohmori, 1990; Ikeda et al., 1991). ATP as a trigger for the Ca^{2+}-influx seems more likely, since sound exposure induces rise of endolymphatic ATP (Munoz et al., 2001; Housley et al., 2013).
ATP-associated Ca^{2+}-oscillations were previously shown in different exitable and non-excitable cells (e.g. Evans and Sanderson, 1999) and Ca^{2+}-signaling is crucial within cells in general (Orrenius et al., 2003; Clapham, 1995). When intracellular Ca^{2+}-levels rise due to Ca^{2+}-influx, they elicit two mechanisms: Ca^{2+}-induced Ca^{2+}-release and Ca^{2+}-induced Ca^{2+}-uptake. Those two mechanisms might be characterized by different time constants and a slight disturbance might lead to underdamped oscillations of cytosolic Ca^{2+}-level.
In OHCs, these oscillations translate into slow motility. OHCs elongate and contract and thereby change the opening probability of MET channels (see Fig. 5.2), which consequently influences hearing sensitivity and OAE amplitude and phase.

5.3.5 Cochlear Homeostasis based on Calcium and Adenosine Triphosphate

Already during cochlear development the signaling molecule ATP and associated Ca^{2+}-waves play an important role to promote cochlear hair cell maturation (Tritsch et al., 2007; Ceriani et al., 2019). In addition, ATP and Ca^{2+} are crucial for cochlear homeostasis after hearing onset.

5.3.5.1 Calcium

Ca^{2+}, presumably involved in the BP, regulates several hair cell functions and is vital for normal hearing (Wood et al., 2004). In humans, disorders lowering the serum and presumably endolymphatic [Ca^{2+}] are often associated with hearing loss (vitamin D deficiency (Brookes, 1985; Ikeda et al., 1989) or hypoparathyroidism (Ikeda et al., 1987)). Ca^{2+} has a dual role:
On the one hand, excess Ca^{2+} can lead to free radicals and even Ca^{2+} mediated cell death (Farber, 1990; Orrenius et al., 2003). On the other hand, tightly controlled Ca^{2+}-homeostasis can protect cells. Transient Ca^{2+}-oscillations like suggested to occur during the BP probably fall into the latter. [Ca^{2+}] changes, which were previously observed in the gerbil cochlea in response to high intensity sounds (Chan and Rouse, 2016), might also be of otoprotective character.
In addition, Ca^{2+}-homeostasis is closely connected to water homeostasis in the inner ear. The presence of Ca^{2+}-sensing receptors within cells of the endolymphatic sac, SV and organ of Corti allows aquaporin trafficking and water flux control (Beitz et al., 1999). In consequence, although Ca^{2+} shows a lower endolymphatic concentration compared to the dominant K^+, it might be a key player of regulating inner ear homeostasis.

5.3.5.2 Adenosine Triphosphate

According to several studies, ATP modulates cochlear sensitivity (Thorne et al., 2002; Housley et al., 1999) and is involved in maintaining EL homeostasis (Marcus et al., 1998). More specifically, ATP modifies K^+-recycling via supporting cells (Zhu and Zhao, 2010), induces EP reduction (Telang et al., 2010; Thorne et al., 2004) and reduces OHC amplification (Yu and Zhao, 2008).
The signaling pathway of the ATP involves ATP secretion, activation of purinergic receptors and subsequent ion channel activity. Possible sites for secretion of ATP in the inner ear are strial marginal cells (White et al., 1995), gap junctions of cochlear supporting cells (Zhao et al., 2005) and cells in the organ of Corti (Wangemann and Schacht, 1996). ATP can activate metabotropic and extensively expressed ionotropic receptors in tissues lining the endolymphatic compartment (Telang et al., 2010; Lagostena and Mammano, 2001; Piazza et al., 2007). The purinergic signaling system reacts to various changes of the extracellular environment such as noise, hypoxia, ischemia, and ototoxic drugs (Vlajkovic et al., 2009). During noise exposure, ATP is released and ATP-levels increase. This might be a mechanism to protect the cochlea from high sound levels (Lee and Marcus, 2008). However, ATP-dependent processes and subsequent adaptive cochlear responses may be reduced with ageing (Telang et al., 2010). Like in other body tissues (Willems et al., 2005), the purinergic receptor system and adenosine metabolism might be impaired with advancing age. This could contribute to an increased susceptibility to noise-induced injury and consequently age-related hearing loss.
The role of ATP in the different cells of the organ of Corti, and the mechanisms regulating ATP release require further investigation. Understanding the ATP signaling pathways holds significant promise to find otoprotective therapies counteracting homeostatic disturbances.

5.4 Conclusion

The mammalian inner ear is a complex anatomical structure consisting of two different sensory systems: the cochlea processes auditory information and the otolith organs and the three semicircular canals process vestibular information. Although both systems contribute to different functions, the underlying physiology such as the transduction process is similar. Hair cell motion is transformed into an electrical signal and thereby depends on mechanical and neurobiological processes. Understanding acoustics and fluid-surface interactions, on the one hand, and electrophysiology, on the other hand, is essential. Additionally, many subtleties like ion movements, cascades of messenger molecules and other homeostatic processes are involved in keeping the cochlea and the vestibular endorgans in balance. These subtleties might make the difference between a disturbed and dysfunctional inner ear or an efficient and sensitive inner ear. The underlying mechanisms causing disturbances like vertigo, hearing loss and tinnitus can best be studied in humans, including patients, and small mammals, including genetically manipulated strains. A combination of behavioral and psychophysical experiments, structural and functional

imaging, acoustical and electrophysiological recordings provides the most precise estimates.
In this thesis, peripheral processes for tinnitus generation under hydropic conditions were addressed. Findings suggested that tinnitus developed in response to alterations of the mechano-electrical transduction of cochlear hair cells and specifically due to changes of the cochlear amplifier.
In contrast, the cochlear amplifier was not affected when transcutaneous electrical stimulation was applied to the inner ear. Stimulation activated the vestibular periphery eliciting vestibular responses and influenced auditory percepts on a retrocochlear level.
On a cochlear level, intense non-traumatic low-frequency sound stimulation temporarily modified sensitivity in a uniform and unique manner. Sensitivity changes were of oscillatory nature, as assessed with otoacoustic emission recordings, suggesting operating point shifts of the cochlear amplifier. Different homeostatic mechanisms involving intra- and extracellular potentials and ion concentrations were considered.
The present results encourage further investigation of the mammalian inner ear homeostasis in the intact inner ear keeping the close relation and the interplay between the auditory system and the vestibular system in mind.

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APPENDIX

DPOAE frequencies can be mathematically calculated. The non-linear behavior, e.g. of the MET transfer function of OHCs (see section 1.2.4) can be approximated by a Taylor series at the origin (Lukashkin and Russell, 2005), with y as output and x as input signal:

$$y(x) = \sum_{n=0}^{\infty} \frac{1}{n!} \cdot \frac{d^n y(x)}{dx^n}\Big|_{x=0} \cdot x^n \tag{6.1}$$

To understand the generation of the DPOAEs, the term is simplified in the following section, excluding the constant component (n=0) and higher order components (n>3), as they contribute only little to the nonlinear output (Abel, 2008). This results in the Taylor polynomial of the 3rd order:

$$y(x) = a_1 x + a_2 x^2 + a_3 x^3 \tag{6.2}$$

Replacing the input signal x with the two wave stimuli $x(t) = L_2 cos(f_2 t) + L_1 cos(f_1 t)$ results in:

$$\begin{aligned} y(t) &= a_1[L_2 cos(f_2 t) + L_1 cos(f_1 t)] \\ &+ a_2[L_2 cos(f_2 t) + L_1 cos(f_1 t)]^2 \\ &+ a_3[L_2 cos(f_2 t) + L_1 cos(f_1 t)]^3 \end{aligned} \tag{6.3}$$

Applying the binomial theory leads to:

$$\begin{aligned} y(t) &= a_1[L_2 cos(f_2 t) + L_1 cos(f_1 t)] \\ &+ a_2[L_2^2 cos^2(f_2 t) + \underline{2L_2 cos(f_2 t) L_1 cos(f_1 t)} + L_1^2 cos^2(f_1 t)] \\ &+ a_3[L_2^3 cos^3(f_2 t) + \underline{3L_2^2 cos^2(f_2 t) L_1 cos(f_1 t)} + \underline{3L_2 cos(f_2 t) L_1^2 cos^2(f_1 t)} + L_1^3 cos^3(f_1 t)] \end{aligned} \tag{6.4}$$

By applying the following trigonometrical calculations:

$$cos(f_2 t) cos(f_1 t) = \frac{1}{2}\{cos[(f_2 + f_1)t] + cos[(f_2 - f_1)t]\} \tag{6.5}$$

and

$$cos^2(f_2 t) = \frac{1}{2}[1 + cos(2 f_2 t)] \tag{6.6}$$

The underlined parts can be reformulated into:

$$2L_2 cos(f_2 t) L_1 cos(f_1 t) = L_2 L_1\{cos[(\underline{f_2 + f_1})t] + cos[(\underline{f_2 - f_1})t]\} \tag{6.7}$$

and

$$3L_2^2 cos^2(f_2 t) L_1 cos(f_1 t) = \frac{3}{2} L_2^2 L_1 \{cos(f_1 t) + \frac{cos[(2f_2 + f_1)t]}{2} + \frac{cos[(2f_2 - f_1)t]}{2}\} \tag{6.8}$$

$$3L_2 cos(f_2 t) L_1^2 cos^2(f_1 t) = \frac{3}{2} L_2 L_1^2 \{cos(f_2 t) + \frac{cos[(2f_1 + f_2)t]}{2} + \frac{cos[(2f_1 - f_2)t]}{2}\} \tag{6.9}$$

These equations show why the frequencies of DPOAEs can be calculated by summing or subtracting multiples of primary frequencies. The quadratic part of the Taylor series ($a_2 x^2$) generates distortion at $f_1 \pm f_1$, therefore DPOAEs with their frequency at f_2-f_1 are called quadratic emissions. The cubic part ($a_3 x^3$) results in distortion at $2f_1 \pm f_2$ and $2f_2 \pm f_1$. Thus, DPOAEs at $2f_1$-f_2 are referred to as cubic DPOAEs. In a similar fashion, higher order DPOAEs can be calculated when considering higher order components of the Taylor series. However, quadratic and especially cubic emissions have the highest "weight" within the Taylor series (see inverse component $1/n$). Of all DPOAEs, the quadratic and cubic DPOAEs show the highest sound pressure levels and are therefore most often used.

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Eidesstattliche Erklaerung/Affidavit

Hiermit versichere ich an Eides statt, dass ich die vorliegende Dissertation ***Mammalian Inner Ear Homeostasis - Out of Balance?*** selbstständig angefertigt habe, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

I hereby confirm that the dissertation ***Mammalian Inner Ear Homeostasis - Out of Balance?*** is the result of my own work and that I have only used sources or materials listed and specified in the dissertation.

24.September 2019	Margarete Überfuhr
.....	.....
München, den (Munich, date)	Unterschrift (Signature)