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# Analysis of Biological Acoustic Waves by Means of the Phase–Sensitivity Technique

Wojciech Michalski<sup>1</sup>, Wojciech Dziewiszek<sup>2</sup> and Marek Bochnia<sup>2</sup>

<sup>1</sup>Technical University of Wrocław,

<sup>2</sup>Medical University of Wrocław

Poland

## 1. Introduction

The analysis of hearing mechanisms and research on the influence of various internal (pathologies, ageing) and external (trauma, vibration, noise) factors on sound perception are usually done using acoustic waves induced in the external ear canal. Stimuli which have been used for this purpose are: clicks, tone bursts, half-sine-waves, single tones or pairs of tones. The Corti organ's responses to the external stimuli have either an electric or acoustic character. In the former case, these are cochlear microphonics (CMs) picked up from the surface or from the inside of the cochlea, which are usually used as an indicator of damage to the organ of Corti in animals. In the latter case, these are acoustic waves that appear in the external ear canal as a result of stimulation. The acoustic waves have an important clinical value. Taking into account the presence of nonlinear distortions in the cochlea, the waves that appear after stimulation with a pair of tones are called distortion product otoacoustic emissions (DPOAE).

In studies on CMs, the origin of stimulating waves is often a single earphone (controlled by a generator of defined, often periodical, electrical signals) placed in the external auditory canal. In studies on DPOAE, a probe with two miniature earphones and one microphone is placed in the external auditory canal. The earphones are controlled by two generators of frequencies  $f_1$  and  $f_2$  and the microphone converts the returned DPOAE wave with a combination frequency, e.g.  $f_3 = 2f_1 - f_2$ , into an electrical signal.

The acoustic wave which induces CM signals is usually a periodic wave, while the waves inducing DPOAE signals consist of two pure tones. Thanks to the easy access to the output(s) of the generator(s) the phase-sensitive detection (PSD) technique can be used to measure both CM and DPOAE signals. Very weak (even below single microvolts) CM and DPOAE signals originating from the unimpaired cochlea can be measured in this way. Thanks to this technique signals obscured by other disturbing sources (even thousand times larger) can be measured accurately. This is possible because the phase-sensitive detector singles out the input signal with a specific reference frequency while signals with frequencies different from the reference are rejected. The fundamentals of this technique and its measuring potential are described in section 2.

In section 3, the authors' own experiments aimed at determining the effect various factors on the electrical function of the Corti organ are described. The factors include: vibration

(3.3), ototoxic medicines (3.4) and laser beams used in ear microsurgery (3.6). The role of the signal phase in the measurements is given special attention.

In section 4, experiments involving acoustic waves being nonlinear products of the Corti organ are presented. The individual subsections describe the way in which the PSD technique is applied (4.1), compare the latter with the previously used methods (4.2) and discuss the authors' own experiments in which the phase-sensitive technique is employed to measure DPOAE signals (4.3) and to measure simultaneously DPOAE signals and CMDP (cochlear microphonic distortion product) signals (4.4). It is shown that the phase of DPOAE signals plays an essential role in otoacoustic emission studies.

All the experiments described in sections 3 and 4 were carried out on coloured guinea pigs, each weighing 500-650 g, being under general ketamine/xylazine (15 mg/kg and 10 mg/kg body weight, respectively) anaesthesia. A Homoth measuring probe was placed in the external auditory meatus of the animals. The probe contained two mini earphones and a standard microphone. Prior to the measurements the probe had been graduated in a Brüel&Kraej artificial ear 4144, using a measuring amplifier 2607 made by the same company. Permission to carry out the experiments had been given by the Bioethical Committee in Wrocław.

Section 5 presents the final conclusions and discusses the future of the phase sensitive detection technique in investigations into the function of the cochlea exposed to various hazards.

## 2. Phase-sensitive detection technique

### 2.1 Fundamentals

The measurement apparatus based on the phase-sensitive detection technique is called a *lock-in amplifier* or a *lock-in nanovoltmeter*. Lock-in measurements require a frequency reference which should be strictly connected with a fixed frequency of the function generator used in the experiment. The reference signal can be either a square wave or a sinusoid. A block diagram of a typical lock-in amplifier is shown in fig. 1.

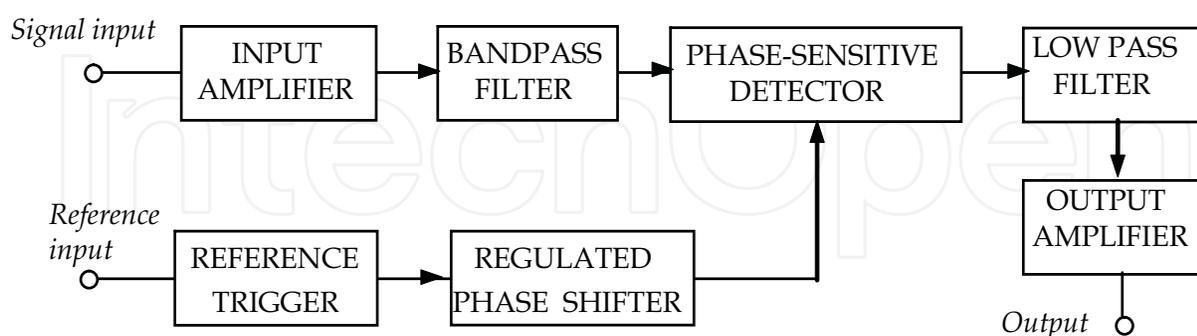


Fig. 1. Block diagram of lock-in amplifier with single phase-sensitive detector

Let us assume that the input signal can be described as:

$$V_{sig} = A_{sig} \cos(\omega_1 t + \alpha_0), \quad (1)$$

and the reference signal as:

$$V_{ref} = A_{ref} \cos(\omega_2 t + \beta_0) \quad (2)$$

The two signals have different amplitudes, frequencies and initial phases. At the phase-sensitive detector inputs there are signals with unchanged frequencies, but with different amplitudes and phases:  $V_{sig} = A_{sig1} \cos(\omega_1 t + \alpha_{01})$  and  $V_{ref} = A_{ref1} \cos(\omega_2 t + \beta_{01})$ . The output of the PSD is simply the product of the two sine waves

$$\begin{aligned} V_{PSD} &= A_{sig1} \cos(\omega_1 t + \alpha_{01}) \cdot A_{ref1} \cos(\omega_2 t + \beta_{01}) \\ &= 0,5 A_{sig1} A_{ref1} \left[ \cos[(\omega_1 - \omega_2)t + (\alpha_{01} - \beta_{01})] + \cos[(\omega_1 + \omega_2)t + (\alpha_{01} + \beta_{01})] \right] \end{aligned} \quad (3)$$

At the output of the PSD there are two signals: a slow-changing signal with differential frequency  $(\omega_1 - \omega_2)$  and a signal with overall frequency  $(\omega_1 + \omega_2)$ . If the PSD output signals are passed through a low pass filter, the fast AC signal will be removed. When the frequencies of the two signals are approximately equal  $(\omega_1 \approx \omega_2)$ , the filtered PSD output is a slowly changing DC signal proportional to the signal amplitude and  $\cos(\alpha_{01} - \beta_{01})$ . When  $\omega_1 = \omega_2$ , the filtered signal is exactly a DC signal. By adjusting the phase of the reference signal one can make  $(\alpha_{01} - \beta_{01})$  equal to zero, in which case only  $B_{sig}$  can be measured. This is true if both initial phases  $\alpha_0$  and  $\beta_0$  do not change over time, otherwise  $\cos(\alpha_{01} - \beta_{01})$  will change over time and  $V_{out}$  of the lock-in amplifier will not be a DC signal.

The phase dependency of the output voltage of the lock-in amplifier with one PSD unit can be eliminated by adding a second PSD multiplying the same measured signal by a reference signal shifted by  $90^\circ$ . A block diagram of the lock-in amplifier with a double PSD is shown in fig. 2.

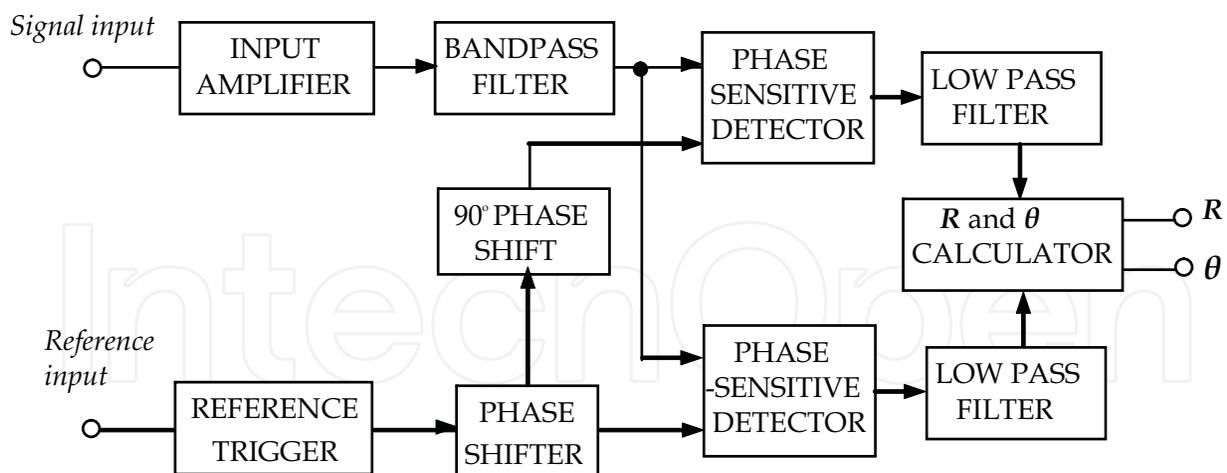


Fig. 2. Block diagram of lock-in amplifier with double PSD

The output of the second PSD, filtered by the low pass filter, is proportional to the signal amplitude and  $\sin(\alpha_{01} - \beta_{01})$ . Now there are two outputs:  $X = A_{sig1} \cos(\alpha_{01} - \beta_{01})$  and  $Y = A_{sig1} \sin(\alpha_{01} - \beta_{01})$ . When

$$R = \sqrt{(A_{sig1} \cos(\alpha_{01} - \beta_{01}))^2 + (A_{sig1} \sin(\alpha_{01} - \beta_{01}))^2} = A_{sig1} \quad (4)$$

the phase dependency is removed. Phase difference ( $\alpha_{01}-\beta_{01}$ ) can be measured according to

$$\alpha_{01} - \beta_{01} = \tan^{-1}(Y/X) \quad (5)$$

The first lock-in amplifiers were based on analogue technology. The measured signal and the reference were analogue voltage signals and they were multiplied in an analogue PSD. The results of multiplication were filtered through a multistage RC filter. In such lock-ins the reference signal phase at the PSD input had to be manually adjusted to the phase of the measured signal so that  $\cos(\alpha_{01} - \beta_{01}) = 1$ . It was technically difficult to perform measurements by means of such lock-ins and it was practically impossible to register the amplitude and phase changes of the measured signals. Digital technology made it possible to build lock-in amplifiers in which both signal and reference inputs were multiplied and filtered digitally. Dual phase-sensitive detection eliminated the need for manual phase adjustments and enabled the simultaneous measurement of signal amplitude and phase. Such simultaneous measurements can be performed in *real time*, practically without any delay to the inducing signal. It also became possible to register short (below 0.1s) and slow changes in amplitude and phase over time.

## 2.2 Application of double-phase detection

The PSD technique offers greater measuring possibilities owing to the fact that:

1. the signal fed to the examined object may have various periodical waveforms,
2. the examined object can be linear or nonlinear,
3. the reference signal frequency can be equal to the frequency of the signal being delivered to the examined object, but it also can be an integral multiplicity (or submultiplicity) of this frequency.
4. two coherent signals can be introduced to the examined (usually nonlinear) object; the reference signal can be used at a frequency that is a linear combination of the frequencies of the inducing signals.

The basic experimental setup is shown in fig.3.

The generator used in the setup has two synchronous outputs. One of them (sync. output) supplies a TTL signal. Depending on the frequency of the reference signal one can measure the first harmonic, higher harmonics and subharmonics. In switch position 1 (fig.3), the reference signal is taken directly from the generator's synchronous output whereby the first harmonic can be measured. In switch position 2, the synchronous signal is multiplied by integral number  $n$  whereby the  $n$ -harmonic can be measured. In order to measure the  $n$ -subharmonic the generator's synchronic output must be divided by integral number  $n$  (the switch in position 3).

In the simplest case, the waveform of the signal directed to the examined object is sinusoidal. When the examined object is linear, using the PSD technique one can very precisely (with an accuracy of 1 nanovolt) measure the electrical response of the object. If the signal is a simple square wave or another periodical wave with frequency  $f$ , the examined linear object does not change the signal spectrum and the filtered PSD output is a DC signal proportional to the root mean square (rms) of the first component of the signal.

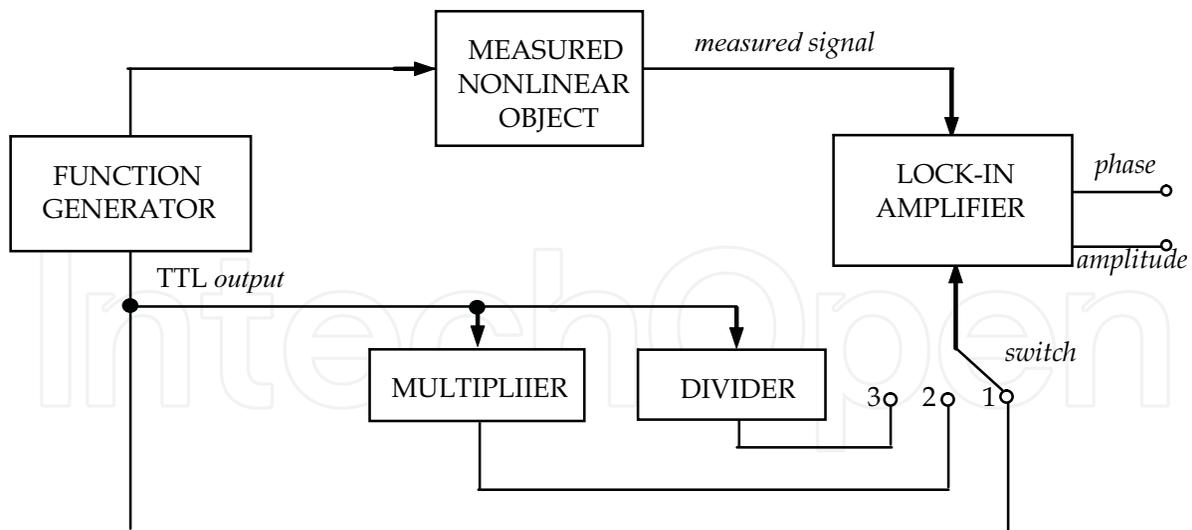


Fig. 3. Basic experimental set-up with lock-in amplifier for measuring first harmonic (1), higher harmonic (2) and subharmonic (3)

The situation becomes more complicated when the examined object is nonlinear. The signal spectrum at the PSD input differs from the one at the generator output (the nonlinear object changes the input signal spectrum). This is true for all the signal waveforms, including the sinusoidal one. Then the lock-in amplifier measures the rms of both the first harmonic and the  $n$ -harmonic ( $n$ -subharmonic). When harmonic or subharmonic distortion is measured, the function generator supplies a pure sinusoidal signal without any harmonics. The basic experimental setup shown in fig. 3 was used to carry out experiments described in section 3.

### 2.3 Nonlinear object testing with two synchronous signals

The double phase-sensitive detection technique and modern digital technologies offered new possibilities of examining nonlinear objects. An example of the measuring systems which have been developed is shown below (fig. 4). The main component of the setup is a digital sinus generator of three signals with synchronous frequencies. Two of the signals are delivered to the examined object while the third one serves as a reference signal. The frequency of the third signal is a linear combination of the frequencies of the other two signals.

Let us assume that the output-input function for the examined object can be described by the formula:

$$V_{out} = C \cdot V_{in}^3, \quad (6)$$

where  $C$  is a constant value. The input signal is the sum of signals with different amplitudes, phases and frequencies and so:

$$V_{out} = C \left[ A_1 \cos(\omega_1 t + \alpha_{01}) + A_2 \cos(\omega_2 t + \alpha_{02}) \right]^3 \quad (7)$$

After trigonometric conversions it is possible to receive a signal frequency spectrum at the object's output. The frequencies, amplitudes and phases of the particular spectral components (assuming that the examined object does not change its phase relations, i.e. it is characterized by pure resistances) are shown in table 1.

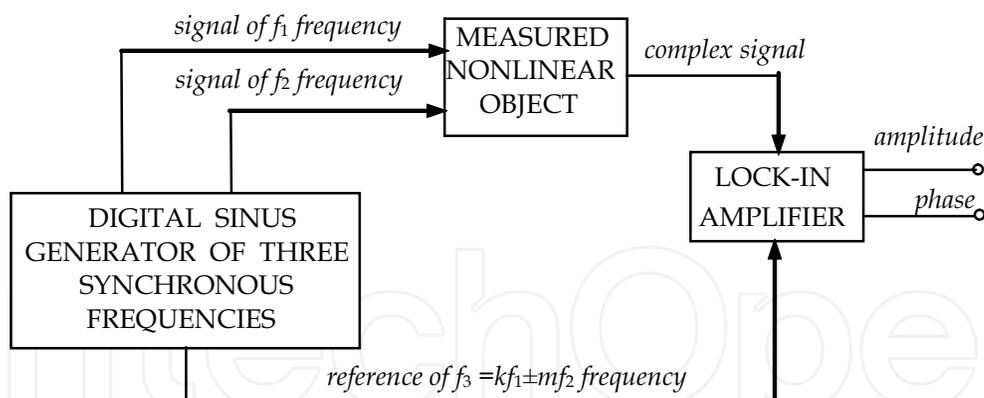


Fig. 4. Block diagram of experimental setup for more complicated studies of nonlinear objects

Frequencies of spectral components	Amplitudes of spectral components	Phase of spectral components
$\omega_1$	$1,5A_1 C (0,5 A_1^2 + A_2^2)$	$a_{01}$
$\omega_2$	$1,5A_2 C (0,5 A_2^2 + A_1^2)$	$a_{02}$
$3 \omega_1$	$0,75 C A_1^3$	$3 a_{01}$
$3 \omega_2$	$0,75 C A_2^3$	$3 a_{02}$
$2 \omega_1 + \omega_2$	$0,75 C A_1^2 A_2$	$2a_{01} + a_{02}$
$\omega_1 + 2 \omega_2$	$0,75 C A_1 A_2^2$	$a_{01} + 2 a_{02}$
$2 \omega_1 - \omega_2$	$0,75 C A_1^2 A_2$	$2a_{01} - a_{02}$
$\omega_1 - 2 \omega_2$	$0,75 C A_1 A_2^2$	$a_{01} - 2 a_{02}$

Table 1. Exemplary spectrum at output of object with 3rd-order nonlinearity, tested by pair of pure tones

The amplitude of each of the spectral components can be measured using this technique if a proper reference signal frequency is selected. The interpretation of phase shifts between the particular spectral components is much more complicated and requires taking into account the phase shifts introduced by the examined object. Moreover, the phase shifts introduced by the examined object may be a function of frequency and so they may be different for each spectral component. The technique was used to examine nonlinear distortions during the stimulation of the cochlea by a pair of pure tones. The results of this research are presented in section 4.

### 3. Using PSD technique to study cochlear potentials

#### 3.1 Measuring techniques

Cochlear potentials are biopotentials of the inner ear. They are described as electrical signals arising in response to the acoustic stimulation (usually by a click or a tone) of the organ of

Corti. For the first time they were registered by Wever and Bray in 1930 (Wever & Bray, 1930). The discovery of the signals made it much easier to examine the function of the inner ear and made it possible to assess the impact of various external and internal factors on this function. It is widely believed that cochlear microphonics (CMs) are generated mainly by outer hair cells (OHCs). Therefore it seems reasonable to use CMs as an indication of the OHC function. On the basis of measurements performed over a long period (e.g. a few weeks or months) one can assess if given hearing damage is temporary or permanent. The CM signal originating from different places in the human ear (or the animal ear) can be recorded. In humans CMs are usually picked up from the round window during surgical procedures performed on patients with various hearing pathologies. There are much fewer reports describing the reception of CM signals from the promontory or the ear canal near the eardrum. The past and present studies of the mechano-electrical cochlear function (based on the reception of CMs) are conducted mainly on animals, using: *in vivo* preparations of anaesthetized animals with positive Preyer's reflex, *in vitro* preparations of the cochlea or *in vitro* preparations of the hair cells. As regards the research into the impact of various external and internal factors on the hearing organs, the *in vivo* studies seem to be most clinically valuable.

In the 1930s and 1940s CMs were measured at the cochlea's round window (Wever & Bray, 1930). In most animals the round window is relatively easily accessible and so measuring electrodes were usually placed on it or in its direct proximity. In the first years after the discovery of inner ear potentials, CM signals were measured by a single active probe. Several years later the first mapping of CMs on the cochlear surface was described (Thurlow, 1943). It was probably the first attempt ever to place the probe so close to the source of cochlear microphonics. CM potentials are continued to be measured at the cochlea's round window today (Brown, 2009). This measuring technique was not abandoned after the introduction of very sensitive (but invasive) procedures (Tasaki et al., 1952). Tasaki monitored CMs using a pair of active intracochlear electrodes in the basal turn (one electrode in scala tympani, the other in scala vestibuli). The electrodes were connected to a balanced differential amplifier. The reference electrode was placed on the neck muscles. This enables the measurement of the potentials very close to the organ of Corti and eliminates the auditory nerve potentials. The largest drawback is the mixing of perilymph and endolymph when the probe is introduced.

A new recording technique has been described by Carricondo (Carricondo et al., 2001). In this technique, CM potentials are recorded by subcutaneous electrodes in animals or by surface electrodes in humans. Two active electrodes are placed on the mandibular muscles while the reference electrode is located on the head's vertex. All the three electrodes are connected to a differential amplifier. The signal coming from the amplifier's output is filtered and subsequently averaged through in-phase synchronization with the sound stimuli.

### 3.2 History of CM studies in Wrocław

In 1960 at the Wrocław University of Technology an oscillograph was built and used as part of an experimental setup for registering cochlear microphonics (CMs). In the following years Jankowski and Giełdanowski started a series of experiments on animals – first on cats, later on guinea pigs (Jankowski et al., 1962). A Biopotentials Research Workshop was founded, where biopotentials were measured after damage to the inner ear or the skull, in acoustic

trauma, in hypothermia or hypoxia, after the administration of certain drugs, and so on. Figure 5 shows a schematic of the experimental setup.

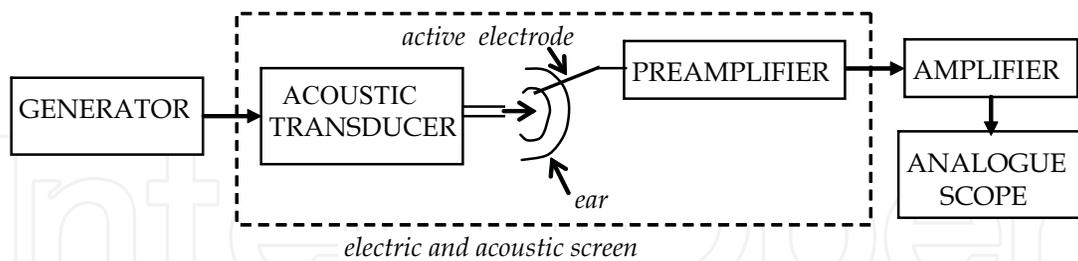


Fig. 5. Schematic of experimental setup used by Jankowski & Giëdanowski

The experimental animals (under urethane anaesthesia, which does not diminish CM voltages) underwent ear surgery: the bones were exposed and drilled until the round window was revealed. Platinum electrodes (platinum wires 0.1 mm in diameter, coated with PMMA) were used for CM measurements. The bare end (not coated with PMMA) of the platinum wire was brought into contact with the round window membrane (without damaging it). An injection needle was used as the other electrode. It was inserted into the muscles around the surgical wound. An example of an CM oscillogram from Ziëmski's work (Ziëmski, 1970) is shown in fig. 6.

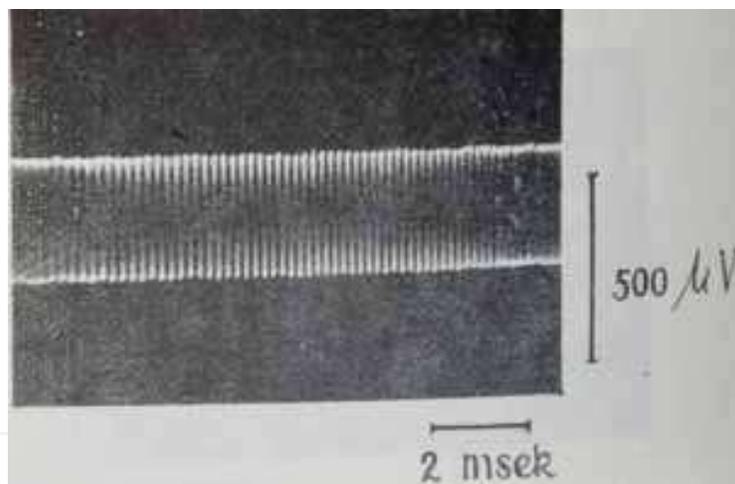


Fig. 6. Example of CM oscillograph record (stimulus tone parameters:  $f=4096$  Hz, acoustic pressure level - 60dB)

In the middle of the 1990s a new surgical approach, making it possible to expose the whole cochlea in guinea pigs, was proposed. Skin was cut from the occipital part of the skull, around the angle of the mandible up to the place of about 0.5 cm above the angle of the animal's snout. The temporal muscles were removed, the mandible partially resected and the stylo-hyoid muscle cut. Bones were drilled, leaving the tympanic membrane intact. In the 1990s the present authors used the phase-sensitive detection technique to measure CMs. A patent was applied for in 1996 and the technique was patented in December 2000 (Patent PL no. 180060). A report on the use of a similar method was published by Kobayashi (Kobayashi, 1997). A schematic of the first setup used to measure CMs by the PSD technique is shown in fig. 7.

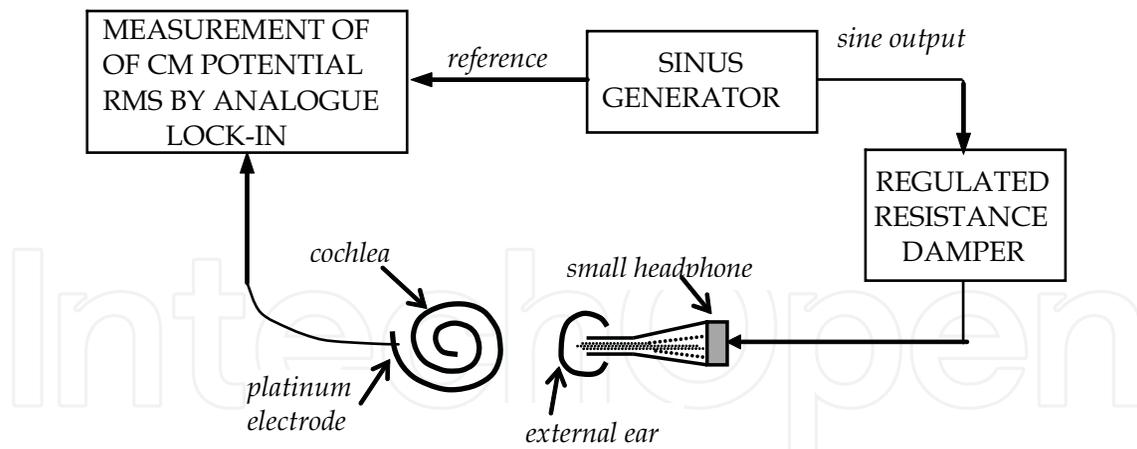


Fig. 7. Schematic of first setup used to measure CMs by phase-sensitive detection technique

Thanks to the new surgical approach combined with the phase-sensitive detection technique it became possible to create a map of CM potential amplitude and phase distributions on the cochlear surface. For this purpose, the active electrode would be fixed in six different points on the cochlea's surface (on the apex, one point at the third turn, two points at the second turn and two points at the basal turn). When the electrode was fixed in any of the six points, the frequencies of the stimulating acoustic wave would be successively selected from the measurable range. Three different acoustic wave pressures (60 dB, 80 dB and 95 dB) would be set for each of the measuring frequencies. This means that 18 different amplitude-phase (A-P) values of the CM signal were measured in each of the six points on the cochlea's surface. In this way 18 different A-P distribution patterns would be obtained for the guinea pig cochlea. Six of them (three for  $f = 260$  Hz and three for  $f = 8$  kHz) are shown in fig. 8. Phase in each measuring point is related to phase on apex at 60 dB.

It was found that CMs had a different phase and amplitude in the different parts of the cochlea, which was due to the fact that each of the six measuring points was located at a different distance from the CM sources inside the organ of Corti.

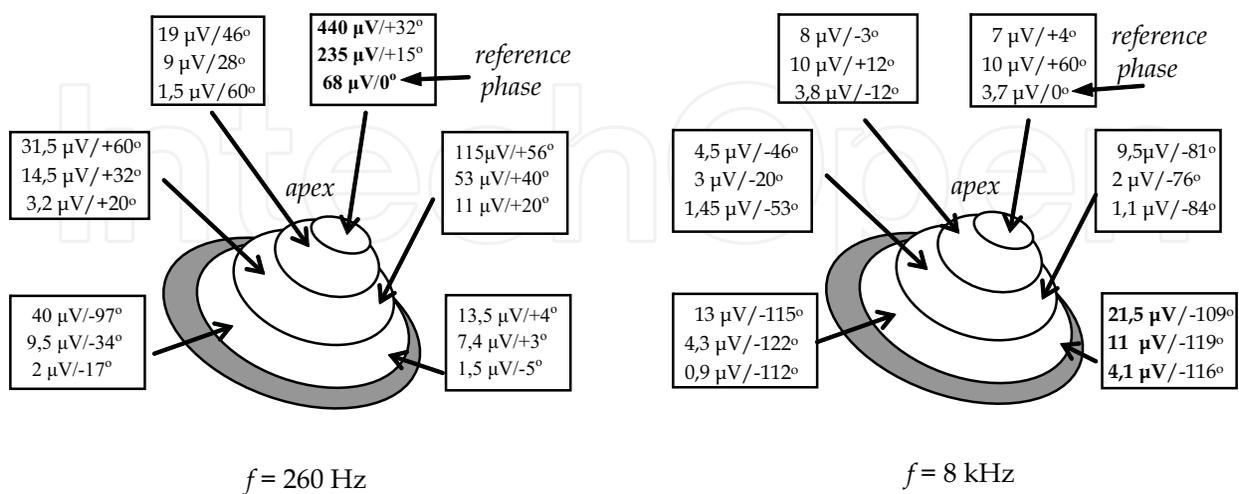


Fig. 8. CM potential amplitude and phase in six different points on cochlea surface, measured at 260 Hz and 8 kHz. At each of six points there are three amplitude/phase values: upper for 90 dB, central for 80 dB and lower for 60 dB

In order to unify CM measurements, two distinctive and universal measurement points were established: the cochlea's apex for the frequencies of 260, 500, 1000 and 2000 Hz and the cochlea's base for 4000 and 8000 Hz. Phase in each measuring point is related to phase on apex at 60 dB.

### 3.3 Influence of whole-body vibration on inner ear

Vibration is one of the most widespread injurious factors in the environment of civilized man (Palmer et al., 2000a, 2000b). The energy absorbed can have a pathological effect on all the tissues and organs of the body, although the consequences of exposure to vibration do not present a uniform clinical picture (Jones, 1996; Seidel & Heide, 1986). Because all machines and vibration devices also produce noise, usually the combined effect of the two factors is examined (Castelo Branco, 1999). There is a prevalent view that mechanical vibrations exert only a weak, additionally traumatic influence on the hearing organ (Seidel, 1993). Several experimental investigations into the harmfulness of vibration were carried out on animals (Hamernik et al., 1980, 1981). Changes in the hearing organ most often would be found in the hair cells (Rogowski, 1987). This made us undertake our own research in the 1990s. In order to determine the impact of long-term general vibration on the inner ear it was necessary to: 1) design and built noiseless vibration apparatus, 2) subject several groups of animals to general vibration (defined by controlled parameters over different periods of time) and 3) evaluate selected parts of the organ of hearing, using norms based on values derived from a control group.

In order to ensure proper experimental conditions, i.e. sinusoidal (10 Hz) vertical (5 mm) shaking, a device consisting of an electric impulse generator, a power amplifier and an impulse exciter was built (fig. 9). Experiments were carried out on young, coloured guinea pigs of both sexes weighing 240-360g. Fifty six animals with the normal Preyer reflex and without otoscopically detectable changes were used. The control group (group m0) consisted of 20 of the animals and served to establish functional and morphological norms. In order to avoid changes due to aging being interpreted as the effects of vibration, the control group was examined after a seven-month stay (6+1 months = duration of the longest experiment + a rest) in an animal house. The study group consisted of 36 guinea pigs divided into two subgroups of 18 animals each. Each subgroup was subjected to vibration over different periods, i.e. 30 (group m1) and 180 (m6) days. These were in fact respectively 22 days (5 days/week, 6 hours/day = 132 hours) and 132 days (792 hours). After the experiment and a one-month (30 day) rest, the animals which were in good general condition and without otoscopically detectable changes were qualified for functional and morphological investigations.

Cochlear microphonics were measured under urethane anaesthesia, using the PSD technique and the setup schematically shown in fig. 3 (the switch in position 1). CMs were picked up from the apex of the cochlea for the frequencies of 250 Hz, 500 Hz, 1 kHz and 2 kHz and from the region of the round window for 4 kHz and 8 kHz, using a platinum needle electrode. For the two study groups and the control group, a total of 6048 data values were taken for the bilaterally examined pulse wave frequencies (260 Hz-8 kHz) and intensities (55 dB-95 dB).

The results of the CM measurements were subjected to statistical analysis. The aim was to find out whether the experiment had any influence on CMs and, if so, what that influence was. The questions asked were: 1) are there statistically significant differences between the

CM voltages obtained from the control groups and the study groups, and 2) are there statistically significant differences in the CM voltages obtained within the study groups? The CM values obtained from the healthy animals showed considerable individual differences, and their distribution showed neither normalcy nor log-normalcy. Therefore all the experimental samples were examined using non-parametric tests. The K-S Lilliefors test showed: 1) for control group m0 compared with study groups m1 and m6, a significant decrease in CMs for the frequencies of 260 Hz, 1 kHz and 2 kHz, and 2) for m1 compared with m6, a decrease in CM for the frequencies of 260 Hz and 2 kHz. The Kruskal-Wallis test confirmed the results of the K-S Lilliefors test as regards the location and nature of the changes.



Fig. 9. Cage with animals exposed to vibrations

The results of the investigations indicated possible greater damage to the hair cells in the forth and third turnings of the cochlea. Further morphological examinations were needed to verify this observation. After the bilateral CM measurements the animals were decapitated and samples were prepared for SEM examinations of the sensorial epithelium. The samples were examined and photographed using a scanning DSM 950 microscope. The influence of general vibration on the organ of Corti was assessed on the basis of the condition of the hair cells, taking into consideration their disorganization, deformation, mutual adhesion and any reduction in the number of cilia.

SEM examinations were carried out on 20 cochleae from the control group animals and on all the animals in the two study groups. In the healthy animals, the sensorial epithelium was found to be normal in every case, but in each of the study groups the above mentioned damage was observed. It usually occurred in the OHC region of the apex, and its extent gradually increased in the direction of the cochlea's base (up to the second turning). OHC3 was found to be most susceptible to vibratory trauma. Cell damage decreased from the circumference to the modiolus, and the OHCs showed considerably greater resistance to vibration (fig.10). Undoubtedly, the observed damage to the sensorial epithelium resulted from mechanical vibration, and its severity clearly increased with the duration of the

experiment. Consequently, the mechanism of deterioration in hearing in all the frequency ranges (especially at low and average frequencies) in persons subjected to whole-body vibration could be discovered by analyzing the observed changes.

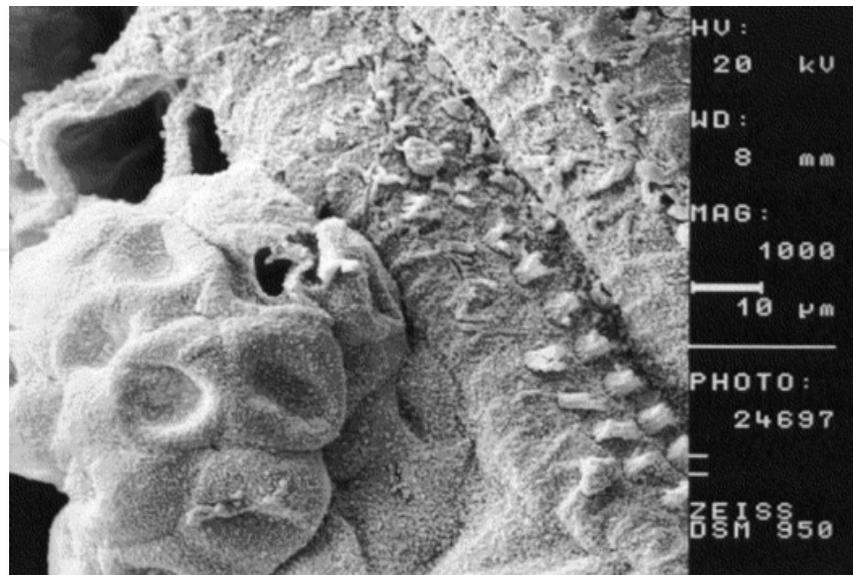


Fig. 10. Group M6, 4<sup>th</sup> cochlear turning: numerous lesions of hair cells and damage to Hensen's cells

### 3.4 Studies of gramicidin ototoxicity

Polypeptide antibiotics are used in a variety of clinical situations. Their molecules contain a specific chain of aminoacids and a non-aminoacidic part (e.g. fatty acids in polymyxins or glycopeptide in vancomycin). They are generally effective against Gram-positive bacteria, except for polymyxins which are effective against Gram-negative bacteria. They act by disrupting the selective permeability of bacterial cellular membranes. Despite their long history, polymyxins have had a limited clinical use due to the large number of side effects. Currently they are used primarily for topical treatment (Wadsten et al., 1985).

Since no descriptions of the effects of the systemic administration of gramicidin on the inner ear could be found in the literature, the authors decided to examine CMs and to compare the ototoxic effects after the systemic and topical administration of gramicidin. Also the inner ear of animals which received i.m. injections of gramicidin were examined using a DSM 950 scanning electron microscope (Bredberg et al., 1970; Davis, 1983).

The research was conducted on 70 young, coloured guinea pigs. All the animals showed the positive Preyer reflex and no pathologies under otoscopic examinations. The experimental animals (G) were divided into 5 subgroups, depending on the drug administration mode and the administered dose. Each experimental subgroup (G1-G5) consisted of 8 randomly chosen animals. Subgroups G1-G3 received respectively 2, 5 and 10 mg of gramicidin/kg i.m., once per day, for 14 consecutive days. The animals from subgroups G4 and G5 were administered a 0.25% and 10% solution of gramicidin suspended on a haemostatic sponge placed on the round window.

The control group (K) consisted of 30 animals randomly divided into 2 subgroups (K1 and K2). The animals in control subgroup K1 were injected with normal saline solution once per day for 14 consecutive days. The animals in subgroup K2 were administered normal saline

solution placed on the round window. One day after the last injection (the 15<sup>th</sup> day of the study) electrophysiological measurements were carried out on the animals in subgroups G1-G3 and K1. Then their cochleae were removed for SEM examinations. In the case of the animals belonging to subgroups G4, G5 and K2, CM measurements were performed after removing the haemostatic sponge from both ears and allowing the round windows with their surroundings to dry (Gale & Ashmore, 1977).

Cochlear microphonics (CMs) were investigated under urethane anaesthesia, using the PSD technique and the setup schematically shown in fig. 3 (the switch in position 1). CMs were picked up from the apex of the cochlea for the frequencies of 260 Hz, 500 Hz, 1 kHz and 2 kHz and from the region of the round window for 4 kHz and 8 kHz by means of a platinum needle electrode. As regards study subgroups G1-G5 and control subgroups K1 and K2, a total of 7560 data values were taken for the examined frequencies (260 Hz-8 kHz) and intensities (55 dB-95 dB). The results of the CM measurements were subjected to statistical analysis (the t-Student test).

Gramicidin administered systemically in a dose of 2 mg/kg led to a significant (38%) decline in CM voltage in K1 subgroup animals for the frequencies of 260 Hz and 2 kHz. For the other frequencies the drop in CMs amounted to about 15%, except for the 4 kHz at which a slight improvement was observed for sound levels between 55 and 70 dB. A significant drop in CMs was observed in subgroup G2 at 2 kHz and sound levels above 70 dB. At 95 dB the decline in CMs was 30% larger than in the G1 animals. The changes in the G2 animals relative to G1 were even more significant at 500 Hz, 1 kHz and 8 kHz. The animals receiving 10 mg/kg of gramicidin showed lower CMs than the ones registered in all the examined frequency ranges for control subgroup K1. The largest drop was registered at 2 kHz (31% lower than in the K1 control subgroup). The smallest changes were observed at 8 kHz. In subgroups G1-G3, the largest differences in CMs were observed at 4 kHz for all the sound levels.

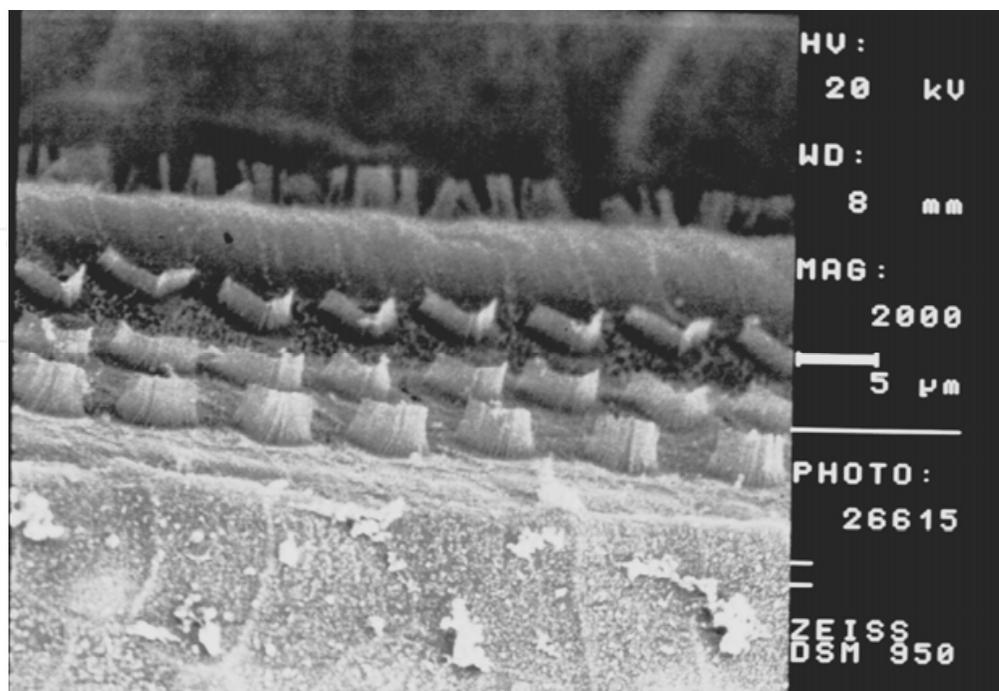


Fig. 11. Group K1, 2nd cochlear turn: unchanged sensory epithelium

In the animals receiving topical 0.25% gramicidin solution (G4), a significant drop in CMs (in comparison with control K2) was observed at 1 kHz and 2 kHz. In group G5 (where the animals were administered 10% gramicidin in solution on the round window) a drop in CMs was observed also at 4 kHz and 8 kHz. At low sound levels the largest falls in CMs were observed in subgroup G4.

In the G1 and G2 animals no damage to the sensory epithelium was found under SEM. The destruction of cochlear hair cells occurred in the G3 animals. The changes were most visible in OHC3 cells in the cochlea's third turning.

To sum up, the systemic administration of gramicidin leads to greater disruptions of the bioelectric functions of the inner ear than local, topical administration (Linder et al., 1995).

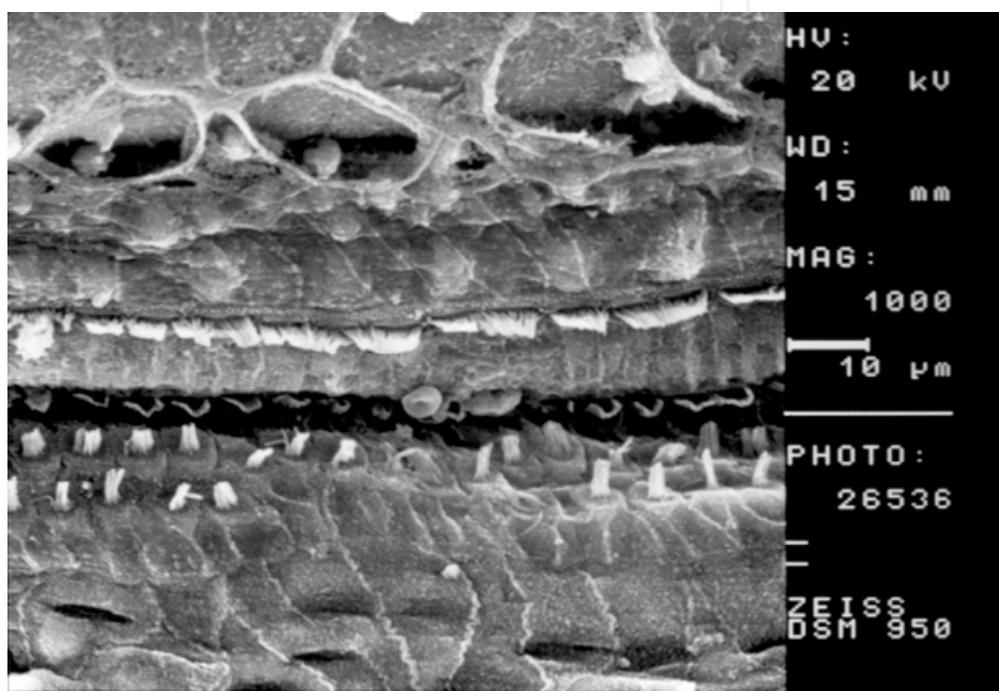


Fig. 12. Group G3, 3<sup>rd</sup> cochlear turn: numerous lesions in OHC3 cells and structural changes in cilia

### 3.5 CM amplitude and phase changes caused by changes in intensity of stimulating acoustic wave

Another important improvement in CM measurement came with the introduction of a lock-in amplifier with double phase-sensitive detection. In December 2003 a device for the phase-sensitive measurement of inner cochlea microphonic potentials was registered at the Patent Office. It was patented in November 2010. The device can measure harmonic, subharmonic and linear distortion products of the cochlea after dual-tone stimulation. Figure 13 shows a schematic of the measuring device.

The amplitude and phase of CMs in a given point on the surface of the cochlea depend on the intensity ( $L$ ) and frequency ( $f$ ) of the sound. When the frequency is fixed, the two CM potential parameters (amplitude and phase) depend on only parameter  $L$ . Typical changes in amplitude and phase over time registered at two different acoustic wave frequencies (260 and 8000 Hz) for the same guinea pig are shown in fig. 14. For this data, graphs of CM potential rms and phase depending on the level of sound intensity are shown in Fig. 15.

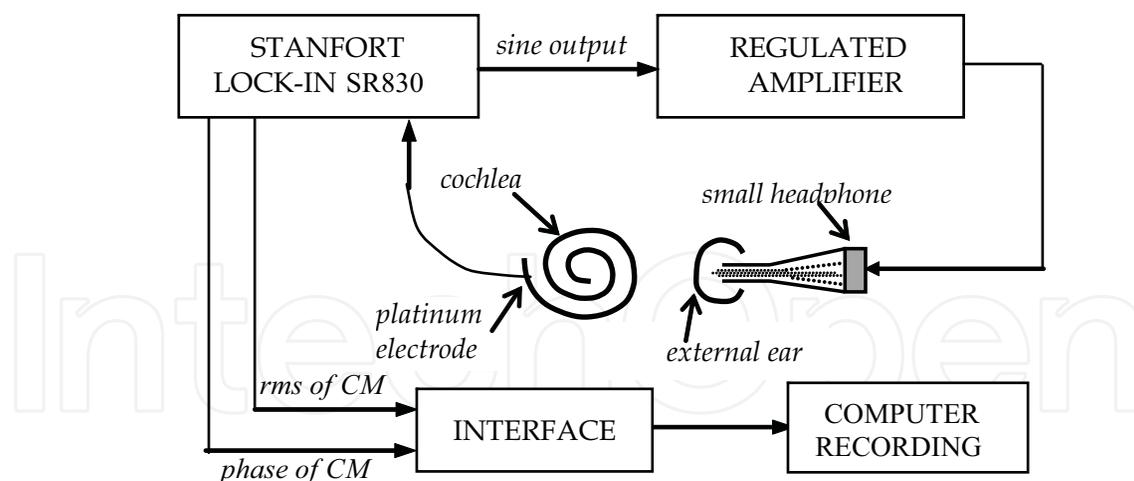


Fig. 13. Experimental setup for measuring CM potentials

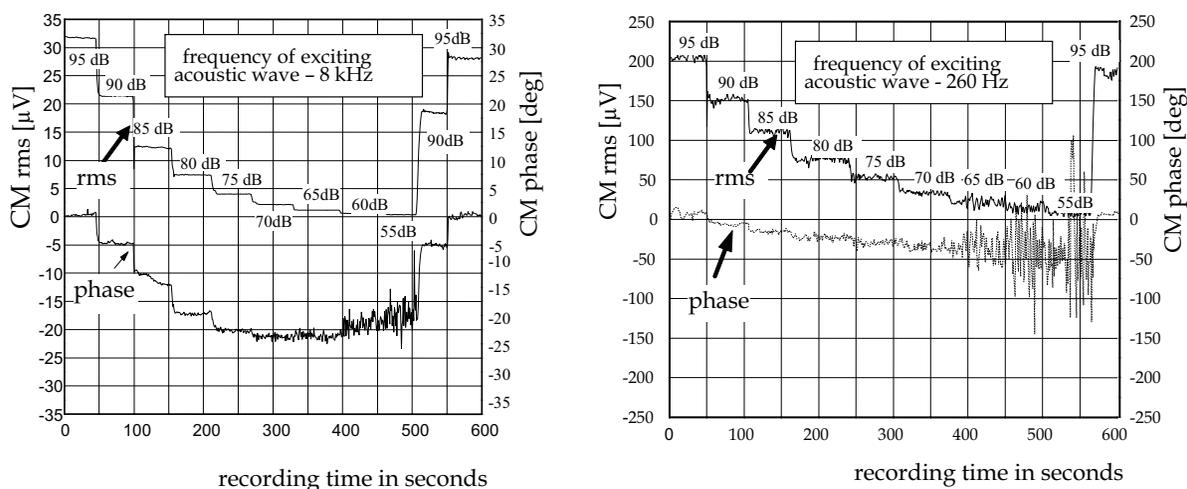


Fig. 14. Exemplary changes in CM rms and phase depending on sound intensity (sound levels were changed by 5 dB every 50 seconds)

Cochlear microphonic potentials are believed to be generated by the outer hair cells (OHCs). The latter are situated in three rows on the basilar membrane. All the OHCs have tiny strands (numbering about a hundred) called stereocilia. The apex of each single stereocillium lies in the tectorial membrane. In the resting state the stereocilia of each single cell form a conical bundle. During the acoustic excitation of the cochlea the stereocilia may dance about wildly. This alternating motion causes the channels in the stereocilia to open and close, providing a route for the influx of  $K^+$  ions. The upper part of the OHCs acts as a resistor whose resistance changes according to the mechanical movements of the stereocilia. Changes in this resistance cause changes in extra-cellular currents. The measured CM potential is the result of the flow of extra-cellular currents through the input resistance of the lock-in amplifier.

The place theory suggests that a tone of a defined frequency excites mainly the OHCs located on the basilar membrane in a place specific for the given frequency (CF). The OHC electrical activity picked up from a given place on the cochlea surface is the vector sum of the extra-cellular currents generated by the particular OHC cells belonging to the given CF area (probably oval in shape). As the excitation wave intensity increases, extra-cellular

currents are generated by an increasing number of OHC cells within the same CF area, which results in an increase in CM amplitudes. The phase changes registered then probably correspond to the shifts of the centre of the extra-cellular currents within the CF area.

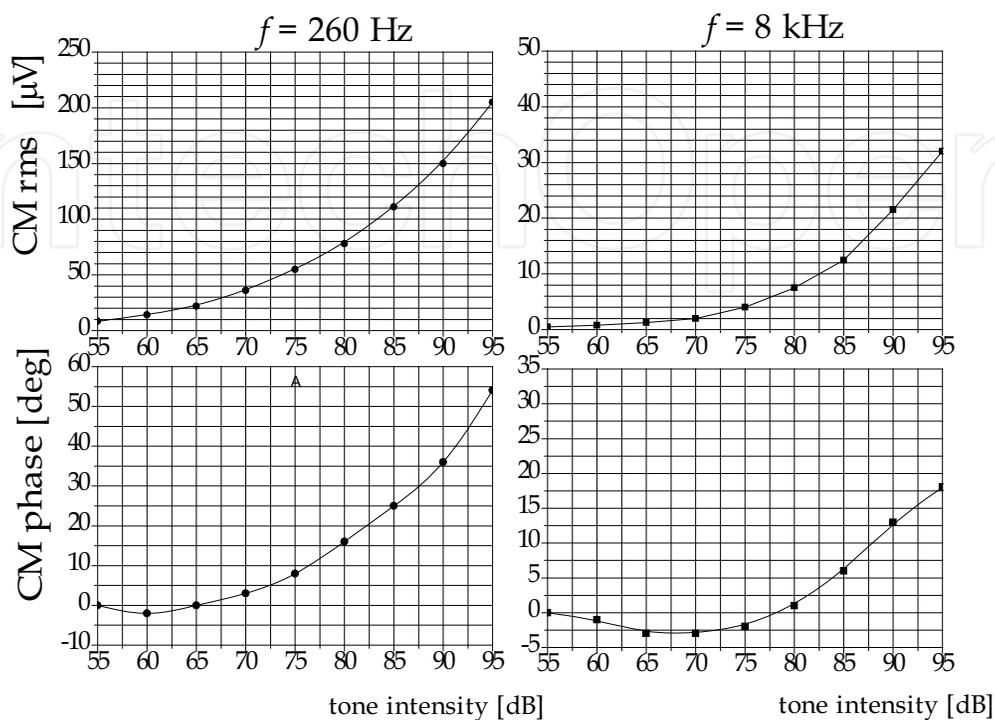


Fig. 15. Output-input characteristic obtained from traces shown in Fig. 14

### 3.6 Changes in amplitude and phase of CM potentials as result of laser irradiation

A focused laser beam can be a precise surgical scalpel. Perkins was the first to describe the use of a laser (an argon laser to be precise) in the surgical treatment of otosclerosis (Perkins, 1980). Since that time several kinds of laser (Ar, KTP, CO<sub>2</sub>, Er) have been used in ear microsurgery. Vollrath and Schreiner were the first to use the rms of cochlear microphonics to estimate the effect of the argon laser beam on the electrical response of the cochlea in guinea pigs (Vollrath & Schreiner, 1982). The PSD technique enables the recording of the simultaneous changes in amplitude and phase of the CM potential during laser irradiation. The information about cochlear activity acquired in this way is more detailed.

Studies of the effect of Ar laser irradiation on the electrical activity of the cochlea have been described by us in several papers. We used the double PSD technique to record CM potentials prior to, during and after argon laser irradiation of the cochlea in guinea pigs. The goal of the studies was to determine safe laser parameters for argon laser stapedotomy, taking into account changes in not only the rms of CM potentials but also in their phase. In our experiments we used a CW argon laser with adjusted output power (0.1 – 3.0 W). An electronically controlled mechanical chopper was used to obtain laser light pulses differing in their parameters (the duration of a single laser pulse, the time interval between the successive pulses, the number of pulses in a series). Via a 200 µm optical lightguide the laser pulses would be delivered to the cochlear bone (near the round window) of an anaesthetized guinea pig with the surgically opened bulla. Exemplary traces selected from many different recordings are shown in fig. 16.

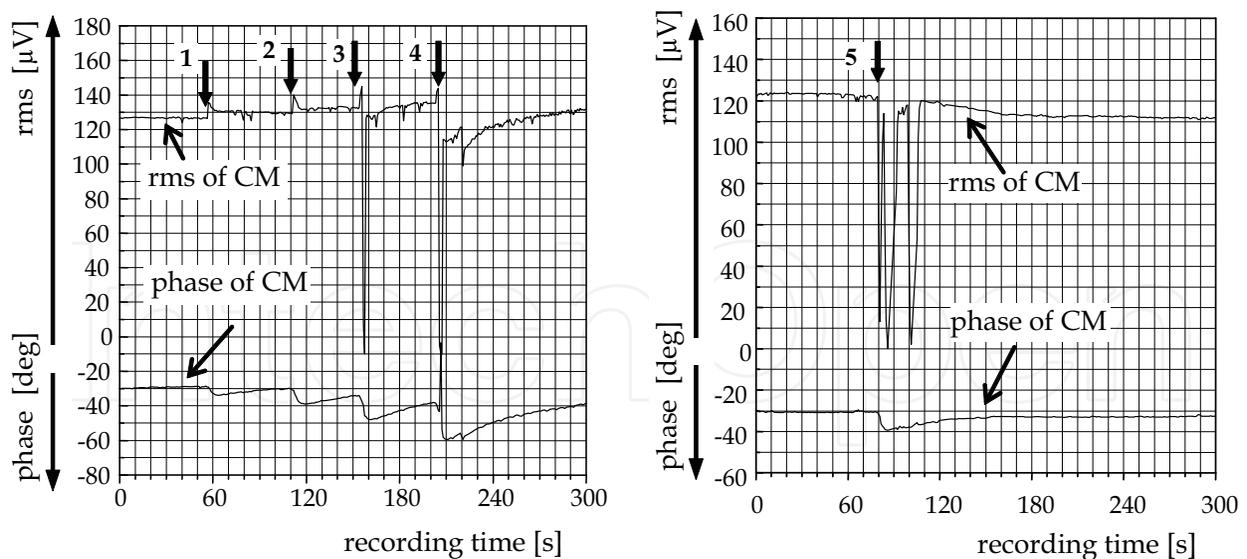


Fig. 16. Changes in rms and phase of CM potentials evoked by 80 dB acoustic wave of 1 kHz frequency during Ar laser pulse irradiation of 0.27 W (left) and 0.48 W (right) peak power. Irradiation parameters: 1 - single pulse of 0.5 s duration, 2 - single pulse of 0.5 s duration, 3 - single pulse of 1 s duration, 4 - two pulses of 1s duration with 1s interval between them, 5 - single pulse of 0.5s duration

It was found that in each registration the phase and amplitude of CM potentials changed during a laser pulse. The characteristic of the phase changes is always the same and diminishes relative to the initial (prior-to-irradiation) phase (in fig. 16 the initial phase was assumed to be equal to  $-30^{\circ}$ ). The character of changes in CM rms depends on the peak power of the pulses used. Two characteristic peak power levels:  $P_1$  and  $P_2$  can be distinguished. When the peak power of the pulses is lower than  $P_1$ , laser irradiation results in a small increase in CM rms. This may be due to the slight increase in the temperature of the cochlea and to a biostimulating effect. After the first peak, but still below the second one ( $P_2$ ), a sharp drop (even down to zero) in CM rms occurs. The drop is temporary and the cochlea quickly recovers its initial activity. Beyond  $P_2$ , changes in the electrophysiological activity of the cochlea are irreversible. As for today, the observed changes in the phase of CM potentials are hard to explain. It remains unknown why low-level laser radiation activates other groups of OHC cells in the CF area.

#### 4. Double PSD technique in studies of DPOAE

##### 4.1 Evoked otoacoustic emission

Evoked otoacoustic emissions (EOAE) are acoustic waves present in the external auditory canal after the cochlea is stimulated with an acoustic excitation wave. Depending on the excitation, different kinds of emission can be distinguished. If the stimulating signal is constant, then the emission is called *simultaneous evoked otoacoustic emission* (SEOAE). When pulse stimulating (clicks) sounds are used and the emission is registered between the clicks, the emission is called *transiently evoked otoacoustic emission* (TEOAE). If dual-tone stimulation (by two sinusoidal waves with respectively frequencies  $f_1$  and  $f_2$  and levels  $L_1$  and  $L_2$ ) is used, then the emission is called *distortion product otoacoustic emission* (DPOAE).

Otoacoustic emission was predicted by Gold as early as in 1948 (Gold, 1948). Thirty years later Kemp published a paper in which he described experiments proving the existence of this phenomenon (Kemp, 1978). He used clicks of 0.2 ms duration at a repetition rate of 16/s. In-between the successive pulses he recorded (with an electret microphone) acoustic wave pressure fluctuations at the outlet of the external acoustic canal. By applying an averaging procedure to the two-minute recordings he was able reduce the noise level to 0 dB SPL and reveal the backward signal which originated from the cochlea stimulated by the click. A few hundreds of works on this subject have been published since the first paper by Kemp. New experimental data are reported but their interpretations are not always explicit and mutually consistent. Despite the fact that the DPOAE mechanism is not yet fully understood, DPOAE signal estimation is a method of testing the human peripheral auditory function. The method is widely used in newborn hearing screening tests.

The presence of components which are absent in the stimulating acoustic wave is distinctive of DPOAE. The components result from the mechanical activity of the organ of Corti and are transmitted in the reverse direction through the middle ear and the tympanic membrane. Among the few possible products of cochlear nonlinearity, the acoustic wave  $f_3 = 2f_1 - f_2$  is most widely examined because of its highest acoustic pressure level.

All the DPOAE acoustic waves are studied after their transduction into electric signals by a microphone. The microphone must be of high sensitivity and with a linear dynamic reserve (about 80 dB). The same requirements apply to the input preamplifier and the lock-in voltmeter amplifier since the measured DPOAE electrical signals cannot result from measuring system nonlinearity. The microphone placed in the external auditory canal transduces acoustic waves into electrical signals: both primary tones of 60-70 dB and reverse DPOAEs of 0 - 20 dB. Also floor noise occurs in the external ear canal. The apparatus used for measuring DPOAE must eliminate all undesirable signals with frequencies different than the frequency of the signal to be measured.

Otoemissions are examined after they have been converted in very accurate electric microphones. The biggest problem faced when examining DPOAEs is their extremely low level in comparison with the excitation waves. The difference may reach 30-60 dB. The phase-sensitive detection of DPOAE is therefore very useful. A basic experimental setup for measuring DPOAE signals is shown in fig. 17.

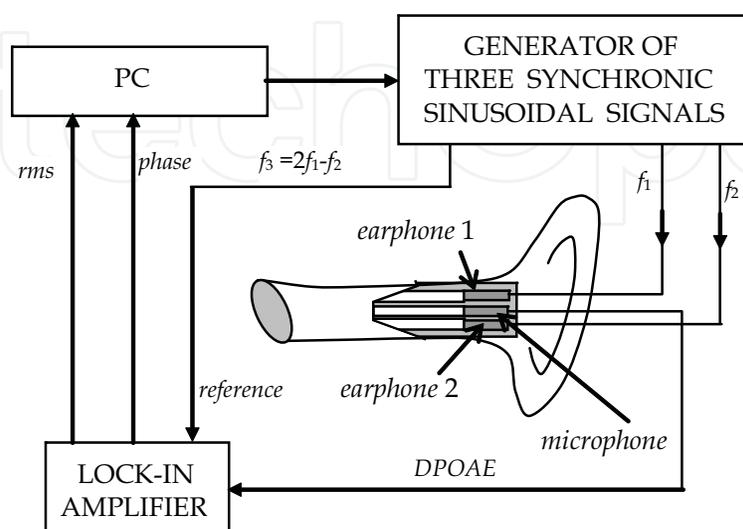


Fig. 17. Basic experimental setup for measuring DPOAE signals, using double PSD technique

The main unit of the experimental setup is a generator of three synchronous sinusoidal signals. The three frequencies are synchronized by a 18 MHz clock, whereby weak DPOAE signals can be measured using the PSD technique. The DPOAE response is measured by means of a probe which contains two miniature earphones and a low noise microphone. From the generator, pure tones with frequencies  $f_1$  and  $f_2$  are fed to the earphones. The two primary tones are digitally synthesized. The amplitudes, phases and frequencies of the tones are regulated by a PC with dedicated software. The software also enables the acquisition of the amplitude and phase of the DPOAE signals during measurements.

#### 4.2 Previous techniques of measuring DPOAE

As mentioned earlier, DPOAE signals are of very low level, even if they are evoked in an unimpaired ear. Several signal processing techniques for the measurement of DPOAE signals under a large amount of noise and primaries of 60-70 dB have been developed. Initially, the Fast Fourier Transform (FFT) was used as the main signal processing tool for improving the signal-to-noise ratio in order to better estimate the level of DPOAE signals. In this method, the signals are first divided into data blocks and then averaged over time. For better reduction of the overall background noise long measurement time is required, which increases the amount of recorded data to be averaged. The FFT method requires about 10 seconds of block data. During long DPOAE recording, transient artefacts (e.g. talking, head movements) may occur, which when averaged together with the measuring signal may degrade the accuracy of the signal. Besides, the averaging method is incapable of measuring rapid changes of DPOAE signals.

In the first decade of the 21st century several novel methods of measuring DPOAE signals were developed (Ziarani & Konrad, 2004; Li et al., 2003). In comparison with the conventional methods, the new methods offer a shorter measurement time, which is of significance for clinical examinations. In addition, these methods are more immune to artefact and background noise. Thanks to the new methods it is possible to continuously record DPOAE signals. Besides offering the above advantages, the double PSD technique enables the simultaneous measurement of amplitude and phase of DPOAE signals. The two DPOAE parameters can be measured in a very short time, even below 10 ms.

In screening protocols typically a few pairs of primary tones with fixed acoustic levels are used and the responses are analyzed one after another (sequentially). In order to reduce the examination time the multiple-tone pairs method can be employed. In this method, DPOAE signals are evoked simultaneously by three or four pairs of two-tones. This method reduces measurement time but has a limited use.

#### 4.3 DPOAE measurement using double PSD technique

Before the PSD technique was introduced to measure DPOAE signals it had been assumed that the amplitude and frequency of DPOAE signals depended on four acoustic parameters of the stimulating signals (primaries), i.e. the amplitude and frequency of each of the two signals. The DPOAE phenomenon itself is investigated according to the procedure described below. First the  $f_2/f_1$  ratio (usually 1.22) and the stimulating signal intensity levels (e.g.  $L_2/L_1 = 60\text{dB}/65\text{dB}$ ) are fixed. For the frequency of one of the stimulating waves (usually  $f_2$ ) several discrete values are set while the frequency of the other wave is changed in small steps, e.g. 1/3 octave-bands centred around the fixed  $f_2$  (Wagner et al., 2008). When examining the effect of different internal (e.g. age, gender) and external (e.g. industrial

noise, medicines) factors, DPOAE is measured in the same stimulation conditions before and after the stimulus acts. Most experimental works in this field describe measurements of solely the amplitude of DPOAE signals. Some works also dealt with the phase of DPOAE signals, but it was measured in an indirect way, using signal processing methods. The phase-sensitive technique enables the simultaneous measurement of the amplitude and phase of DPOAE signals, with no need to use complex signal processing methods. The measurement takes place in real time.

Figure 18 shows an exemplary record of the simultaneous changes in the amplitude and phase of DPOAE signals caused by changes in the acoustic parameters of the stimulating waves. The recording was made in real time using the measuring setup shown in fig. 17. In the whole course of recording the combination frequency ( $f_3$ ) remained constant at 3749 Hz while the other parameters were changed every 20 seconds in a specified sequence. The whole 980 second long recording time had been divided into seven 140 long time intervals in which parameter  $k = f_2/f_1$  assumed the consecutive values: 1.10, 1.15, 1.20, 1.25, 1.39, 1.35, 1.40. The following seven combinations of stimulating wave levels: 1 - (55 dB, 55 dB), 2 - (55 dB, 60 dB), 3 - (60 dB, 55 dB), 4 - (60 dB, 60 dB), 5 - (65 dB, 60 dB), 6 - (60 dB, 65 dB) and 7 - (65 dB, 65 dB) were fixed for each value of parameter  $k$ . In each of the combinations, the dB SPL of primary  $f_1$  is in the first place. During the 980 second long recording the parameters of the primaries were changed 49 times in total.

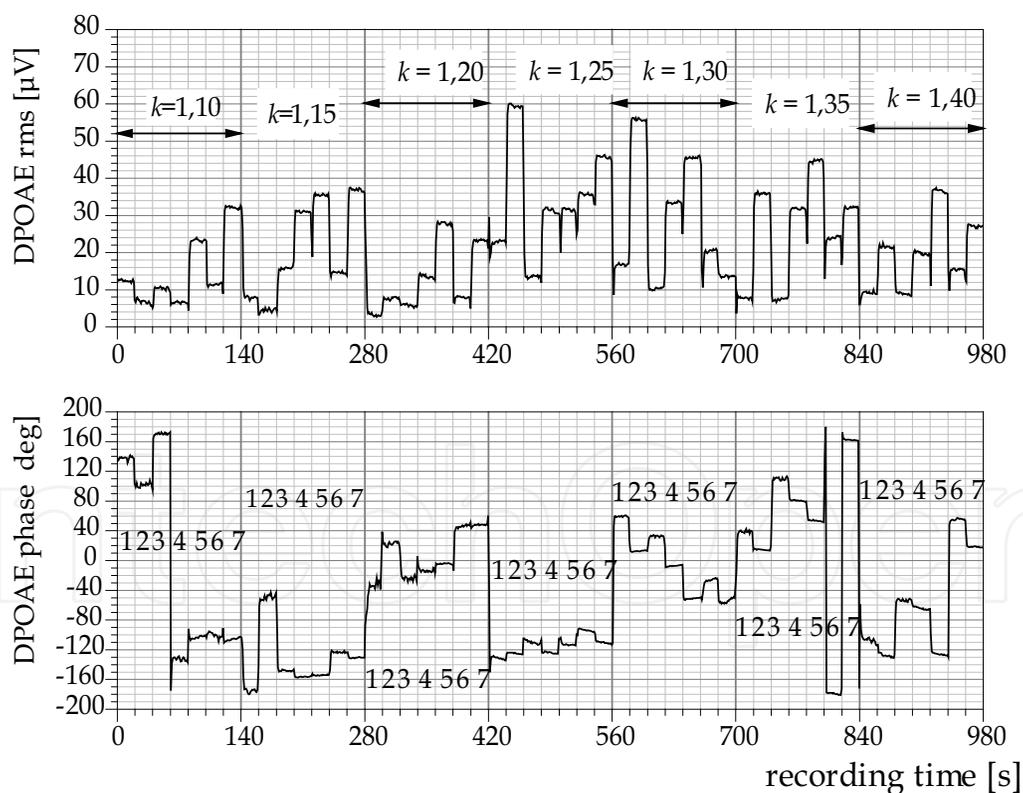


Fig. 18. Simultaneous changes in rms and phase of DPOAE signals, caused by fixed sequence of changes in parameters of primaries. Numbers 1 – 7 denote following combinations of primary frequencies  $L_{1dB} / L_{2dB}$  levels: 1 - (55 dB, 55 dB), 2 - (55 dB, 60 dB), 3 - (60 dB, 55 dB), 4 - (60 dB, 60 dB), 5 - (65 dB, 60 dB), 6 - (60 dB, 65 dB) and 7 - (65 dB, 65 dB). The same combinations of levels were used for each value of parameter  $k=f_2/f_1$

The measurements showed that each change in the value of one of the parameters of the primaries results in a change of both the amplitude and phase of the DPOAE signal. Moreover, the character of the changes depends on the ontogenetic traits.

Thanks to the phase-sensitive technique one can determine the effect of the initial phase of each of the primaries on the amplitude and phase of DPOAE signals. For this purpose a generator of three synchronous sinusoidal signals was incorporated into the setup shown in fig. 17. The generator offers the possibility of fixing not only the amplitude and frequency of each of the primaries, but also the initial phase of each of the signals.

Five parameters of the primaries, i.e. the amplitude and frequency of each of the signals and the initial phase of one of the signals were fixed. The sixth parameter, i.e. the initial phase of the second primary was changed in a range of 0 - 360 degrees. The phase was changed in steps of 22.5 degrees. Exemplary measurements are shown in figs 19 and 21. Each of the figures comprises four panels. On the left side of each of the figures there are two panels showing experimentally determined changes in the amplitude and phase of DPOAE signals, caused by changes in the initial phase of primary  $f_1$  (fig.19) or primary  $f_2$  (fig.21). The data were obtained for combination frequency  $f_3 = 3749$  Hz, parameter  $k = f_2/f_1 = 1.25$ , intensity levels  $L_1/L_2 = 65/55$  dB and the zero initial phase of primary  $f_1$  (fig.19) or  $f_2$  (fig.21).

The graphs in the panels on the right side of each of the figures were plotted on the basis of formulas (8) - (10), but the values of some of the constants in the formulas were matched to obtain agreement with the experimental traces.

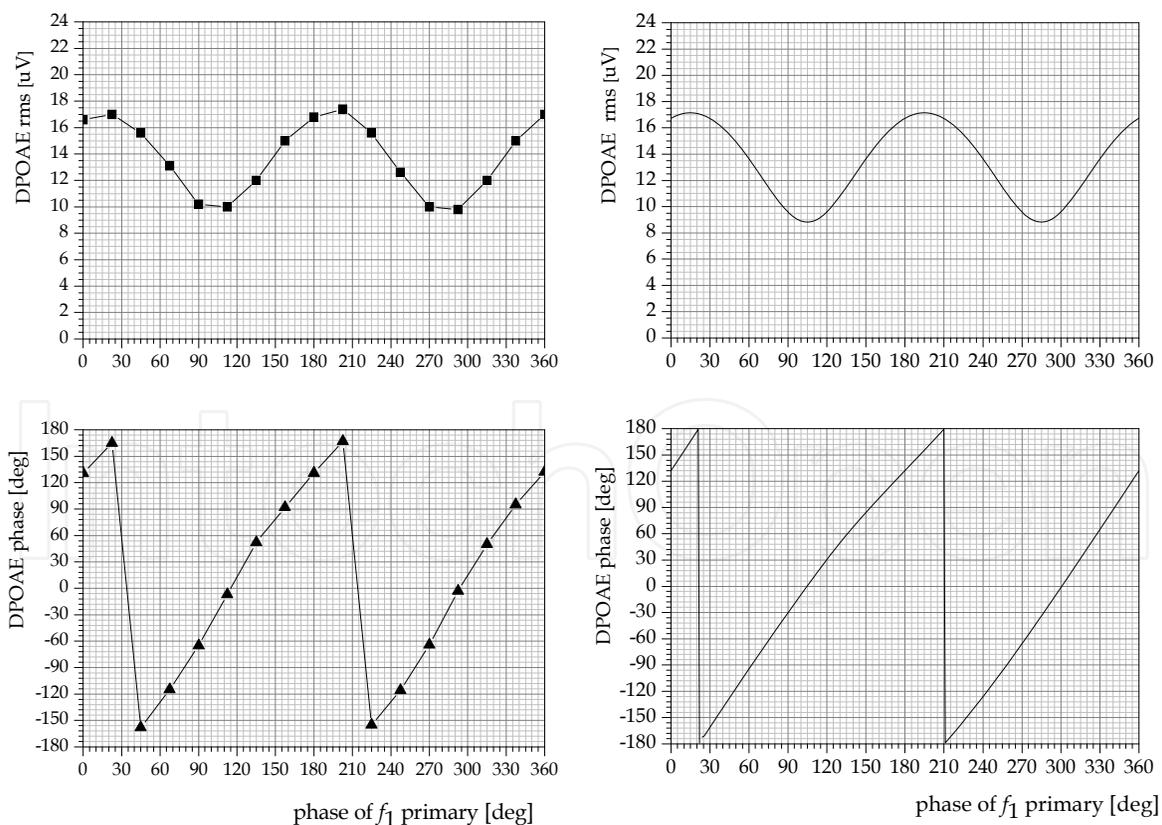


Fig. 19. Simultaneous changes in amplitude (upper panels) and phase (lower panels) of DPOAE signals, caused by changes in initial phase of primary  $f_1$ , obtained from experiment (left) and theoretically (right) (details in text)

Currently, it is generally believed that the DPOAE signal induced in the external acoustic canal by a double-tone is composed of two backward travelling waves (e.g. Knight & Kemp, 2000). The primary wave arises in the place where the two regions ( $CF_1$  and  $CF_2$ ) characteristic of frequency  $f_1$  and  $f_2$  overlap (but much more closer to  $CF_2$ ). The wave propagates in the basilar membrane towards both the cochlea's base and its apex. The wave directed towards the apex bounces off in the region characteristic of frequency  $f_3$  ( $CF_3$ ) and propagates towards the base. Thus two waves with the same frequency  $f_3$ , but shifted in phase relative to each other, propagate towards the cochlea's base. Depending on the difference between the two waves, destructive or constructive amplitude interference occurs.

There are two different theories in the literature, concerning how the waves propagate backward from their generation places (He at all, 2007). According to one theory, the two waves propagate as compression waves to the cochlear base via the cochlear fluids. According to another theory, the two waves are transverse waves slowly propagating along the basilar membrane. Currently the prevailing view is that two backward waves, being transverse waves in the basilar membrane, arise in the cochlea excited by two tones. Taking into consideration this view and the previously determined sites where the backward waves arise, the schematic shown below (fig. 20) was drawn.

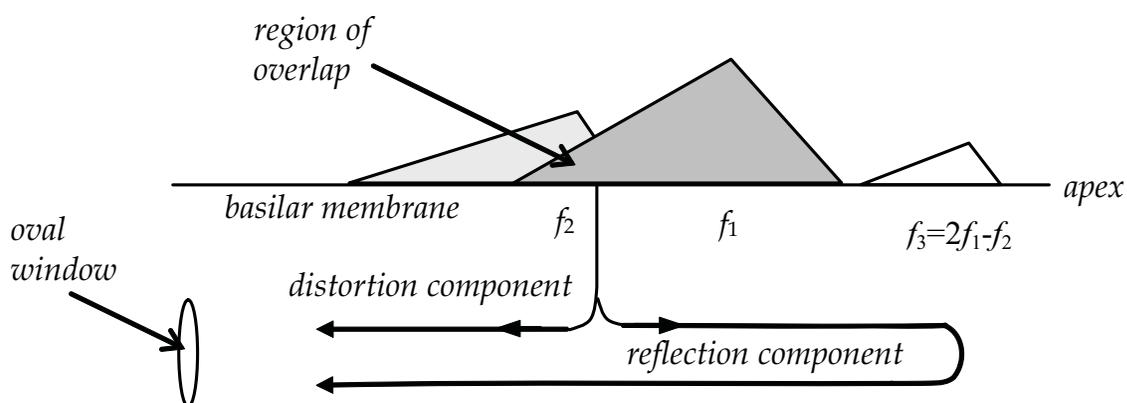


Fig. 20. Schematic diagram of source of two backward travelling waves whose interference produces DPOAE wave in auditory canal

The resultant wave near the oval window can be written as

$$A_w(t) = A_m \cos(\omega_3 t + \alpha_{10} + \kappa + 2\beta_1 - \beta_2), \quad (8)$$

where

$$A_m = A_{1m} \sqrt{1 + K^2 + 2K \cos \alpha_r}, \quad (9)$$

$$\kappa = a \tan \left( \frac{\sin \alpha_r}{K^{-1} + \cos \alpha_r} \right) \quad (10)$$

$A_{1m}$  - the amplitude of the primary wave,

$K = A_{2m}/A_{1m}$ ,

$\alpha_{10}$  - the initial phase of the primary wave,

$\beta_1, \beta_2$  - the phases induced by the initial phases of the primaries,

$\alpha_r$  - a phase difference between the primary and secondary wave, due to the path length distance.

It follows from formula (9) that the amplitude of the resultant wave does not depend on the the initial phases of the primaries, and the phase of the resultant wave:

$$\Omega = \alpha_{10} + \kappa + 2\beta_1 - \beta_2 \quad (11)$$

(directly measured by the lock-in amplifier) is a linear function of the stimulating waves phase. However, experimental results do not corroborate the above dependence. The amplitude of the DPOAE signal turns out to be a function of the initial phases of the stimulating signals, and the measured phase is only approximately a linear function of the initial phases (panels on the left side of figs 19 and 21). If it is assumed that angle  $\alpha_r$  changes in the same way as the initial phase of the primary, full agreement between the experimental traces and the ones determined from formulas (8) and (10) is obtained. This is shown in the panels on the right side of figs 19 and 21. The graphs on the right side of fig. 19 were plotted on the basis of formulas (8) and (10), assuming  $\alpha_r = 2\beta_1$ , while the graphs on the right side of fig. 21 were plotted assuming  $\alpha_r = -\beta_2$ .

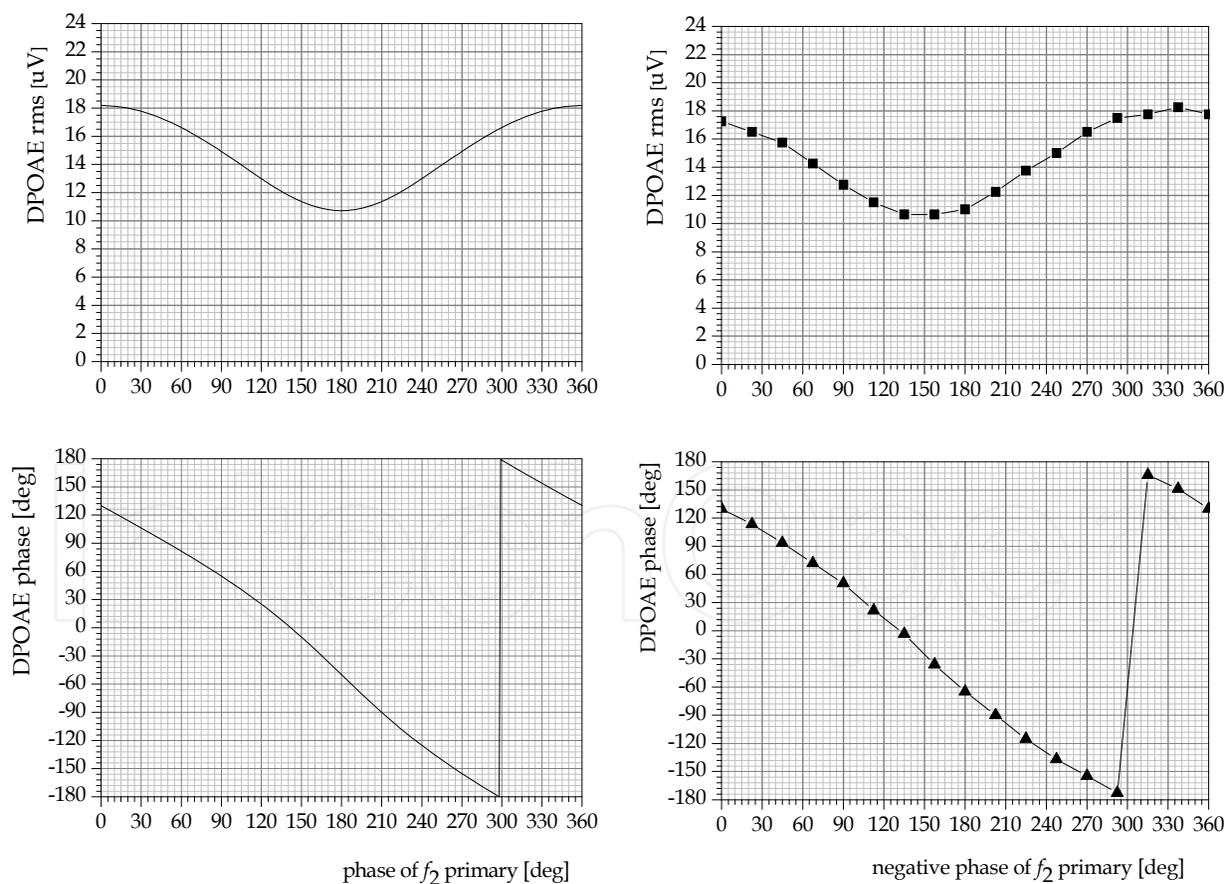


Fig. 21. Simultaneous changes in amplitude (upper panels) and phase (lower panels) of DPOAE signals, caused by changes in initial phase of primary  $f_2$ , obtained in experiment (left) and theoretically (right) (details in text)

It follows from formula (9) that the ratio of the maximum amplitude of the DPOAE wave ( $A_{wmax}$ ) to the minimum value ( $A_{wmin}$ ) amounts to

$$\frac{A_{wmax}}{A_{wmin}} = \frac{1+K}{1-K} \Rightarrow K = \frac{A_{2m}}{A_{1m}} = \frac{A_{wmax} - A_{wmin}}{A_{wmax} + A_{wmin}} \quad (12)$$

For the measurement conditions for which the traces shown in figs 19 and 21 were determined, it was calculated from formula (12) that  $K = 0.34$  and  $K = 0.27$  when respectively initial signal phase  $f_1$  and  $f_2$  is changed. This means that about 11.5% and 7.3% of the primary wave energy is reflected from region CF<sub>3</sub> in respectively the former and latter case.

The preliminary measurements shows that there is a certain mechanism in the Corti organ, which is responsible for the fact that a change in the phase of one of the stimulating signals (i.e. phase modulation) causes the amplitude modulation of the DPOAE signal. Further research is needed to explore this mechanism, but already at this stage one can say that the PSD technique proposed by the authors will play a major role in the exploration of this mechanism.

#### 4.4 Simultaneous measurements of DPOAE and CMDP, using double PSD technique

Much of the experimental research reported in the world literature indicates that the main source of CM signals and DPOAE waves are OHCs. Thanks to the use of phase-sensitive detection in the measurement of each of the signals one can observe the simultaneous changes in the amplitude and phase of the two signals, resulting from changes in the parameters of the primaries. The measuring setup used for this purpose is shown in fig. 22. The setup incorporates two patents developed by the authors.

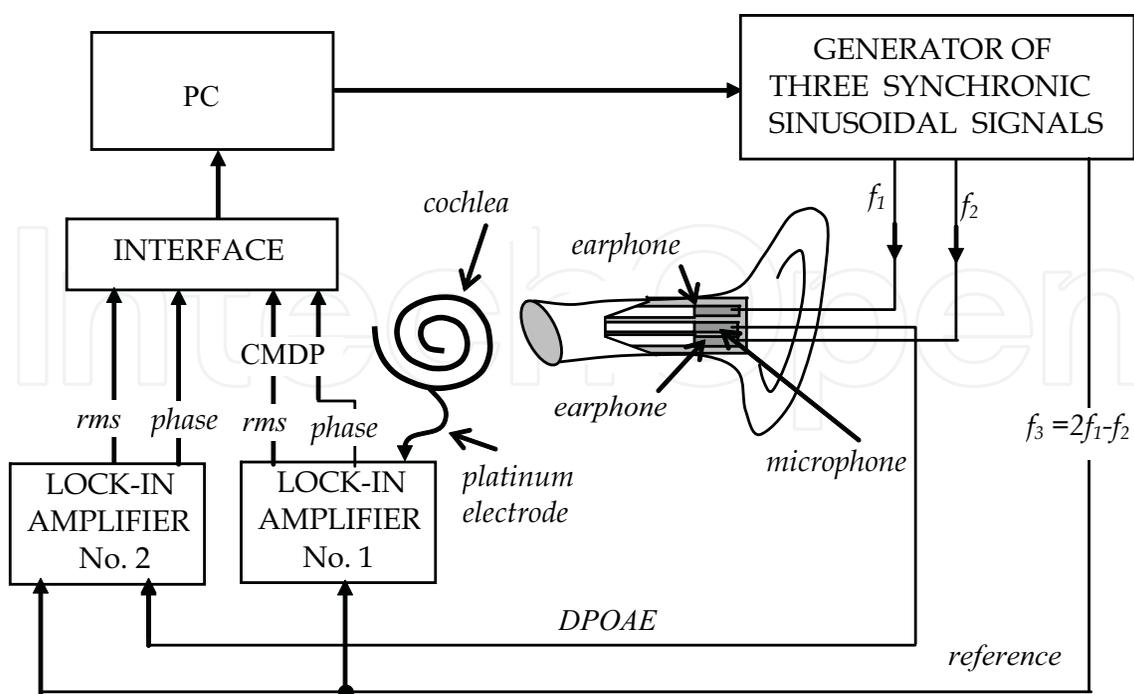


Fig. 22. Experimental setup for simultaneous measurement of amplitude and phase of DPOAE and CMDP signals

Two lock-in amplifiers, one for measuring the rms and phase of the DPOAE signal (amplifier No. 2) and the other for measuring the rms and phase of the CMDP signal (amplifier No. 1), have been incorporated into the setup. The CMDP signal is the distortion product in cochlear microphonics. The same signal (with combination frequency  $f_3$ ) from the generator is fed to the reference inputs of each of the amplifiers. The reference input of lock-in No.2 can also be successively fed signals with stimulation frequencies  $f_1$  and  $f_2$  and combination frequency  $f_3$  and the rms and phase of three CM signals with different frequencies can be measured. Measurements made in this way may provide a fuller picture of the cochlea functions.

The above setup was used to measure changes in the rms and phase of both DPOAE and CMDP signals, caused by changes in the excitation parameters. Four anaesthetized guinea pigs with the positive Preyer reflex were subjected to the experiments. Recordings were made at the following combination frequencies: 1312, 1875, 2671, 3749 and 5342 Hz. The parameters of the primaries were changed as in sect. 4.3, i.e. one of the parameters of the primaries was changed every 20 seconds in a specified way. Exemplary traces recorded for frequency  $f_3 = 1875$  Hz are shown in fig. 23.

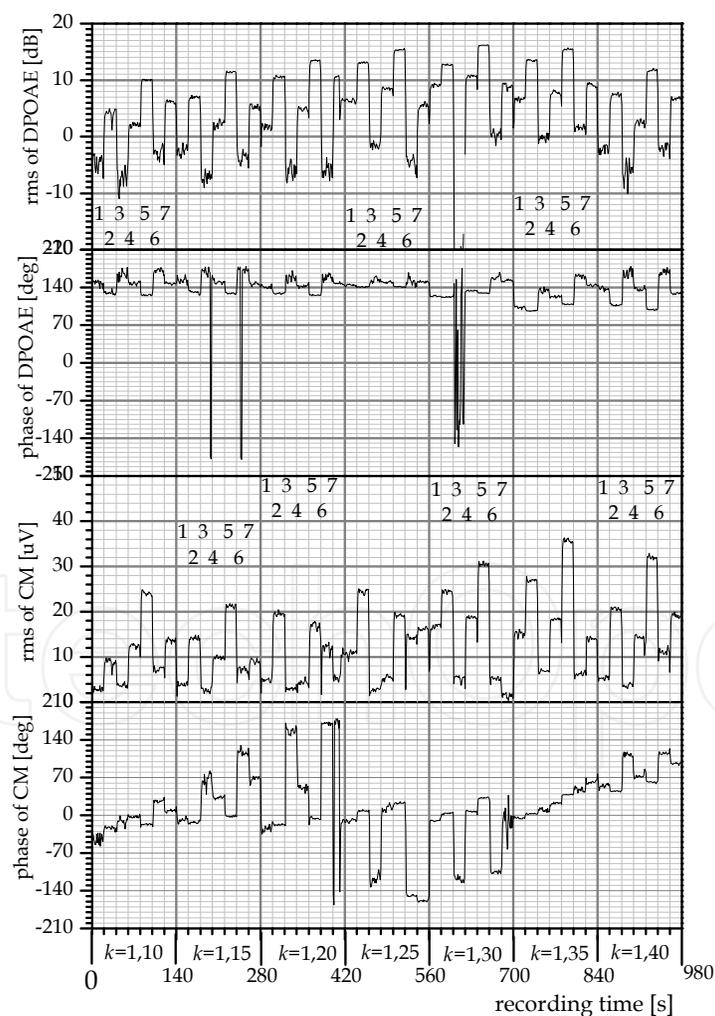


Fig. 23. Simultaneous changes in rms and phase of both DPOAE and CMDP, induced by changes in parameters of primaries

Nearly 100% correlation between the DPOAE rms and the CMDP rms was found, i.e. when after a change in one of the parameters of the primaries the DPOAE rms increased, then CMDP rms would also increase. Unfortunately, there was no such correlation between the phases of the two signals. This situation is well illustrated by the records of the changes, shown in fig. 23.

## 5. Conclusion

Practically all the ways of measuring biological acoustic waves in the cochlea, in which the phase-sensitive detection technique can be applied, have been described. Exemplary experimental results coming from many different measurement cycles carried out by the authors on guinea pigs in the last nearly 20 years were presented to demonstrate the measuring possibilities offered by the PSD technique. The latter's main advantage is that very weak (even below the ambient noise level) electrical signals can be measured in a very short time (in the order of milliseconds). A minor limitation of this technique is that it is applicable to objects to whose input periodical signals are fed from the outside.

In many investigations into the electrophysiological function of the cochlea it is essential not only to simultaneously measure the amplitude response, but also the phase response to the stimulation. This is undoubtedly another advantage of the PSD technique.

Much more difficult than the measurement of the phase is the interpretation of its changes. As for now, it is not always possible to interpret the observed changes, which particularly applies to DPOAE. This phenomenon has been known for over 30 years, but it still has not been fully explored. The great worldwide interest in this subject is reflected in the large number of publications devoted to it. The interest stems from the fact that for many years DPOAE measurements have been part of hearing screening tests during which DP-grams are recorded. This especially applies to newborns and people with mental disabilities, in which cases it is impossible to record audiograms. Besides gaining an insight into the nature of the DPOAE phenomenon, it is essential to determine the correlation between the DP-gram and the audiogram. In the authors' opinion, the phase-sensitive detection technique represents a new tool for investigating electrophysiological phenomena in the cochlea and it will contribute to the better understanding of the phenomena taking place in this organ.

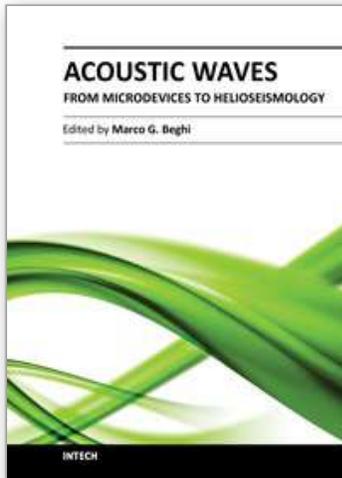
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## **Acoustic Waves - From Microdevices to Helioseismology**

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The concept of acoustic wave is a pervasive one, which emerges in any type of medium, from solids to plasmas, at length and time scales ranging from sub-micrometric layers in microdevices to seismic waves in the Sun's interior. This book presents several aspects of the active research ongoing in this field. Theoretical efforts are leading to a deeper understanding of phenomena, also in complicated environments like the solar surface boundary. Acoustic waves are a flexible probe to investigate the properties of very different systems, from thin inorganic layers to ripening cheese to biological systems. Acoustic waves are also a tool to manipulate matter, from the delicate evaporation of biomolecules to be analysed, to the phase transitions induced by intense shock waves. And a whole class of widespread microdevices, including filters and sensors, is based on the behaviour of acoustic waves propagating in thin layers. The search for better performances is driving to new materials for these devices, and to more refined tools for their analysis.

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No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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